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Cognitive Neuroscience and the Study of Memory

Review

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The neurosciences have grown rapidly over the last half century. This growth has been stimulated by two important developments. First, molecular biology has transformed cellular neurobiology and has led to a new conceptual framework for signaling, a molecular framework that encompasses not only signaling in nerve cells but in all the cells of the body. Second, work on brain and cognition, which was traditionally associated with a number of different disciplines, has merged into a single discipline: cognitive neuroscience. This has provided a new framework for the study of memory, perception, action, language, and perhaps even conscious awareness.

In this review, we will consider the second development by focusing on one aspect of cognitive neuroscience: recent progress in memory research. In so doing, we also want to consider the broader question: to what degree can these two independent and disparate strands—molecular neurobiology and cognitive neuroscience—be united? Can molecular biology enlighten the study of cognitive processes, such as learning and memory, as it has other areas of biology, such as development? In turn, can cognitive neuroscience define novel phenomena that will lead to a completely new set of molecular mechanisms and insights?

The Emergence of Cognitive Neuroscience

Cognitive neuroscience originated in two disciplines: in *psychology*, in the development of rigorous methods for analyzing behavior and cognition, and in *systems neurobiology*, in the effort to understand the structure and function of neuronal circuits of the sensory and motor systems of the brain. The fusion of these two disciplines was facilitated as well by the emergence of a coherent neuroscience—an interdisciplinary approach to the nervous system that encouraged the idea that the techniques and concepts of neurobiology and systems neuroscience might be usefully applied to the analysis of cognition.

Until the beginning of the nineteenth century, the study of normal mental activity was a part of philosophy, and the chief method for understanding the mind was introspection. By the middle of the nineteenth century, introspection began to give way to experimental approaches that eventually led to the independent discipline of experimental psychology. In its early years, experimental psychology was concerned primarily with the study of sensation, but by the turn of the century the interests of psychologists turned to behavior itself—learning, memory, attention, perception, and voluntary action.

The development of simple experimental methods for studying learning and memory—first in humans by Hermann Ebbinghaus in 1885 and a few years later in experimental animals by Ivan Pavlov and Edgar Thorndike—led to a rigorous empirical school of psychology called *behaviorism*. Behaviorists, notably James B. Watson and Burrhus F. Skinner, argued that behavior could be studied with the precision achieved in the physical sciences, but only if students of behavior abandoned speculation about what goes on in the mind (the brain) and focused instead on *observable* aspects of behavior. For behaviorists, unobservable mental processes, especially abstractions like perception, selective attention, and memory, were deemed inaccessible to scientific study. Instead, behaviorists concentrated on examining—objectively and precisely—the relationship between specific physical stimuli and observable responses in intact animals. Their early successes in rigorously studying simple forms of behavior, including learning, encouraged them to treat all processes that intervene between the stimulus (input) and behavior (output) as *irrelevant* to a scientific study of behavior. Thus, behaviorism largely ignored mental processes. As a result, the science of behavior was defined in terms of the limited techniques used to study it. This emphasis reduced the domain of experimental psychology to a restricted set of problems, and it excluded from study some of the most fascinating features of mental life.

By the 1960s, it was not difficult for the founders of *cognitive psychology*—George Miller, Ulric Neisser, Herbert Simon, and others—to convince the scientific community of the narrowness of behaviorism. These early cognitive psychologists, building on the earlier evidence from Gestalt psychology, European neurology, and work by the British psychologist Frederic Bartlett, sought to demonstrate that our knowledge of the world is based on our biological apparatus for perceiving the world, and that perception is a *constructive* process dependent not only on the information inherent in a stimulus but also on the mental processing of the perceiver. Thus, cognitive psychology was concerned not simply with specifying the input and output for a particular behavior but also with analyzing the process by which sensory information is transformed into perception and action—that is, with evaluating how a stimulus leads to a particular behavioral response. In redirecting scientific attention to mental operations, cognitive psychologists focused on *information processing*, on the flow of sensory information from sensory receptors to its eventual use in memory and action. It was implicit in the cognitive approach to behavior that each perceptual or motor act has an *internal representation* in the brain: a representation of information in patterns of neural activity.

Once cognitive psychologists acknowledged that internal representations are an essential component of

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behavior, they had to come to grips with the fact that most mental processes were still largely inaccessible to experimental analysis. Without direct access to the neural substrates of internal representations it was difficult, if not impossible, to understand the path from perception to action. At about this time, the work of Vernon Mountcastle on somatic sensation, David Hubel and Torsten Wiesel on vision, and Edward Evarts on the control of movement inaugurated the neuronal analysis of perception and voluntary action. Moreover, during the 1970s, Evarts and Mountcastle developed techniques for studying the activity of single cells in the brains of awake, behaving monkeys. In their hands, and in work that followed by Robert Wurtz, Apostolos Georgopoulos, William Newsome, and others, single-unit studies in monkeys led to the first correlations between cognitive processes (such as perception, attention, and decision making) and patterns of firing of individual cells in specific brain regions. This work changed the way behavior was studied both in experimental animals and in humans; the focus now was on the information processing in the brain that leads to behavior.

The need for greater anatomical knowledge led to a renaissance of neuroanatomy, evident in the development of new techniques for tracing connections between neurons by Sanford Palay at the NIH, Walle Nauta at MIT, Matthew and Jennifer LaVail at Harvard, and Max Cowan at Washington University. The search for new neuroanatomical methods and the need to bridge anatomy and function led to the application of neuroimaging techniques (positron emission tomography [PET] scanning and functional magnetic resonance imaging [MRI]) to cognitive problems. This major advance, pioneered by Marcus Raichle and Michael Posner and by Seiji Ogawa, Ken Kwong, and others, made it possible to relate changes in activity in large populations of neurons to specific cognitive acts in living humans. By comparing the results of cellular recordings in nonhuman primates and the results of neuroimaging in humans, it has become possible to study directly the neural correlates of sensory processing, motor actions, and cognitive processes.

In the 1960s and 1970s, there was also renewed interest in the traditional discipline of neuropsychology. Early students of brain and behavior like Karl Lashley and Donald Hebb used the term neuropsychology broadly to encompass studies of experimental animals as well as studies of humans. In this sense, cognitive neuroscience is the modern forum for the same topics and issues that engaged Lashley and Hebb earlier in this century. Studies of patients with brain injury or disease that affects mental function have always been a vital part of neuropsychology, and such studies formed one of the foundations of cognitive neuroscience.

As first clearly shown for language by Pierre Paul Broca in 1863, patients with lesions of specific regions of the brain exhibit quite specific cognitive deficits. Following Broca and Wernicke, the neuropsychological attempt at regional localization remained strong in Europe and in Canada but was in good part neglected in the United States, with the exception of the work of Arthur Benton, Hans-Lukas Teuber, and Norman Geschwind. As we shall see, continuing study of the behavioral consequences of brain lesions proved to be a rich source of information about the organization and anatomy of higher functions, including memory. Lesion studies have shown that cognition is not unitary but that there are several cognitive systems, each with independent information-processing modules. For example, the visual system of primates, a prototypical cognitive system, has specialized anatomical pathways for processing information about color, form, and movement.

Finally, computational science has made a distinctive contribution to cognitive neuroscience. Computers made it possible to model the activity of large populations of neurons and to begin to test ideas about how specific components of the brain contribute to particular cognitive processes. To understand the neural organization of a complex behavior like speech, we must understand not only the properties of individual cells and pathways but also the *network properties* of functional circuits in the brain. While network properties arise from the properties of individual neurons in the network, they need not be explainable in terms of the behavior of individual cells. Computational approaches are helpful for characterizing the system as whole, for obtaining formal descriptions of what the system is capable of doing, and for determining how the interacting constituent elements account for system properties.

This review focuses on the topic of memory, but one aspect of cognitive neuroscience. We have not attempted to document fully the remarkable progress that has been achieved in our understanding of how the nervous system learns and remembers. Rather, we focus on two key components in the study of memory, as viewed through the work that the three of us have carried out with our colleagues during the past several decades. The first component is concerned with analyzing what memory is, where it is stored, and what brain systems are involved. This is the *systems problem* of memory. The second component of memory is concerned with analyzing *how* memory is stored. This is the *molecular problem* of memory.

Where Are Memories Stored?

The question of where memory is stored emerged at the beginning of the 19th century as part of the larger question—to what degree can *any* mental process be localized within the brain? The first person to address this question was Franz Joseph Gall, who made two major conceptual contributions. First, Gall attempted to abolish mind–brain dualism. He argued, based on his anatomical studies, that the brain is the organ of the mind. Second, he appreciated that the cerebral cortex is not homogenous but contains distinctive centers that control specific mental functions. Gall therefore proposed the idea of cortical *localization*. Gall asserted that the brain does not act as a unitary organ but is divided into at least 27 faculties (others were added later), each corresponding to a specific mental faculty. He thought that even the most abstract and complex of human traits, such as generosity and secretiveness, are localized to discrete areas of the brain.

Gall was not an experimentalist. He rejected the study of neurological lesions and the surgical manipulation of experimental animals and instead attempted to locate

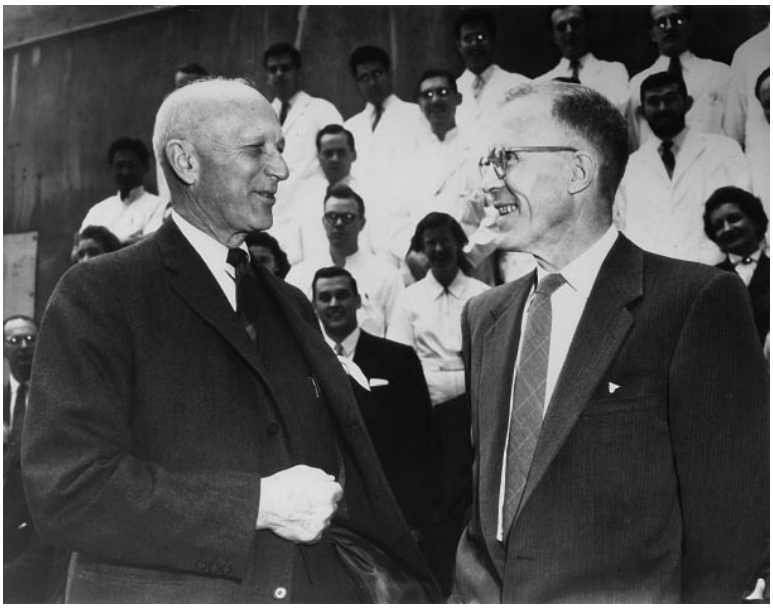

Figure 1. Hebb and Penfield

D. O. Hebb (right) and Wilder Penfield (left) in 1958 on the occasion of Hebb delivering the 24th Annual Hughlings Jackson lecture at the Montreal Neurological Institute.

mental faculties by examining the surface of the skulls of individuals well endowed with particular functions. Perhaps not surprisingly, with this approach he misidentified the function of most parts of the cortex. This anatomically oriented approach to personality Gall called *organology*. Later, Gall's associate, Gaspard Spurzheim, adopted the better-known term *phrenology* to describe this approach.

Gall's ideas were subjected to experimental analysis by Pierre Flourens in France in the late 1820s. Flourens attempted to isolate the contributions of different parts of the nervous system to behavior by removing from the brains of experimental animals the functional centers identified by Gall. From these experiments, Flourens concluded that individual sites in the brain are not sufficient for specific behaviors such as sexual behavior and romantic love and that all regions of the brain—especially the cerebral hemispheres of the forebrain—participate in every mental function. He proposed that any part of the cerebral hemisphere is able to perform all the functions of the hemisphere. Injury to a specific area of the cerebral hemisphere should therefore affect all higher functions equally.

Despite the findings of Broca and Wernicke on the localization of language, the ensuing debate between cortical localization and equipotentiality in cognitive function dominated thinking about mental processes, including memory, well into the first half of the twentieth century. For example, in the period from 1920 to 1950, this dispute could be followed in the work of Karl Lashley, perhaps the dominant figure in American neuropsychology in the first half of this century. Lashley explored the surface of the cerebral cortex in the rat, systematically removing different cortical areas. In so doing, he failed repeatedly to identify any particular brain region that was special to or necessary for the storage of memory. Based on these experiments, Lashley formulated the law of *mass action*, according to which the extent of the memory defect was correlated with the size of the cortical area removed, not with its specific location (Lashley, 1929). Many years later, with additional experimental work, it was possible to arrive at a different understanding of Lashley's famous conclusion.

Perhaps the first effective answer to Lashley came from Donald Hebb (Figure 1, right). In his book *The Organization of Behavior*, Hebb (1949) convinced many that it was possible to think seriously about the brain processes underlying memory. He developed concrete proposals based on biological facts, taking into consideration the neuronal circuitry that might contribute to memory storage. To explain Lashley's result that learning could not be localized to a single brain region, Hebb suggested that assemblies of cells work together to represent information and that these assemblies are distributed over large areas of cortex. Sufficient numbers of interconnected cells will survive most lesions to ensure that information can still be represented. The idea of a distributed memory store was far sighted. With the accumulation of additional evidence, it has become apparent that no single memory center exists, and many parts of the nervous system participate in the representation of any single event.

Hebb influenced many students and colleagues—in particular, Brenda Milner, who in 1957 described the remarkable patient H. M. (Scoville and Milner, 1957). H. M. had sustained a bilateral resection of the medial structures of the temporal lobe in 1953 to relieve severe epilepsy. It was immediately evident following the surgery that H. M. had a very profound impairment of recent memory in the apparent absence of other intellectual loss (Scoville, 1954). He could not remember what he had for breakfast, and he could not find his way around the hospital or recognize members of the hospital staff (except Scoville, whom he had known for many years). It seemed as though his life from the surgery onwards was not contributing to his store of knowledge. He was able to hold immediate impressions in his mind, but as

soon as his attention was diverted they were lost. In contrast, old memories from his childhood seemed to be intact.

In fact, the encounter with H. M. was not the first encounter with this kind of memory impairment. During the early 1950s, Wilder Penfield (Figure 1, left) began to carry out unilateral removals of parts of the frontal or temporal lobe as a treatment for patients with localized injury causing seizures. The temporal-lobe removals typically included the anterior temporal neocortex together with the uncus, amygdala, and anterior parahippocampal gyrus and hippocampus on the medial aspect of the hemisphere. Milner and Penfield found that these removals produced at most mild material-specific memory deficits that varied in kind with the side of the lesion. But, unexpectedly, Milner and Penfield encountered two patients with a severe, persistent, and generalized impairment of recent memory, following a removal limited to the left temporal lobe. Because both patients had undergone extensive preoperative testing, it was easy to establish that this was a selective impairment of memory, with no accompanying intellectual loss (Penfield and Milner, 1958). The impairment was manifested clinically as a profound anterograde amnesia, such that the experiences of daily life were forgotten as soon as the focus of attention shifted to a new topic. In addition, one patient showed a retrograde amnesia covering salient events of the preceding few months and the other showed a retrograde amnesia covering the 4 preceding years.

To account for this unexpected memory loss, Milner and Penfield (1955) hypothesized that in each case there must have been a pre-existing, but undetected, atrophic lesion in the hippocampal region of the opposite hemisphere, so that when the surgeon removed the anterior hippocampus and parahippocampal gyrus in the left hemisphere, he effectively deprived the patients of medial temporal-lobe function bilaterally. The reason that Milner and Penfield focused on the hippocampal region was that one patient, P. B., had had his temporal lobectomy in two stages, and it was only after removal of the medial structures of the temporal lobe that the memory loss was seen. Their hypothesis was confirmed 9 years later, when P. B. died of a pulmonary embolism and the autopsy findings revealed the presence of long-standing extensive right hippocampal atrophy, whereas the rest of the right temporal lobe, including the amygdala and the parahippocampal gyrus, showed no significant abnormality. In contrast, on the operated (left) side, the 22 mm of the hippocampus that remained appeared to be normal (Penfield and Mathieson, 1974).

Milner and Penfield reported these two cases at the 1955 meeting of the American Neurological Association in Chicago, and Scoville read their abstract. He called Penfield and said that he thought he had seen a similar memory disturbance in a patient of his (H. M.) in whom he had carried out a bilateral medial temporal-lobe resection, also in an attempt to control epileptic seizures. Penfield asked Milner if she would like to go down to Hartford, Connecticut to study the patient, and that is how the memory deficit in H. M. became more widely known.

Clinically, H. M.'s memory disorder appeared identical to that of Penfield's two patients, except that it was more severe. Again, there had been no intellectual loss; in fact, H. M.'s IQ had risen postoperatively, from 104 to 117, presumably because he was having far fewer seizures. His capacity for sustained attention was also remarkable. Thus, Milner showed that he could retain the number 584 for at least 15 minutes by continuous rehearsal, combining and recombining the digits according to an elaborate mnemonic scheme, but the moment his attention was diverted by a new topic, the whole event was forgotten.

H. M.'s success in remembering a three-digit number for 15 minutes in the absence of distraction was at first sight consistent with Drachman's view that amnesics can hold a simple memorandum indefinitely provided that no interfering activity claims their attention (Drachman and Arbit, 1966). Yet it was already clear that for H. M. verbal rehearsal played a key role in this holding process. In contrast, certain simple nonverbal stimuli were forgotten by him within less than a minute. The evidence for this comes from delayed paired comparison and delayed matching studies.

In 1959, Konorski described a method for testing memory of single events, which was later adapted for work with human subjects by Stepien and Sierpinski (1960). This technique, called by Milner "delayed paired comparison," consists of presenting two stimuli in succession, separated by a short time interval. The subject must then indicate whether the second stimulus is the same as or different from the first. This means that subjects must retain an impression of the first stimulus in order to compare the second one with it. Task difficulty may be increased by lengthening the intratrial interval or by introducing an intratrial distraction. Prisko (1963; cited by Milner, 1972) used the Konorski method to demonstrate H. M.'s rapid forgetting of simple perceptual material. She sampled five different sets of stimuli (three visual and two auditory), each set constituting a separate task. The stimuli used were clicks, tones, shades of red, light flashes, and nonsense patterns. At least five values were assigned to each variable, to prevent as far as possible the use of verbal mediation to bridge the retention interval. All paired stimuli were easily discriminable at zero intratrial delay. These proved to be extremely easy tasks for normal subjects, who rarely made errors even with a 60-second delay and an interpolated distraction. In contrast, H. M. performed all tasks well at zero delay, but with increasing intratrial intervals his performance deteriorated sharply, so that at the 60-second delay scores were approaching the chance level and were not further impaired by distraction.

Subsequently, Sidman, Stoddard, and Mohr (1968) confirmed Prisko's findings, using a delayed matching-to-sample technique that allowed the plotting of discrimination gradients to show how far the subject's choice of a matching stimulus deviates from the sample stimulus as the intratrial interval lengthens. In the nonverbal form of their task, H. M. was required to indicate which one of eight ellipses matched the sample stimulus. With zero delay he chose correctly most of the time, showing a normal discrimination of axis-ratios, but with increasing delays his performance deteriorated until, at 32 seconds, the sample no longer exerted any control over his

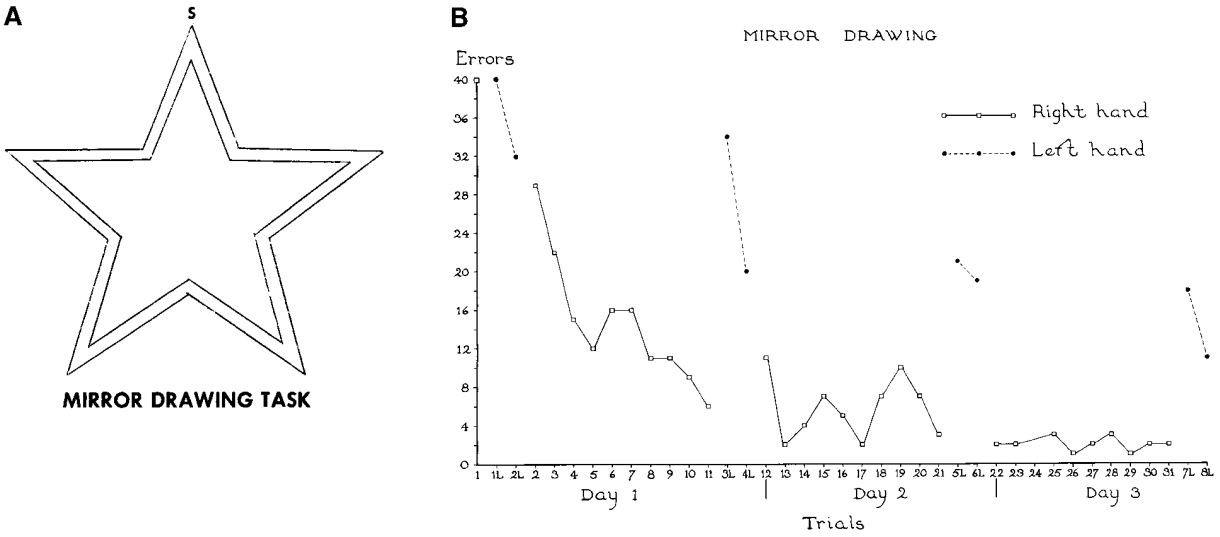

Figure 2. H. M. Showed Improvement in a Task Involving Learning Skilled Movements

In this test, he was taught to trace a line between the two outlines of a star, starting from the point S (Figure 2A), while viewing his hand and the star in a mirror. He showed steady improvement over the 3 days of testing, although he had no idea that he had ever done the task before. (The graph in Figure 2B plots the number of times, in each trial, that he strayed outside the boundaries as he drew the star.) Adapted from Milner (1962).

choice. In contrast, H. M. had no difficulty with a verbal version of the task, which required the matching of consonant trigrams. However, as with other short-term verbal memory tasks, he succeeded only by constant rehearsal; his lips could be seen moving throughout the delay period.

These and other related studies (Milner and Taylor, 1972) concur in showing that H. M. can register perceptual information normally, but that the information ceases to be available to him within about 30–40 seconds. Milner (1972) suggested that such results support the distinction between a primary memory process with a rapid decay and an overlapping secondary process (impaired in H. M.) by which the long-term storage of information is achieved.

There Are Multiple Memory Systems in the Brain

H. M.'s failure on delayed matching and delayed comparison tasks, which assess memory after a single presentation, did not rule out the possibility that he might be capable of some learning with intensive practice, or indeed that certain kinds of learning might take place at a normal rate. Accordingly, Milner and her students embarked on a variety of learning studies with H. M., including stylus maze tasks, both visual (Milner, 1965) and tactual (Corkin, 1965). With one notable exception, these studies merely served to demonstrate H. M.'s extreme difficulties with new learning, as evident also in his daily life. The exception was in the domain of motor skills, where, in 1962, Milner showed that H. M. could learn a mirror-drawing task efficiently with stable retention from day to day (Figure 2A).

If one is shown a picture of a double-margin star (Figure 2) and asked to draw a line between the two margins, one can do that very easily. However, if one has to do it while seeing one's hand and the star reflected in a mirror, then it becomes quite difficult. When one reaches the points of the star, one tends to move the hand in the wrong direction. Eventually, with practice, we can all learn to draw the outline of a star in a mirror. It is a new sensorimotor skill, a visual-motor skill, and it is acquired across many trials. Milner was able to show that H. M. could learn that kind of task quite well. She took H. M. through 30 trials of mirror drawing spread over 3 days, and he exhibited a typical learning curve (Figure 2B). Yet at the end he had no idea he had ever done the mirror drawing task before: this was learning without any sense of familiarity. Nowadays, we are well aware that such dissociations are possible following a discrete brain lesion, but for Milner, looking at it for the first time, it was quite astonishing. Her finding contributed some of the early evidence that there is more than one memory system in the brain.

Interestingly, even before the study of patient H. M. inaugurated empirical work on the different memory systems of the brain, similar ideas had been proposed by philosophers and psychologists on the basis of intuition and introspection. For example, in 1949, Gilbert Ryle, a philosopher of mind at Oxford, proposed the existence of two types of knowledge: *knowing how*, as in knowledge of motor skills, and *knowing that*, as in the knowledge of facts and events. Some years later Jerome Bruner, one of the founders of cognitive psychology, called "knowing how" a memory *without record*. Memory without record, Bruner argued, occurs in the case of experiences that "change the nature of the organism, change his skills, or change the rules by which he operates, but are virtually inaccessible in memory as specific encounters." Here, the neural machinery that supports a behavior is presumably modified directly. He called "knowing that" *a memory with record*, a repository of information about the facts and events of everyday life.

The demonstration of intact motor skill learning in patient H. M. marked the beginning of a period of experimental work that eventually established the biological

reality of multiple memory systems. This later work made it clear that the spared memory capacities of H. M. and other amnesic patients with bilateral medial temporal-lobe lesions are not limited to motor skills. Motor skills are a subset of a large collection of learning and memory abilities, all of which are spared in amnesia and independent of the medial temporal lobe. In 1968, Warrington and Weiskrantz demonstrated what turned out to be another kind of preserved learning ability in a group of six amnesic patients, one after a right temporal lobectomy and five with alcoholic Korsakoff's psychosis. Using a version of the Gollin Figures task (Gollin, 1960), Warrington and Weiskrantz asked their patients to try to identify line drawings of common objects and animals (such as a chair or an elephant) from which most of the contour lines had been removed. This is initially quite difficult with the most fragmented drawings, but over successive presentations the contour is gradually filled in until the subject can name the item depicted. On a second presentation of the task, 1 hour later, normal subjects show considerable savings, requiring fewer contour cues to name the items. On this incomplete figures task and on an analogous fragmented words task, Warrington and Weiskrantz found marked savings in their amnesic patients, with good retention 4 weeks later, although the patients did not remember doing the tasks before. It is true that the amnesic group showed less savings than the age-matched control group, but this was only to be expected, given that the control subjects could recall most of the items and anticipated seeing them again.

Milner subsequently replicated the findings for the Gollin figures with H. M. Interestingly, H. M.'s initial performance on the first exposure to the material was above the control mean, illustrating his superior perceptual abilities. On retesting, 1 hour later, he reduced his error score by 48%, although he did not remember seeing any of the drawings before. Moreover, he showed residual savings 4 months later (Milner et al., 1968). This long-term effect of a prior visual experience, which Milner called "perceptual learning," is an instance of what is now known as priming, a form of learning distinct from motor skill and which, in this case, is probably mediated by higher visual cortical areas.

The Declarative and Nondeclarative Memory Systems

In 1980, Neal Cohen and Larry Squire showed that amnesic patients could learn the task of reading mirror-reversed print as well as normal subjects. These findings broadened further the scope of what amnesic patients could do and suggested a fundamental distinction in the way all of us process and store information about the world. The major distinction is between declarative memory and a collection of nondeclarative, nonconscious forms of memory.

Declarative memory (Figure 3) is what is ordinarily meant by the term memory. It depends on the integrity of the medial temporal lobe and affords the capacity for conscious recollections about facts and events. Declarative memory is propositional—it can be either true or false. It is involved in modelling the external world and storing representations about facts and episodes. Nondeclarative memory is neither true nor false. It underlies changes in skilled behavior and the ability to respond appropriately to stimuli through practice, as the result of conditioning or habit learning. It also includes changes in the ability to detect or identify objects as the result of recent encounters, a phenomenon known as *priming*. In the case of nondeclarative memory, performance changes as the result of experience, which justifies the term memory, but performance changes without providing conscious access to any prior episodes (Squire et al., 1993; Schacter and Tulving, 1994). Many forms of nondeclarative memory, such as habituation, sensitization, and classical conditioning, are phylogenetically ancient and well developed in invertebrate animals that do not have a medial temporal lobe or hippocampus.

A number of nondeclarative forms of memory have been subjected to intensive study. In humans, perhaps the best studied example of nondeclarative memory is priming, first explored by Warrington and Weiskrantz (1968) and by Milner et al. (1968). Endel Tulving, Daniel Schacter, Larry Squire, and others have explored several paradigms in which subjects see lists of words, pictures of objects, or nonverbal material such as novel objects or designs (Weiskrantz, 1990; Tulving and Schacter, 1990). Subsequently, subjects are tested with both old and new items and asked to name words or objects as quickly as possible, to complete fragments to form whole items, or to make rapid decisions about items. For example, when the first few letters (MOT__) of a recently studied word (MOTEL) are presented, priming is evidenced in the tendency to complete the word fragment to form the study word instead of other possible words. Severely amnesic patients exhibit fully intact priming, despite being unable to recognize as familiar the items that had been presented previously.

Other forms of nondeclarative memory also have been studied. These include habit memory, which refers to gradually acquired dispositions or tendencies that are specific to a set of stimuli and that guide behavior. Habit learning survives hippocampal damage in humans and experimental animals but is impaired by damage to the caudate nucleus (Packard et al., 1989; Knowlton et al., 1996). Emotional learning, as in the development of phobias or in fear conditioning, is dependent on the amygdala. An enormous amount has been learned about the essential structures and connections involved in emotional learning, particularly from studies in which rats learn to fear a neutral stimulus such as a tone (fear conditioning and fear-potentiated startle) (LeDoux, 1995; Davis et al., 1997). The amygdala has also been shown to be important for emotional learning in humans (Damasio, 1995; Cahill et al., 1996). Moreover, the amygdala is essential not only for emotional learning itself; it also exerts modulatory effects on other memory systems (McGaugh et al., 1996). For example, the amygdala is responsible for the enhancement of declarative, conscious memory, which normally occurs with emotional arousal (Adolphs et al., 1997).

Perhaps the best studied example of nondeclarative memory in mammals is classical *Pavlovian* conditioning of discrete behavioral responses. A body of work initiated in the early 1980s by Richard Thompson and his

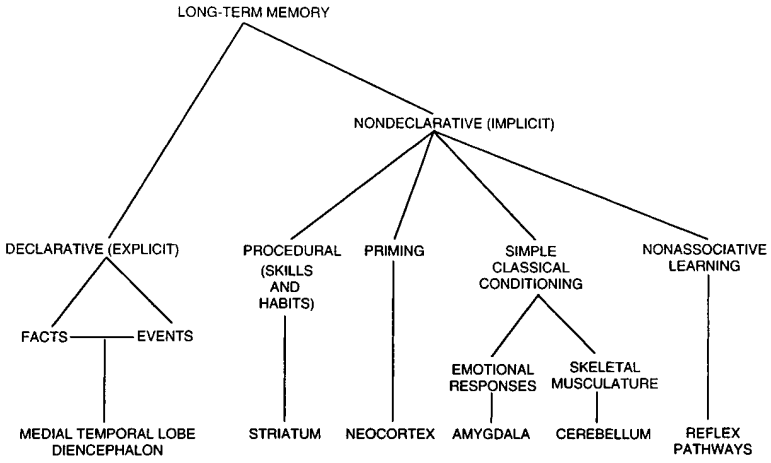

Figure 3. A Taxonomy of Mammalian Memory Systems

This taxonomy lists the brain structures and connections thought to be especially important for each kind of declarative and nondeclarative memory.

colleagues has focused on basic delay conditioning of the rabbit eyeblink response (conditioned stimulus = tone; unconditioned stimulus = airpuff; conditioned response = eyeblink). Based on anatomical findings, electrical stimulation, and reversible lesion techniques, the results provide strong evidence that the essential memory trace circuit includes the cerebellum and related brain stem circuitry and that the memory traces themselves are formed and stored in the cerebellum (Thompson and Krupa, 1994). To date, eyeblink conditioning provides the clearest information about the localization of a memory within the mammalian brain.

In humans, several kinds of nondeclarative memory have been studied, which are likely based on perceptual learning. These include adaptation-level effects, the ability to resolve random-dot stereograms, the ability to learn the regularities of "artificial grammars" by studying lawfully ordered letter strings, and the ability to acquire knowledge about categories. In category learning, one extracts and stores information about the prototype (or representative instance) of a series of items by studying many different items that, when averaged together, describe the prototype. All these forms of memory are intact in amnesic patients (Squire et al., 1993; Squire and Zola-Morgan, 1996). These kinds of memory likely involve changes within the same cortical areas responsible for perceiving and analyzing the materials that are studied.

What Parts of the Medial Temporal Lobe Are Important for Memory?

The behavioral studies reviewed above provide compelling evidence that the human declarative memory system is critically dependent upon the medial temporal region. Yet we still have much to learn about the relative importance of specific structures within the region for memory processes and the mode of interaction of these structures with other brain areas. Although Scoville and Milner (1957) drew attention to the hippocampus in the title to their paper, this was only because in their experience bilateral removals limited to the amygdala and uncus did not result in amnesia; they never claimed that the hippocampal lesions alone were responsible for H. M.'s severe memory loss.

David Amaral has recently reviewed the results of a magnetic resonance imaging study of H. M. (Corkin et al., 1997). He finds that Scoville's removal was in fact exactly as he had described it, except that the resection only extends about 5 cm posteriorly in both hemispheres, instead of the radical 8 cm originally reported. Thus, in both hemispheres the removal included the amygdala, the perirhinal and entorhinal cortex, and the anterior hippocampus. The parahippocampal cortex was largely spared. Most importantly, the temporal neocortex and the temporal stem were spared. If the roles of these various structures were to be understood, an animal model clearly was needed.

Nonhuman Primate Models of Declarative Memory

As soon as H. M. was described in 1957, efforts began to establish an animal model of his condition in the rat and monkey. If the concept of conscious recollection is central to declarative memory, how can declarative memory be studied in experimental animals? Several characteristics have been useful in extending the notion of declarative memory to mice, rats, and monkeys (Eichenbaum, 1997). These include its flexibility and the ability to use it inferentially in novel situations. It took considerable time to achieve such a model, and the first results of lesion studies in the monkey were puzzling. Animals with bilateral medial temporal-lobe resections similar to what was described in H. M. showed normal performance on visual discrimination learning tasks, even when concurrent trials on a different task were interpolated as potential "distractors" for the discrimination learning. This led many investigators to question either the human findings or the validity of cross-species comparisons. It was not until the early 1980s, with the concept of multiple memory systems and the idea that amnesia impaired only one kind of memory, that it became clearer which memory tasks were appropriate to give to experimental animals. The beginning of the solution came in 1978 when Mort Mishkin described severe deficits in monkeys with bilateral medial temporal-lobe lesions, when the monkeys were given a one-trial task of object recognition memory (delayed nonmatching to sample). This finding was consistent with the severe impairment shown by H. M. on single-trial delayed matching tasks.

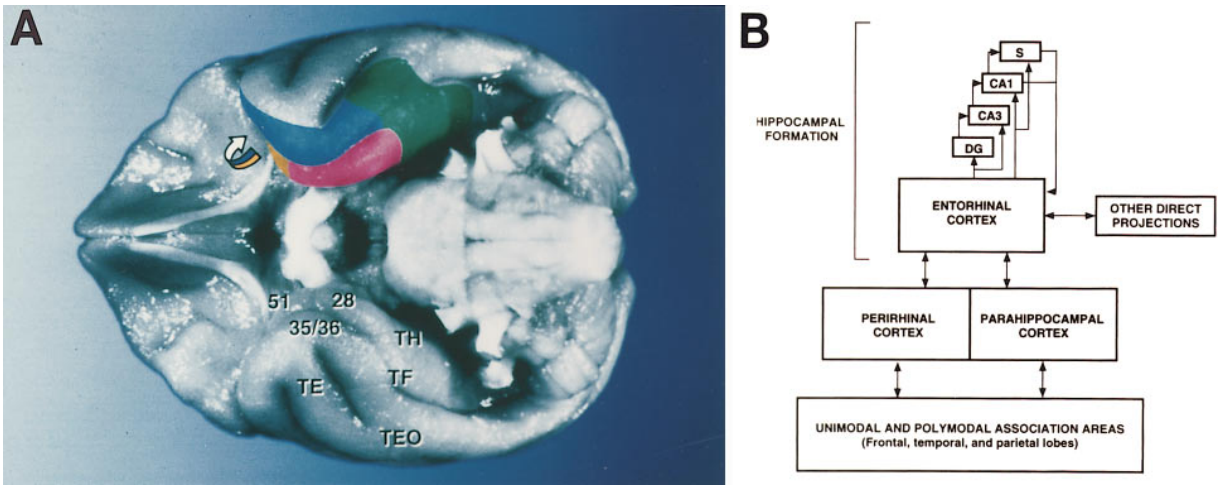

Figure 4. The Medial Temporal-Lobe Memory System in the Monkey

(A) Ventral view of a monkey brain illustrating the cortical areas underlying the hippocampus that are part of the medial temporal-lobe system. Blue, perirhinal cortex; pink, entorhinal cortex; green, parahippocampal cortex. The periamygdaloid cortex (yellow) is not thought to be a part of the system.

(B) Schematic view of the memory system. The entorhinal cortex is a major source of projections to the hippocampal region, which includes the dentate gyrus (DG), the cell fields of the hippocampus, and the subicular complex (S). Nearly two-thirds of the cortical input to entorhinal cortex originates in the adjacent perirhinal and parahippocampal cortices, which in turn receive projections from unimodal and polymodal areas in the frontal, temporal, and parietal lobes. The entorhinal cortex also receives other direct inputs from orbital frontal cortex, insular cortex, and superior temporal gyrus. All these projects are reciprocal.

The 1978 paper did not settle matters all at once but by the early 1980s, after additional work by Mishkin, Zola-Morgan, and others, an animal model of human amnesia in the monkey was established. With this model, the question of precisely which structures within the medial temporal lobe were important could be systematically explored. The identification of the anatomical components of the medial temporal-lobe memory system required about 10 years of experimental work (Squire and Zola-Morgan, 1991). The important structures are the hippocampus proper, the dentate gyrus, the subicular complex, and the entorhinal cortex (which together comprise the hippocampal formation) and the adjacent, anatomically related cortex: the perirhinal and parahippocampal cortices (Figure 4). The amygdala proved not to be a component of the declarative memory system, although it can exert a modulatory action on declarative memory.

A lesion restricted to any of the major components of this system has a significant effect on declarative memory. Indeed, two amnesic patients have been described (R. B. and G. D.) who, following an ischemic event, had bilateral lesions limited to the CA1 region of the hippocampus (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996). Their deficit was qualitatively similar to H. M.'s impairment, though quantitatively it was much milder. It is now clear that the severity of H. M.'s memory impairment depends not only on his hippocampal damage but on the fact that his surgery included the hippocampal region together with the perirhinal and entorhinal cortices (Corkin et al., 1997).

A key feature of medial temporal-lobe function is that the medial temporal lobe is involved in memory for a limited period of time after learning. The initial evidence for this idea came from the observation that patient H. M., as well as the two patients described by Penfield and Milner (1958), appeared to have intact memory for remote events that occurred years before their surgery. Subsequently, formal tests that asked about past public events also showed amnesic patients to have impaired memory for events leading up to the amnesia but intact memory for more remote events (Squire et al., 1989; Rempel-Clower et al., 1996). This loss of premorbid memory (retrograde amnesia) can cover months or even years, depending on the extent of medial temporal-lobe damage (Rempel-Clower et al., 1996).

Studies of remote memory and retrograde amnesia in amnesic patients necessarily rely on retrospective methods and imperfect tests. As a result, it is difficult to compare performance across past time periods. For these reasons, the phenomenon of retrograde amnesia has begun to be examined prospectively in experimental animals. To date, eight different studies have been carried out in which equivalent amounts of training were given at two or more times before bilateral damage to the hippocampal formation, and retention was assessed shortly after surgery (Figure 5). The work has involved mice, rats, rabbits, and monkeys and a variety of memory tasks including object discrimination learning, context-specific fear conditioning, maze learning, and trace conditioning of the eyeblink reflex. In seven of the eight studies, clear evidence was obtained for temporally graded retrograde amnesia, which covered a period ranging from a few days to about a month before surgery. In the eighth study (Bolhius et al., 1994), memory was affected similarly at the time points tested, although performance was always at chance levels so that no difference between the two time points could have been detected.

Recent accounts of temporally graded retrograde amnesia propose that medial temporal-lobe structures

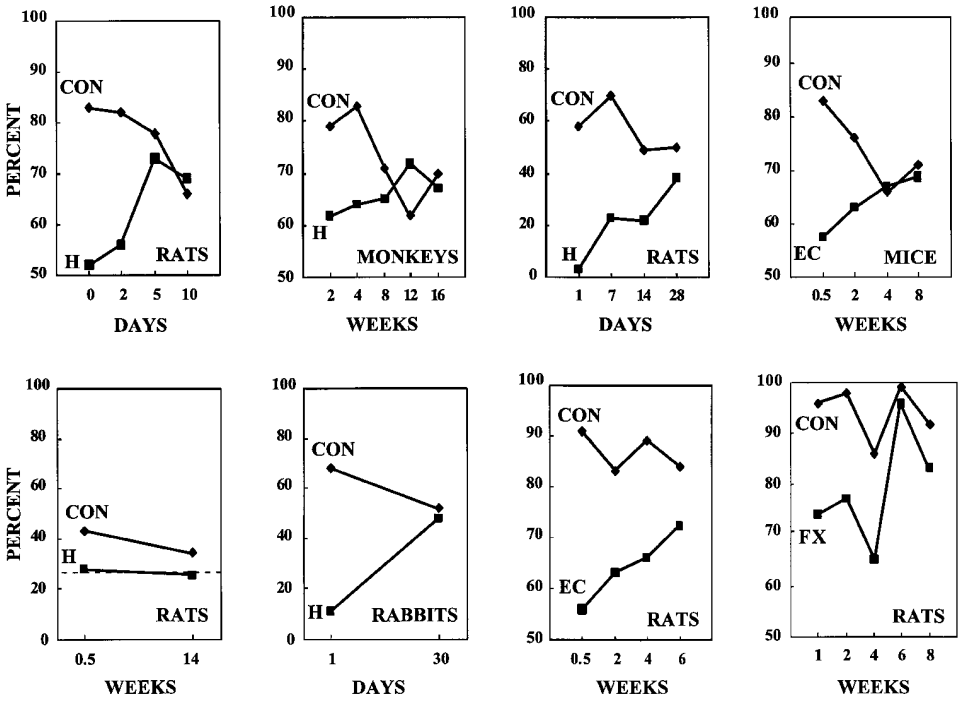

Figure 5. Summary of Findings from Eight Studies that Have Examined Retrograde Amnesia Prospectively

In these studies, an equivalent amount of training was given at each of two or more times before hippocampal formation damage, and retention was assessed shortly after surgery. In each case, the data show the performance of control (CON) and operated animals (H = hippocampus, EC = entorhinal cortex, FX = fornix) as a function of the interval between training and surgery. Control animals typically exhibited forgetting as the interval between training and surgery increased. In seven of the eight studies, operated animals exhibited temporally graded retrograde amnesia. They were impaired at retaining material they had learned recently, but they retained remotely learned material as well as control animals. In addition, the operated animals typically retained remotely learned material better than recently learned material. In the lower left panel, the dotted line denotes chance performance. From left to right, beginning on the top row, the studies are by Winocur (1990); Zola-Morgan and Squire (1990); Kim and Fanselow (1992); Cho, Beracochea, and Jaffard (1993); Bolhuis, Stewart, and Jaffard (1993); Kim, Clark, and Thompson (1995); Cho and Kesner (1996); and Wiig, Cooper, and Bear (1996).

direct a gradual process of reorganization and stabilization by changing the organization of cortical representations, for example, by gradually binding together the multiple, geographically separate cortical regions that together store memory for a whole event (Alvarez and Squire, 1994; McClelland et al., 1995). After sufficient time has passed, the hippocampal formation is not needed to support storage or retrieval of declarative memory, and long-term memory is fully dependent on the neocortex (reviewed by Squire and Alvarez, 1995).

The different components of the medial temporal lobe need not have equivalent roles in declarative memory; different structures within the medial temporal lobe are likely to carry out different subfunctions. As damage increases, fewer strategies may be available for storing memory, with the result that memory impairment becomes more severe. To study the functions of the individual regions in humans would require many patients with very specific brain lesions. Fortunately, recent anatomical and behavioral studies indicate that, even though there are differences in detail, the anatomical and functional organization of the medial temporal-lobe system is similar in humans, nonhuman primates, and simpler mammals such as rats and mice (Squire, 1992; Mayford et al., 1996). Moreover, even the mouse requires this memory system for the storage of memory about places and objects, and this type of memory has many of the characteristics of human declarative memory, affording, for instance, the flexible use of relational information about multiple distal cues. As we shall see in the sections that follow, the possibility of studying declarative memory in mice has opened this form of memory to a molecular genetic approach.

The Molecular Biological Approach to Memory Storage

How are we to think about the cellular and molecular mechanisms of memory storage? By the end of the nineteenth century, biologists had come to appreciate that mature nerve cells have lost their capacity to divide. This fact prompted Santiago Ramón y Cajal to propose that learning does not result in the proliferation of new nerve cells but instead causes existing nerve cells to grow more branches and to strengthen their connections with other nerve cells so as to be able to communicate with them more effectively (Ramon y Cajal, 1894). This prescient idea raised three sets of questions.

First, does memory involve persistent changes in synaptic strength? If so, what are the molecular underpinnings of these synaptic changes?

Second, how do short-term synaptic changes differ from the changes that support long-term storage? Do

they occur at different loci, or can the same neuron store information for both short- and long-term memory?

Third, if memory storage results from changes in specific synaptic connections, do declarative memory and the various nondeclarative forms of memory use different molecular mechanisms for storage, or are the storage mechanisms used by these two memory systems fundamentally similar?

To explore these ideas, neurobiologists developed a number of model systems for the specific purpose of optimizing the ability to study synaptic change in the context of behavioral memory storage, with the ultimate goal of identifying the cellular and molecular basis of the synaptic changes responsible for the storage (see, for example, Kandel and Tauc, 1964; Thompson and Spencer, 1966; Kandel and Spencer, 1968). The reductionist approach to nondeclarative memory storage began with the cell biological studies of the marine snail *Aplysia* by Kandel (Kandel and Tauc, 1964) and with the genetic studies of *Drosophila* by Benzer (Benzer, 1967).

Cell Biological and Molecular Insights into Nondeclarative Memory Storage

The cell biological studies in *Aplysia* (Kandel and Tauc, 1964; Kupfermann and Kandel, 1969; Castellucci et al., 1970) were soon joined by studies of other invertebrates including other opisthobranch snails, specifically *Hermissenda* (Alkon, 1974) and *Pleurobranchaea* (Davis and Gillette, 1978), the land snail *Limax* (Gelperin, 1975), crayfish (Krasne, 1969), and honey bees (Menzel and Erber, 1978). The idea underlying these cell biological studies was that the simple brains of certain experimentally tractable invertebrates combined the advantages of having a relatively small number of nerve cells in the brain with cells that (with the exception of the honey bee) are unusually large and readily identifiable. These features made their behavior and their ability to modify behavior by learning accessible to cellular and molecular analysis. Analogous reductionist approaches were also applied to the mammalian brain, in particular to the isolated spinal cord (Spencer et al., 1966), to brain slices of the hippocampus (Schwartzkroin and Webster, 1975), and to learned behavior dependent on the cerebellum (McCormick and Thompson, 1984) and the amygdala (Davis, 1995; LeDoux, 1995).

The first insight to emerge from this *simple systems approach* to nondeclarative memory was purely behavioral. Studies of invertebrates revealed that even animals with limited numbers of nerve cells—approximately 20,000 to 100,000 central neurons in the nervous systems of *Aplysia*, *Hermissenda*, *Pleurobranchaea*, and *Limax* and approximately 300,000 in *Drosophila*—had rather remarkable behavioral and learning capabilities (reviewed by Carew and Sahley, 1986). In fact, even the gill withdrawal reflex, perhaps the simplest behavioral reflex of *Aplysia*, could be modified by several different forms of learning—habituation, dishabituation, sensitization, classical conditioning, and operant conditioning (reviewed by Carew and Sahley, 1986). Moreover, each of these forms of learning could give rise to both short- and long-term forms of nondeclarative memory as a function of the amount of repeated training. These studies suggested that an animal does not need a large brain or even many thousands of nerve cells for perfectly good long-term storage of a variety of different memories.

These early behavioral studies in invertebrates led to the delineation of a family of psychological concepts that paralleled those first described in vertebrates by both the classical behaviorists—Pavlov and Thorndike—and their modern counterparts—Kamin, Rescorla, and Wagner. These concepts included the distinction between various forms of associative and nonassociative learning, the role of contingency as opposed to mere contiguity, short-term memory consolidation, storage, retrieval of long-term memory, and forgetting. Subsequent cellular studies of these simple forms of learning illustrated that these concepts, initially inferred from purely behavioral studies, could now be approached directly in terms of their underlying cellular and molecular mechanisms (reviewed by Kandel, 1976; Hawkins and Kandel, 1984; Carew and Sahley, 1986). Thus, the ability to analyze learning-related synaptic mechanisms brought to light not only a new set of mechanistic insights into the plastic properties of individual synaptic connections, but in so doing these studies brought concreteness and clarity to the psychological concepts themselves. For example, by identifying significant components of the neural circuits underlying simple behaviors such as the gill-withdrawal reflex in *Aplysia*, the tail flick in the crayfish, feeding in *Limax* or *Aplysia*, and phototaxis in *Hermissenda*, studies in invertebrates delineated how elements in the behavioral circuits themselves changed when behavior was modified by various forms of learning (reviewed by Carew and Sahley, 1986). These findings illustrate that nondeclarative memory storage does not depend on specialized memory neurons or systems of neurons whose only function is to *store* rather than process information. Rather, simple nondeclarative memory storage results from changes in neurons that are themselves *components* of the reflex pathway. The storage of nondeclarative memory is embedded in the neural circuit that produces the behavior. These studies therefore provided the first clear insight that the organization and implementation of nondeclarative memory is different from declarative memory where a whole neural system, the medial temporal-lobe memory system, is needed to ensure the remembrance of things past.

Moreover, these cell biological studies illustrated several general principles about memory-related synaptic plasticity. To begin with, the studies provided the first direct evidence for two of Cajal's prescient suggestions: that the synaptic connections between neurons mediating behavior are not fixed but can become modified by learning, and that these modifications persist and can serve as elementary components of memory storage (Figure 6; Castellucci et al., 1970, 1978; Zucker, 1971; Castellucci and Kandel, 1974). In addition, these studies showed that the same set of synaptic connections was found to be able to participate in several different learning processes and to be modified by them in opposite directions. For example, the synaptic strength of a single synaptic connection could be increased with sensitization and classical conditioning, and it could be decreased with habituation (Castellucci et al., 1978; Carew et al., 1979; Hawkins et al., 1983; Frost et al., 1985; Murphy and Glanzman, 1997; Bao et al., 1997, 1998).

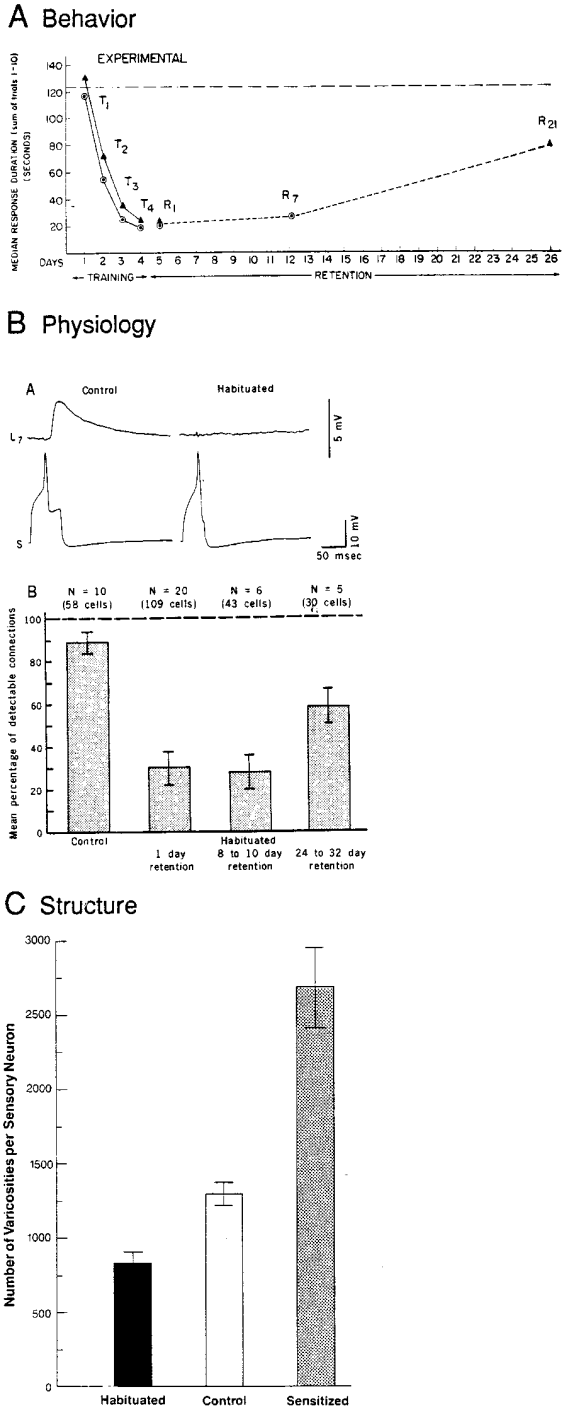

Figure 6. Long-Term Habituation of the Withdrawal Reflex in *Aplysia* Is Represented on the Cellular Level by a Dramatic Depression of Synaptic Effectiveness between the Sensory and Motor Neurons

(A) Time course of behavioral habituation. T1 to T4 represents the average of 10 trials a day for 4 days of training. R1, R7, and R21 are retention tests 1 day, 1 week, and 3 weeks after training. (From Carew et al., 1972.)

(B[A]) Comparison of the synaptic potentials in a sensory neuron and a motor neuron in a control (untrained) animal and in an animal that has been subjected to long-term habituation. In the habituated animal, the synaptic potential in the motor neuron is still undetectable 1 week after training. (From Castellucci et al., 1978.)

(B[B]) The mean percentage of physiologically detectable connections in habituated animals at several points in time after long-term habituation training. (From Castellucci et al., 1978.)

(C) Long-term habituation and sensitization involve structural changes in the presynaptic terminals of sensory neurons. (Adapted from Bailey and Chen, 1983.) This histogram compares the number of presynaptic terminals in control animals with those in long-term habituated and sensitized animals. The number is highest in the sensitized animals.

Furthermore, in the gill-withdrawal and tail-withdrawal reflex of *Aplysia* (Hawkins et al., 1981a, 1981b; Frost et al., 1988; Cleary et al., 1995), and in the escape reflex of *Tritonia* (Katz and Frost, 1995), there were changes in synaptic strength with habituation and sensitization not only in the connections between the sensory neurons and their motoneuron target cells, but also in the connections made by interneurons onto the target cells. Thus, within the neural pathways controlling the reflex, the storage of even a simple nondeclarative memory is distributed and involves multiple storage.

Finally, just as behavioral studies of memory in the gill-withdrawal reflex had found that memory storage has stages—a short-term form lasting minutes and a long-term form lasting days to weeks—so did the cellular studies find that the synaptic changes contributing to memory storage also have stages (Castellucci et al., 1978; Carew et al., 1979; Frost et al., 1988). Thus, both the acquisition of learning and its retention as short- and long-term memory were found to have a representation at the level of individual synaptic connections.

The initial analyses in the 1970s focused on short-term changes. These showed that one mechanism for the synaptic plasticity induced in both the gill-withdrawal reflex of *Aplysia* and in the tail flick response of crayfish was through the modulation of transmitter release. A depression of transmitter release occurred with short-term habituation and an enhancement with short-term sensitization (Zucker et al., 1971; Castellucci et al., 1970, 1974, 1976).

A variety of cell biological studies on the monosynaptic connections between the sensory neurons and motor neurons of the gill- and tail-withdrawal reflexes in *Aplysia* outlined one class of molecular mechanisms for the short-term enhancement of transmitter release produced by sensitization (Figure 7; Brunelli et al., 1976; Kandel and Schwartz, 1982; Byrne and Kandel, 1995). A single sensitizing stimulus to the tail led to the activation of three classes of modulatory neurons, the most important of which uses serotonin (5-HT) as its transmitter. Serotonin acts on a G protein–coupled receptor to activate adenylyl cyclase and increase the level of cAMP in the sensory neurons. The increase in cAMP activates the cAMP-dependent protein kinases (PKA), which then enhance transmitter release in two ways: (1) by closure of K^+ channels leading to a broadening of the action potential, thereby enhancing Ca^{2+} influx necessary for vesicle exocytosis; and (2) by acting directly in ways that are not yet understood, on one or more steps in vesicle mobilization and exocytotic release (Castellucci et al., 1980, 1982; Byrne and Kandel, 1995). A similar second messenger signaling pathway for learning and

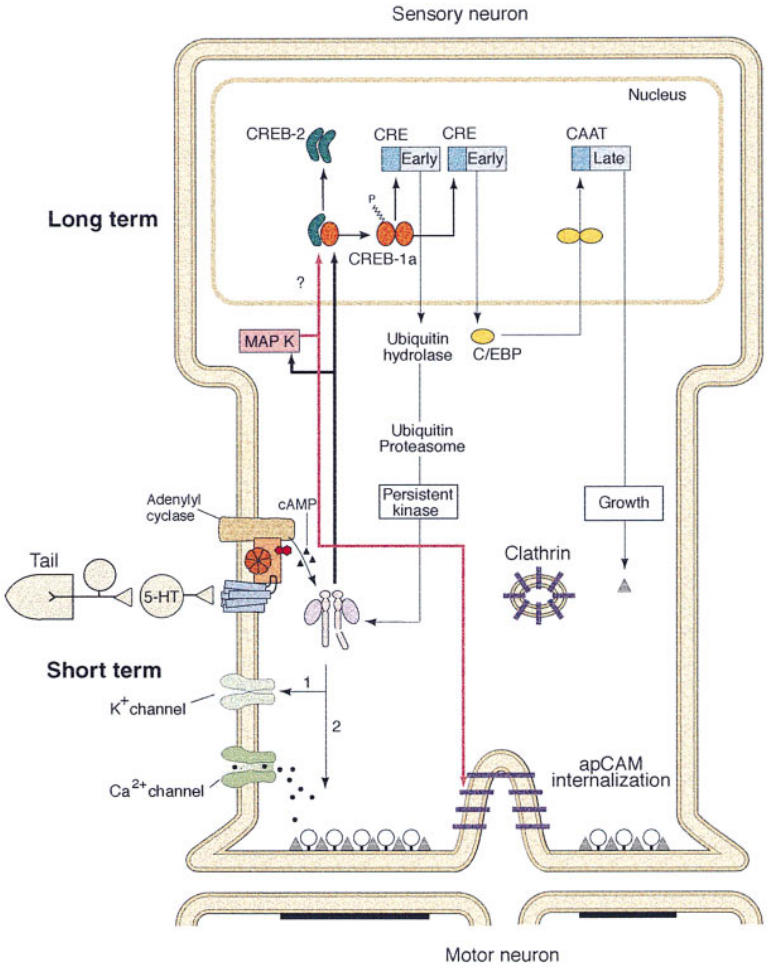

Figure 7. Schematic Outline of Changes in the Sensory Neurons of the Gill-Withdrawal Reflex that Accompany Short- and Long-Term Memory for Sensitization in *Aplysia*

Sensitization is produced by applying a noxious stimulus to another part of the body, such as the tail. Stimuli to the tail activate sensory neurons that excite facilitating interneurons, which form synapses on the terminals of the sensory neurons innervating the siphon skin. At these axo–axonic synapses, the interneurons are able to enhance transmitter release from the sensory neurons (presynaptic facilitation). Serotonin (5-HT), a transmitter released by facilitatory neurons, acts on a sensory neuron to initiate both the short-term and the long-term facilitation that contribute to the memory processes.

Short-term facilitation (lasting minutes) involves covalent modification of preexisting proteins (pathways 1 and 2). Serotonin acts on a transmembrane receptor to activate a GTP-binding protein that stimulates the enzyme adenylyl cyclase to convert ATP to the second messenger cAMP. In turn, cAMP activates protein kinase A, which phosphorylates and covalently modifies a number of target proteins. These include closing of K^+ channels, which prolongs the action potential and increases the influx of Ca^{2+}, thus augmenting transmitter release (pathway 1) as well as steps involved in transmitter availability and release (pathway 2). Can involve the joint action of PKA and protein kinase C (PKC). The duration of these modifications represents the retention or storage of a component of the short-term memory.

Long-term facilitation (lasting one or more days) involves the synthesis of new proteins. The switch for this inductive mechanism is initiated by the protein kinase A. This kinase translocates to the nucleus (long-term pathway) where it phosphorylates the cyclic AMP response element–binding (CREB) protein. The transcriptional activators bind to cyclic AMP regulatory elements (CRE) located in the upstream region of two types of cAMP-inducible genes. To activate CREB-1, protein kinase A needs also to remove the repressive action of CREB-2, which is capable of inhibiting the activation capability of CREB-1. Protein kinase A is thought to mediate the derepression of CREB-2 by means of another protein kinase, MAP kinase. One gene (closed square) activated by CREB encodes a ubiquitin hydrolase, a component of a specific ubiquitin protease that leads to the regulated proteolysis of the regulatory subunit of PKA. This cleave of the (inhibitory) regulatory subunit results in persistent activity of protein kinase A, leading to persistent phosphorylation of the substrate proteins of PKA, including both CREB-1 and the protein involved in the short-term process. The second set of proteins (closed triangles) is important for the growth of new synaptic connections. (From Kandel, Schwartz, and Jessell.)

short-term memory was identified in *Drosophila* using genetic approaches (Byers et al., 1981; Aceves-Pina et al., 1983; Davis, 1996).

Behavioral studies in vertebrates had shown earlier that long-term memory differed from short-term memory not only in time course but also mechanistically (reviewed by Davis and Squire, 1984). Long-term memory requires synthesis of new proteins, whereas short-term memory does not. These behavioral studies raised a number of questions that could now be explored on the cellular level. Can the same set of synaptic connections mediate both short-term and long-term synaptic plasticity? Can PKA induce the long-term as well as the transient changes, or does the long-term process recruit a new signaling system? Finally, is this requirement for protein synthesis evident at the level of single cells involved in memory storage? If so, how is protein synthesis activated for long-term memory?

Studies in *Aplysia* during the last decade have found that the same set of connections that undergo the short-term changes also undergo long-term changes, and the long-term changes in synaptic plasticity parallel behavioral memory in also requiring new protein synthesis (Goelet et al., 1986; Montarolo et al., 1986). Thus, a single synaptic connection can not only be modified in opposite ways by different forms of learning, but it can be modified for periods ranging from minutes to weeks by the different stages of a memory process. Whereas one training trial to the tail in the intact animal or one pulse of 5-HT to the sensory neurons initiates the short-term process through covalent modification of preexisting proteins, five repeated training trials or five pulses of 5-HT initiate the protein synthesis–dependent long-term process. With repeated training, the cAMP-dependent protein kinase recruits another kinase, a mitogen-activated protein kinase (MAP kinase), and both

of these kinases translocate into the cell's nucleus where they activate the transcriptional activator CREB-1 (the cAMP response element–binding protein) (see Bacskai et al., 1993; Kaang et al., 1993; Martin et al., 1997).

In *Aplysia* and *Drosophila*, both PKA and CREB-1 are not only necessary but are also sufficient for the long-term enhancement of synaptic strength (Schacher et al., 1988; Yin et al., 1994, 1995; Davis et al., 1996; reviewed by Martin and Kandel, 1996). In *Aplysia*, CREB-1 leads to the activation of a cascade of immediate-early genes. One of these, the gene for ubiquitin hydrolase, is the first neuron-specific step in this signaling cascade (Hedge et al., 1997). This enzyme is a rate-limiting step in the activation of the ubiquitin proteosome (Figure 7). The proteosome in turn cleaves the regulatory subunit of PKA. This frees the catalytic subunit and establishes a persistently active PKA, which can continue to phosphorylate substrate proteins necessary for the maintenance of facilitation but *now* without requiring either 5-HT or cAMP.

This neuron-specific memory mechanism is active for about 10 hours (Hedge et al., 1997). What gives the long-term facilitation self-maintained properties is the action of a second immediate-early gene, the transcriptional factor C/EBP. This factor acts on downstream genes, which leads to the synthesis of proteins and the growth of new synaptic connections (Alberini et al., 1994; Hedge et al., 1997). This growth of new synaptic contacts appears to be the stable, anatomically self-maintained reflection of stable long-term memory (Bailey and Chen, 1988; Glanzman et al., 1989; Bailey et al., 1992). Thus, synapses not only express plasticity by modulating transmitter function; synapses also express plasticity in terms of their structural morphology and by increasing or decreasing the number of release sites.

The initial studies of the switch from short-term to long-term memory focused on positive regulators that favor memory storage. Recent studies in *Drosophila* and *Aplysia* have revealed the surprising finding that the switch to the long-term synaptic change and to the growth of new synaptic connections are normally constrained by inhibitory factors—*memory suppressor genes*—that oppose long-term memory storage and determine the ease with which short-term memory can be converted to long-term memory (reviewed by Abel et al., 1998). One important constraint is an inhibitory transcription factor, the repressor CREB-2 (Bartsch et al., 1995). Overexpression of the repressor selectively blocks long-term facilitation in *Aplysia*. Removal of the repression allows a *single* exposure of serotonin, which normally produces short-term facilitation lasting only minutes, to produce long-term facilitation lasting days and to induce the growth of new synaptic connections.

These several findings on the cell biology of nondeclarative memory storage in invertebrates carry with them the important implications that the cellular representation of short-term memory involves co-valent modifications of pre-existing proteins and the strengthening of pre-existing connections. By contrast, the cellular representation of long-term memory involves CREB-mediated expression of genes, new protein synthesis, and the formation of new synaptic connections.

Simple Systems for Genetic Studies of Nondeclarative Memory Storage

These cell and molecular biological studies of memory in invertebrates were designed to address two issues: (1) to localize some of the sites of neuronal change within a neural circuit that is modified by learning and memory storage; and (2) to specify the molecules important for these changes. This cell and molecular biological approach has been paralleled and complemented by genetic studies designed to identify specific genes important for learning and memory.

The critical first step for studying the genetics of behavior, learning, and memory was taken by Seymour Benzer. In 1967, Benzer began to apply genetic techniques to *Drosophila* behavior by examining the effects on behavior of changing one gene at a time. Having identified a number of interesting mutants with phenotypes in courtship, vision, and circadian rhythms, Benzer turned to learning and memory storage. Together with his students Chip Quinn and Yadin Dudai, Benzer first demonstrated that flies can acquire associative classical conditioning (Dudai et al., 1974; Quinn et al., 1974). They can remember to avoid an odor that has been paired with an electric shock. Using this learning assay, Benzer's students next screened thousands of flies to find mutants that were impaired and could not remember that a particular odor was paired with shock. In this way, Duncan Byers and Ronald Davis isolated *dunce*, the first mutant fly with a defect in short-term memory storage (Byers et al., 1981). The mutant gene was found to encode a cAMP-dependent phosphodiesterase, an enzyme that destroys cAMP—the same pathway that had been delineated for short-term sensitization in *Aplysia*. As a result of the mutation, the flies accumulate too much cAMP, which interferes with their ability to acquire and store new information.

Quinn, Dudai, Davis, Tully, Yin, and others then searched for other mutants and found that a number of other genes that interfere with short-term memory also are involved in the cyclic AMP pathway (Figure 8). (1) *amnesiac* is deficient in a gene for a neuropeptide that binds to a G protein–coupled receptor that stimulates adenylyl cyclase (Feaney and Quinn, 1995). (2) *Gs* encodes a stimulatory α subunit of a G protein (Connolly et al., 1996). (3) *rutabaga* has a specific deficiency in the enzyme adenylyl cyclase, the enzyme that synthesizes cyclic AMP from ATP (Dudai et al., 1983; Livingston et al., 1984; Levin et al., 1992). (4) *dunce*, as we saw, is a mutation in a cAMP phosphodiesterase. (5) *DCO* (Davis, 1996) is deficient in the catalytic subunit of PKA. In addition, Quinn found that the transient expression of a gene that shuts down PKA interferes with memory storage (Drain et al., 1991; Davis, 1996). (6) *PKA-RI* encodes a regulatory subunit of PKA (Goodwin et al., 1997).

More recently, Tim Tully has found that *Drosophila* also has long-term memory, and that this long-term memory requires repeated training at spaced intervals and is dependent upon new protein synthesis (Tully et al., 1994, 1996). Yin et al. (1994) went on to clone the *Drosophila* form of CREB and found that the gene encodes two forms, an activator and a repressor (Figure 8A). Yin et al. (1994) then overexpressed the inhibitory form of CREB in flies under a heatshock promoter and

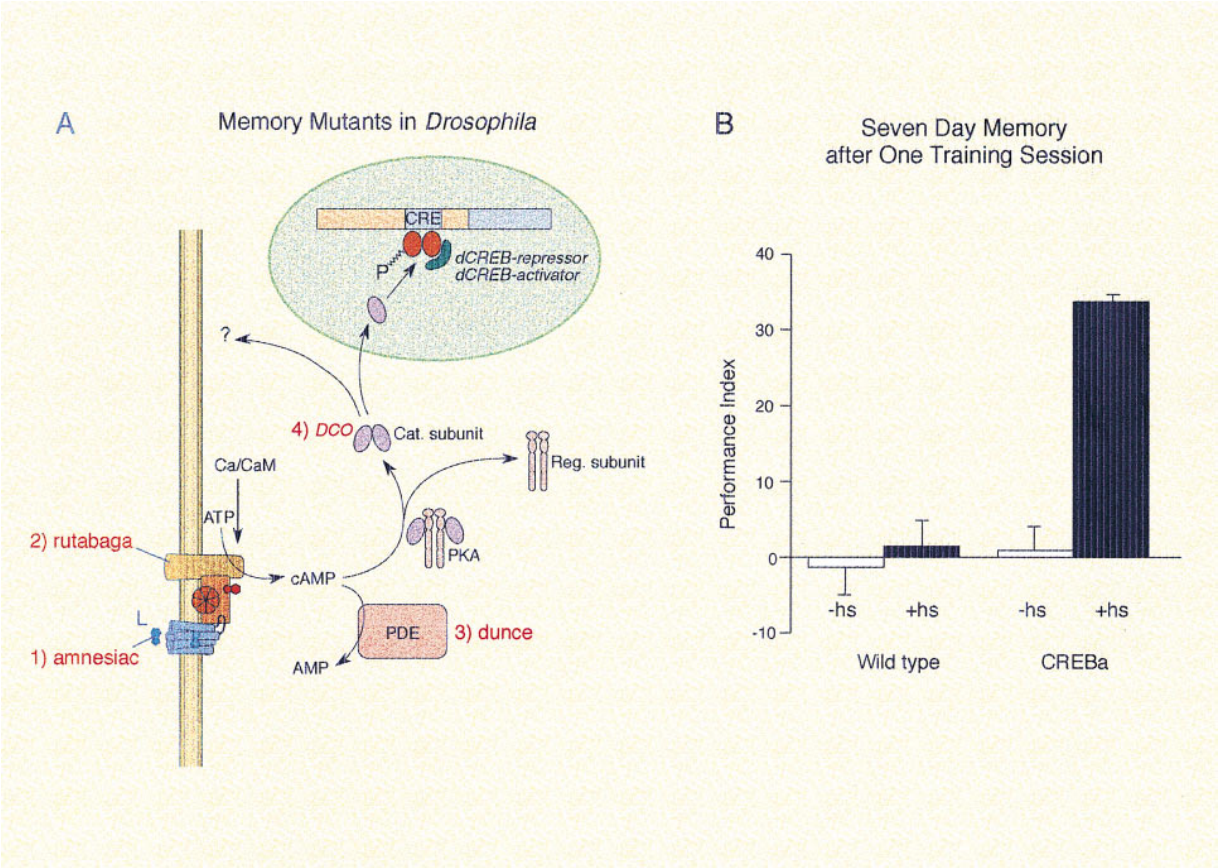

Figure 8. Short-Term and Long-Term Memory in *Drosophila*

(A) Memory mutants in *Drosophila*. Using reverse genetic methodology, seven genes have been isolated that affect olfactory associative learning and that are involved in different steps of the cAMP signaling. Disruptions of each of these genes produce deficits in olfactory learning or memory formation without affecting the sensorimotor responses necessary for the learning task. (1) *amnesiac* encodes a neuropeptide ligand similar to vertebrate Pituitary Adenylyl Cyclase Activating Peptide (Feany and Quinn, 1995). This ligand acts on a seven transmembrane spanning G protein–coupled receptor. (2) *Gs* encodes a (stimulatory) alpha subunit of G protein (Connolly et al., 1996). (3) *rutabaga* encodes a Type I adenylyl cyclase (Levin et al., 1992). (4) *dunce* encodes a Type II phosphodiesterase (Chen et al., 1986). (5) *DCO* encodes a catalytic subunit of cAMP-dependent protein kinase (PKA) Skoulakis et al., 1993; Li et al., 1996). (6) *PKA-RI* encodes a Type I PKA regulatory subunit (Goodwin et al., 1997). The nuclear end-point of this pathway, (7) *dCREB2* encodes both a transcription repressor and an activator.

(B) Induction of the dCREB2 activator isoform accelerates the rate of long-term memory formation without affecting the amount. CREB activator transgenic flies (dCREB2-a) produce a protein synthesis-dependent memory (LTM) after one training sessions, which lasts for at least 7 days. Such minimal training usually does not induce LTM in normal flies (wild-type), with or without heat shock, or in CREBa transgenic flies in the absence of heat shock (−hs) when they are exposed to heat shock (+hs), allowed to recover for 3 hours, and given a single training trial. The CREB activator transgene is under the control of the heat-shock promoter. Normal flies (wild-type), or transgenic flies that are not exposed to heat shock, do not generate any significant levels of memory after a single training trial. The amount of memory that the transgenic flies have is similar to levels that wild-type flies have after 10 spaced training trials. (Data from Yin et al., 1995.)

found that this manipulation blocked the formation of long-term memory in transgenic flies without disrupting short-term memory. Moreover, overexpressing the activating form of *Drosophila* CREB greatly reduced the number of training trials needed to establish long-term memory. Thus, after the induction of the CREB-activator, one training session, which normally produces only short-term memory, gives rise to long-term memory lasting over a week (Yin et al., 1995) (Figure 8B). These data suggest that the ratio of CREB activator to repressor is critical for the activation of the long-term process.

In a number of these memory mutants, the critical gene is expressed preferentially in the mushroom bodies (Davis, 1996). The mushroom bodies are essential for olfactory learning not only in *Drosophila* but also in the honeybee, which also uses PKA for learning (Menzel and Muller, 1996). Based on these studies, Davis has suggested that the mushroom bodies serve as centers for integrating sensory information about odors and electric shock during olfactory conditioning.

Nondeclarative Memory Storage Uses Conserved Signal Transduction Pathway

Both cellular studies of *Aplysia* and genetic studies of *Drosophila* indicate that the cAMP cascade is one of the core signal transduction pathways important for certain elementary forms of short-term and long-term memory storage. Moreover, the data in *Drosophila* and in *Aplysia* are complementary in providing molecular evidence that the CREB genes are important components of the switch

from short-term to long-term memory; and that in addition to several activators, there are functional repressors that prevent information from being converted into long-term memory storage. Consistent with this evolutionary conservation, features of the cAMP–PKA–CREB switch have recently been found in a variety of long-term adaptive changes in brain such as drug addiction and alcohol abuse to convert a short-term change—social usage—to a long-term change—addiction (Nestler and Aghajanian, 1997).

In a broader sense, these studies revealed the interesting finding that the evolution of memory storage mechanisms in brain has been achieved not by recruiting a new set of molecules that is specialized for memory per se. Rather, memory has co-opted and modified, by combining it with additional components, a well-used and efficient signaling system used for *other* purposes in *other* cells of the body. Indeed, the cAMP system is one of the most primitive and evolutionarily conserved. It is the only major second messenger system found in unicellular organisms like bacteria, where it serves as a system to signal hunger.

As these arguments suggest, what may make the cAMP–PKA–CREB pathway suitable as a core pathway for memory storage is the addition of certain additional components. For example, in neurons, unlike in other cells, PKA acts synergistically with MAPK, a kinase often involved in the regulation of growth and in the removal of inhibitory constraints to growth. PKA and MAPK lead to the activation of CREB and to the induction of immediate-early genes, one of which—the ubiquitin hydrolase—is neuron specific. The hydrolase in turn leads to the activation of ubiquitin-mediated proteolysis and the establishment of a persistently active PKA, the first step downstream from CREB-1 in the long-term sequence.

In *retrospect*, what we are seeing in memory storage illustrates a key principle in biological regulations. The dominant idea to emerge from the molecular study of cellular regulation—the cell cycle, signal transduction, apoptosis, cell growth, and oncogenesis—is that biological processes are remarkably conserved. Perhaps the most remarkable example has emerged from studies of development. The genes involved in the formation of the body plan of vertebrates derives from genes and genetic pathways evident in *Drosophila* and *C. elegans*—even though the vertebrate body plan bears little resemblance to that of the fly and even less to that of the worm. Indeed, these same genes are utilized again in the formation of the vertebrate brain.

Molecular Insights into Declarative Memory Storage

Do the mechanisms for declarative memory in mammals differ from those for nondeclarative memory in invertebrates? As we have discussed above, experimental animals cannot declare anything; nevertheless, methods have been developed to explore in simple mammals, such as mice, forms of memory storage that have many of the critical features of declarative memory. Declarative memory storage is concerned with the ability to recall or recognize people, places, and objects. Rodents can readily be tested about their memories for places, objects, and odors, and these studies have revealed that lesions of the hippocampus and related structures interfere with long-term storage of these kinds of memory. One major focus of research on declarative memory in rodents has concerned the role of the hippocampus in spatial memory.

LTP and Hippocampal-Dependent Memory Storage

In the 1970s, two independent findings helped shape thinking about the role of the hippocampus in spatial memory: first, in 1971, O'Keefe and Dostrovsky discovered that hippocampal pyramidal cells can encode information about space (O'Keefe and Dostrovsky, 1971). Second, in 1973, Bliss and Lømo discovered that the synaptic connections within the hippocampus undergo long-term potentiation (LTP). We will consider LTP first.

Working in Per Andersen's laboratory in Oslo, Norway, Timothy Bliss and Terje Lømo first demonstrated that the synapses of the hippocampus have remarkable plastic capabilities of the kind that would be required for memory storage (Bliss and Lømo, 1973). A brief high frequency train of action potentials in any one of the three major anatomical pathways within the hippocampus produces a long-term potentiation (LTP), an increase in synaptic strength in that pathway that has been shown to last for hours in an anesthetized animal and for days and even weeks in an alert, freely moving animal. LTP has several features that make it suitable as a storage mechanism. First, it is found to occur within each of the three principal pathways in the hippocampus (Figure 9): the perforant pathway, the mossy fiber pathway, and the Schaffer collateral pathway (Bliss and Collingridge, 1993). Second, it is rapidly induced: it can be induced by a single, high frequency train of electrical stimuli. Third, once induced, it is stable for 1 hour to many hours or even days depending upon the number of repetitions of the inducing stimulus. Thus, as is the case for long-term facilitation in *Aplysia*, LTP has features of the memory process itself. It can be formed quickly at appropriate synapses and it lasts a long time. LTP in the three canonical synapses of the hippocampus has two forms. Mossy fiber LTP is nonassociative; it does not require coincident activity in both the pre- and postsynaptic elements of the synapse. By contrast, LTP in the perforant pathway and in the Schaffer collateral pathway is associative; it requires coincident pre- and postsynaptic activity. Because genetic lesions that interfere selectively with mossy fiber LTP in mice do not affect the animal's capability for spatial or contextual memory (Huang et al., 1995), we focus here on the Schaffer collateral pathway between the presynaptic CA3 neurons and the CA1 postsynaptic target cells. We do so because it is the best studied synaptic pathway in the hippocampus and because genetic lesions of LTP in this pathway can lead to memory deficits.

The Schaffer collateral axons in the hippocampus use glutamate as their transmitter. Glutamate produces LTP by acting postsynaptically on at least two types of receptors: NMDA receptors and non-NMDA receptors. Non-NMDA receptors mediate basal synaptic transmission

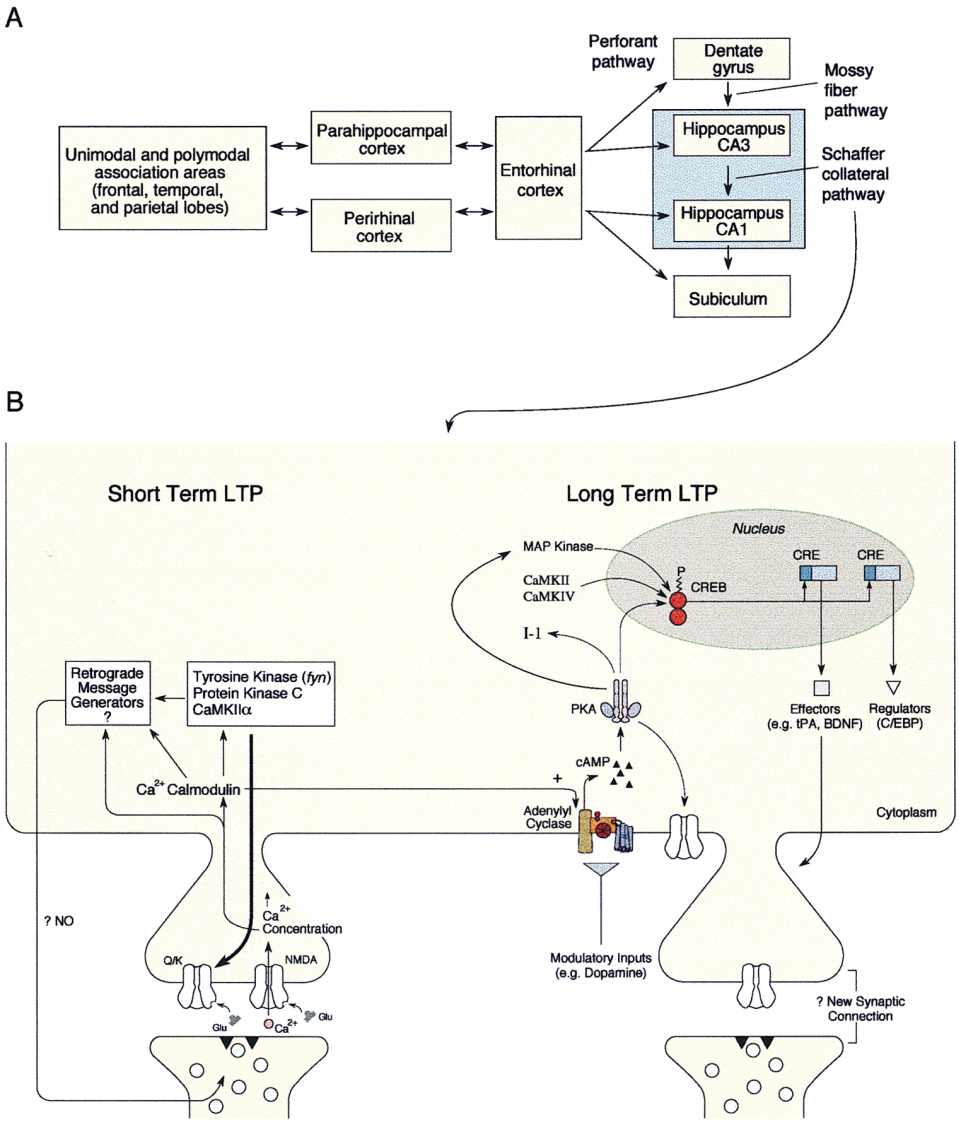

Figure 9. LTP and the Medial Temporal Lobe

(A) Flow diagram for the flow of information in the medial temporal lobe.
(B) Model of early and late phase of LTP in Schaffer collateral pathway.

because the ion channel associated with the NMDA receptor is blocked by magnesium at the resting potential. The NMDA receptor is unblocked only when the postsynaptic cell is depolarized. Thus, the NMDA receptor has associative or coincidence-detecting properties. Optimal activation of the NMDA receptor channel requires that the two signals—the binding of glutamate to the receptor and the depolarization of the postsynaptic cell—occur simultaneously (Bliss and Collingridge, 1993). Once the NMDA receptor is activated, it allows calcium influx into the postsynaptic cell. As was first shown by Gary Lynch and then by Roger Nicoll (reviewed by Bliss and Collingridge, 1993), calcium influx into the postsynaptic neuron is critical for the induction of LTP (Figure 9). The Ca^{2+} influx initiates LTP by activating, directly or indirectly, at least three different protein kinases: (1) calcium/calmodulin protein kinase II (Malenka et al., 1989; Malinow et al., 1989), (2) protein kinase C (Malinow, 1988), and (3) the tyrosine kinase fyn (O'Dell et al., 1990; Grant et al., 1992).

The *induction* of LTP clearly depends on postsynaptic depolarization, the influx of calcium, and the subsequent activation of second-messenger kinases in the postsynaptic cell. By contrast, the site for the *expression* or *maintenance* of LTP, be it presynaptic, postsynaptic, or both, is still debated (Bliss and Collingridge, 1993; Kullman and Siegelbaum, 1995).

As with memory storage in intact animals, LTP has both short-term and long-term phases as does facilitation in *Aplysia* (Figure 9). One stimulus train produces a short-term early phase of LTP (called E-LTP) lasting 1–3 hours; this phase does not require protein synthesis. Four or more stimulus trains induce a more persistent late phase of LTP (called L-LTP) that lasts for at least

24 hours (Frey et al., 1993; Abel, 1997). Late phase LTP requires the synthesis of new mRNA and protein, and it recruits the cAMP, PKA, MAPK, and CREB signaling pathway. Thus, although the molecular mechanisms for E-LTP differ from those used for short-term facilitation in *Aplysia*, the hippocampus uses a conserved set of mechanisms for converting E-LTP to L-LTP. Recent cellular physiological studies suggest that the late phase of LTP might also involve the formation of new synapses (Greenough and Bailey, 1988; Geinisman et al., 1991; Bolshakov et al., 1997).

Genetic Interference with LTP Interferes with Spatial Memory

Even though LTP has features that make it attractive as a memory mechanism, it is not yet clear that this is the mechanism that the hippocampus uses to store declarative memories such as spatial memory (Barnes, 1995; Goda and Stevens, 1996). To begin with, LTP is not unique to the hippocampus, or to declarative forms of memory. As we shall consider below, it is used for the storage of emotionally charged nondeclarative memory in the amygdala and it contributes to classical conditioning in invertebrates such as *Aplysia* that do not have (as far as we know) declarative forms of memory (Dale and Kandel, 1993; Lin and Glanzman, 1994; Murphy and Glanzman, 1997; Bao et al., 1997, 1998). Second, the high frequencies typically used to induce LTP are arbitrary and artificial. It is not at all clear whether the Schaffer collateral pathway (or any hippocampal pathway) ever is exposed to these particular frequencies during learning. Rather than assuming that LTP is specific to declarative memory it is more likely that LTP, as studied experimentally, represents an example of a class of mechanisms for changing synaptic strength that might be used for memory storage.

To examine whether this range of capabilities in the Schaffer collateral pathway is involved in spatial memory, it is important to show that blocking LTP in this pathway blocks long-term memory and that storing of a long-term memory leads to LTP in these hippocampal pathways. If LTP represents a class of synaptic mechanisms for establishing declarative spatial memory, then defects in LTP should interfere with spatial memory.

The initial evidence for this correlation was provided by Morris and his colleagues, who found that when NMDA receptors were blocked pharmacologically LTP was blocked and an animal could navigate a water maze successfully but could not form spatial memories. More direct evidence for this correlation came from genetic experiments. For many years, mutational genetic analyses of behavior were not feasible in mammals. However, in the 1980s and 1990s, methods were developed for expressing or deleting specific genes in mice. As a result, it became possible to determine how changes in the expression of a single gene affect LTP in the hippocampus, and how such a change in LTP affects spatial memory in the intact, freely behaving animal. These technical advances were first applied to studies of memory by Silva et al. (1992a, 1992b) and Grant et al. (1992). These early studies showed that interference with LTP in the Schaffer collateral pathway by knocking out specific genes interfered with spatial memory. However, the studies were limited both spatially and temporally (Mayford et al., 1996): (1) the gene was eliminated not just in the Schaffer collateral pathway but in all parts of the brain, and (2) the gene was eliminated throughout all development and could in principle have interfered with the formation of the basic wiring of the hippocampus.

More recently, a second generation of genetically modified mice has been used to address these two problems. For example, Tsien et al. (1996a) developed a method for producing a knockout of genes restricted to the pyramidal cells of the CA1 region. Tsien et al. (1996b) then used this method to knock out the R1 subunit of the NMDA receptor. These mice had normal basal synaptic transmission, but LTP in the Schaffer collateral pathway was completely disrupted. Although the disruption of LTP is restricted to the Schaffer collateral pathway, these mice nevertheless have a deficit in spatial memory. These findings provide compelling evidence that NMDA receptors and NMDA-mediated synaptic plasticity in the Schaffer collateral pathway are important for declarative memory.

However, gene knockouts, no matter how limited in their anatomical distribution, have the potential problem that the defect in LTP and spatial memory could conceivably result from a developmental defect in the wiring of the Schaffer collateral pathway. Although unlikely in the study of Tsien et al. (1996), this possibility can be eliminated by regulating expression of a transgene that interferes with LTP. Thus, with this idea in mind, Mayford et al. (1996) expressed in different lines of mice a persistently active form of the calcium/calmodulin-dependent protein kinase II in a regulated manner that allowed it to be turned on and off. The mutated gene product interfered with the LTP produced by low stimulation in the theta frequencies (1–10 Hz), a physiological frequency recruited in the hippocampus when a mouse explores an environment. These lines of mice were also deficient in spatial learning and memory. When the transgene was turned off, however, both LTP and the animal's capability for spatial learning and memory were restored. These two findings make it clear that LTP in the Schaffer collateral pathway is essential for spatial memory. The role of LTP in this pathway is quite specific. As mentioned above, selective genetic lesions of mossy fiber LTP had no effect on spatial memory (Huang et al., 1994).

As we discussed earlier, LTP has both early and late phases. Defects in the various phases of LTP are surprisingly selective. Expression in the hippocampus of a transgene that blocks protein kinase A selectively disrupted the late phase of LTP in the Schaffer collateral pathway (Abel et al., 1997). A similar defect is evident in animals that have a selective lesion in the CREB gene (Bourtchouladze et al., 1994). Animals with these deficits had normal learning abilities and normal short-term memory when tested at 1 hour after learning, but they did not convert short-term memory into stable long-term memory. Essentially similar results were obtained when normal wild-type mice were given inhibitors of protein synthesis just before training. Taken together, these experiments show that interference with the early component of LTP in the Schaffer collateral pathway,

as with knockout of *NMDAR* or with blockers of the NMDA receptor, also blocks the late component of LTP and therefore is correlated with deficits in both short- and long-term memory. By contrast, interference with the late component of LTP is correlated only with impaired long-term memory.

Genetic Interference with LTP Is Reflected in the Properties of Place Cells in the Hippocampus

Long-term potentiation in the hippocampus is an artificially induced change in synaptic strength produced by electrical stimulation of synaptic pathways. Is this form of synaptic plasticity used physiologically in the storage of spatial memories? Is it used for the development of a map of space? Following on O'Keefe's original observation, a variety of studies have shown that pyramidal cells can encode relationships between features of the environment that are critically relevant to a learning task. Specifically, the same pyramidal cells that undergo LTP when their afferent pathways are stimulated electrically can also encode the location of an animal in a particular space. Thus, a mouse's location is represented by the discharge of a unique population of hippocampal place cells, each of which discharges when the animal is in a particular area (the cell's "place field"). When the animal enters a new environment, new place fields are formed within minutes, and they are stable for weeks to months. The same pyramidal cells may signal different information in different environments and can therefore be used in more than one spatial map.

The rapid formation and persistence of place fields offers an opportunity to ask: how are place fields formed, and once formed, how are they maintained? Is LTP important for the formation or maintenance of place fields? To address these questions, place cells were examined by McHugh et al. (1996) and by Rotenberg et al. (1996) in the two types of mutant mice generated by Tsien et al. and Mayford et al., which we considered above. As we saw, each of the mutations interferes with LTP in a different way. LTP was not required for the formation of place fields in either type of mutant. By contrast, LTP was required for the fine tuning of place cells and for their stability across time. This instability of place cells is reminiscent of the memory defect in severely amnesic patients with lesions of the medial temporal lobe. Each time these patients enter the same place (so long as the place was not known to them before they became amnesic), they behave as if they have never been there before. Taken together, these two studies of knockout mice lacking the R1 subunit of the NMDA receptor in CA1 and transgenic mice overexpressing a persistently active form of calcium/calmodulin-dependent protein kinase II suggest that LTP is important for maintaining a coherent spatial map.

The Study of Emotionally Charged Nondeclarative Memories May Prove to Be Particularly Advantageous for Determining which Aspects of LTP Are Most Directly Required for Memory Storage in the Mammalian Brain

As we have seen, the medial temporal-lobe memory system that supports declarative forms of memory storage is complex and has several anatomical components: the various association cortices that process information for perception project to the perirhinal and parahippocampal cortices, which in turn project to the entorhinal cortex, which projects through several pathways in the dentate gyrus and the hippocampus. From the dentate gyrus, the main pathway within the hippocampus flows to the CA3 region and then to the CA1 region of the hippocampus, and then to the subiculum, the output component, which in turn projects back to the association cortices through the entorhinal cortex. Thus, the task of understanding how sensory information is processed for any given memory, such as memory for spatial location, is daunting. Beyond that, there is the challenge for each of these relays of relating LTP to memory storage.

Although region-specific promoters and regulated gene expression should help in undertaking an analysis of the contributions of each of these regions to memory storage, the precise form of LTP that is required—the pattern of stimulation that is critical in a given region of the system—will be difficult to infer. As a result, the analysis of what role LTP serves in memory storage in the mammalian brain should benefit from a reductionist approach similar to the kind that has proven informative in invertebrates. As a first step, it will prove useful to analyze LTP in the context of simple, nondeclarative forms of memory, such as conditioned eyeblink or conditioned fear. We here describe recent studies of conditioned fear.

One of the major advances in the study of emotion has been the realization that the amygdala is critical for its expression. In humans, electrical stimulation of the amygdala produces feelings of fear and apprehension. Moreover, functional MRI studies have revealed that stimuli that elicit fear affect blood flow to the amygdala in humans (reviewed by LeDoux, 1995). In experimental animals, the amygdala similarly is essential for both instinctive and learned (conditioned) expressions of fear (Davis et al., 1994; LeDoux, 1995, 1996).

In experimental animals, conditioned fear is produced by pairing a neutral tone (CS) with a fear-inducing electric shock to the foot pads (US). Auditory information critical for conditioning of fear reaches the lateral nucleus of the amygdala via two routes: from the medial geniculate nucleus of the thalamus and from the auditory cortex (LeDoux, 1995, 1996; Maren and Fanselow, 1995). The synapses of both of these projections to the lateral nucleus are thought to be important for memory storage related to fear (Chapman et al., 1990; Rogan et al., 1995, 1997; McKernan and Shinnick-Gallagher, 1997). Both auditory pathways undergo NMDA-dependent LTP (Clugnet and LeDoux, 1990; Maren and Fanselow, 1995), and blocking the NMDA receptor blocks conditioned fear (Miserendino et al., 1990; Fanselow and Kim, 1994). Moreover, recent studies have shown that fear conditioning induces LTP in the auditory input to the basolateral nucleus (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). This LTP is mediated by NMDA receptors. Thus, as is the case for nondeclarative memory storage in invertebrates, a structure essential for memory storage—the amygdala—is directly in the pathway of the conditioned stimulus, the auditory input. Applying a molecular genetic approach to this system may prove to be a good initial strategy for analyzing

exactly which aspects of LTP are important for memory storage.

Molecular Similarities between Declarative and Nondeclarative Memory

On a cognitive level, declarative and nondeclarative memory differ dramatically. They use a different logic (conscious versus unconscious recall), and the memories are stored in different brain areas. However, despite these differences on the cognitive and systems level, the two ways of storing memory share several molecular steps in common.

To begin with, both nondeclarative memory—as studied in *Aplysia*, *Drosophila*, and for emotional memories in rodents—and declarative forms of memory demonstrate stages of memory storage: on a behavioral level there is both a short-term memory that does not require new protein synthesis and a long-term memory that requires new protein synthesis. The cellular representation of short-term memory in each case involves covalent modifications of pre-existing proteins by one or another second messenger kinase. By contrast, the cellular representation of long-term memory involves new protein synthesis. Moreover, at least some examples of both forms seem to share a common switch for converting short-term to long-term memory, the switch's components being cAMP, PKA, MAPK, and CREB-mediated transcription of downstream genes. Finally, both forms appear to use morphological changes at synapses to stabilize long-term memory (Abel et al., 1995).

These several findings have given us a new set of insights into both memory storage and into the evolutionary conservation underlying the molecular underpinnings of mental processes. Although memory involves a variety of different declarative and nondeclarative processes, what is conserved in many of these storage processes is not simply a set of genes and proteins but entire signaling pathways and programs for inducing and stabilizing long-term memory storage. Moreover, the homology does not simply extend from nondeclarative to declarative memory—it extends from invertebrates such as *Drosophila* and *Aplysia* and to mammals such as mice. Taken together, the studies in *Drosophila*, *Aplysia*, and rodents suggest that these quite different types of memory processes, involving distinct neuronal systems for storage, share a common set of molecular mechanisms for the consolidation of short-term to long-term memory.

Looked at from another perspective, studies of synaptic plasticity emphasize still another feature of molecular and functional conservation. There are as yet no forms of plasticity evident in the vertebrate brain that are not already found in invertebrates.

Structural Changes May Prove to Be a General Mechanism for Stabilizing Functional Changes in Both Nondeclarative and Declarative Memory Systems

Both the work on structural changes in nondeclarative memory and that on the possible structural changes in the hippocampus related to declarative memory have been paralleled by studies of neocortical neurons, demonstrating a considerable capacity of these neurons to change their response properties as a result of behavioral experience (Merzenich and Sameshima, 1993). It was long believed that the structure of our sensory cortical areas must be fixed to guarantee the stability of perception. However, recent work on plasticity in the sensory cortices has introduced the idea that the structure of the brain, even in sensory cortex, is unique to each individual and dependent on each individual's experiential history. For example, structural changes induced by behavioral training in the rat occur in task-relevant areas of cortex or cerebellum and include increases in the amount of dendrite per neuron and in the number of synapses per neuron (Greenough et al., 1996). Although the findings are correlational and cannot be definitively linked to learning per se, they document the considerable capacity of the mature nervous system to modify its anatomical circuitry. Similarly, monkeys trained to use their fingers actively in a tactile discrimination task reorganized the sensory cortical map (area 3b) of their hand area and expanded the area that represented the stimulated fingers (Merzenich and Sameshima, 1993). Finally, it also appears likely that the gradual growth of cortical axons, including a proliferation of new synaptic terminals, is involved in some phenomena of perceptual learning (Gilbert, 1998).

Cognitive Neuroscience in the Context of the Last Six Decades

As *Neuron* prepares to enter the twenty-first century, the neurosciences, whose six decades of achievement we celebrate in this issue of the journal, have matured. With this maturation, the neurosciences now have moved from the peripheral position they occupied in the 1940s to a central position within the biological sciences. There has been remarkable progress in understanding neuronal and synaptic signaling. These advances now invite a structural approach to visualize the static and dynamic structures of ion channels, receptors, and the molecular machinery for vesicle transport, fusion, and exocytosis.

Similarly, an understanding in outline of the development of the nervous system has been achieved by a molecular approach. Specific molecules have been identified as inducers and morphogens, constructs that previously were shrouded in mystery. Progress in this area has in turn made possible a molecular-based neurology, a neurology that will, one hopes, finally be able to address the degenerative diseases of the brain that have for so long eluded our best scientific efforts.

The remarkable advances in the cellular understanding of the organization of the somatosensory and visual system by Vernon Mountcastle and Hubel and Wiesel have helped turn our interest to perception and in the broader sense to cognitive psychology. In turn, contact between cognitive psychology and neuroscience has given us a new approach to the classic problems of the mind such as memory—on which we have here focused.

But, of all the fields in neuroscience, in fact, of all the fields in all of science, the problems of cognitive neuroscience—the problems of perception, action, memory, attention, and consciousness on an intellectually satisfying biological level—offer the most difficult and the greatest challenge for the next millennium. In the

fullness of time, advance in these areas may grant us insight into and perhaps solutions for some of the most debilitating diseases confronting medical science—schizophrenia, depression, and Alzheimer's disease.

We have here focused on only one component of cognitive neuroscience, that of memory. As we indicated in the introduction, the problem of memory has two components—the *molecular problem* of memory and the *systems problem* of memory. In the last four decades substantial progress has occurred in both areas.

On the molecular level, a core signaling pathway has been identified that is used in a variety of nondeclarative and declarative forms of memory to convert short-term to long-term memory. Thus, these two major forms of memory use common elementary mechanisms for storage. Most likely the cAMP–PKA–MAPK–CREB pathway represents only one of what is likely to be several core mechanisms for achieving this transformation. The tasks ahead for a deeper understanding of molecular mechanisms are fairly clear. Although we now know something about the switch from short-term to long-term memory, we know only a small percentage of the downstream genes and proteins, for example, the proteins required for the growth of new synaptic connections. *Drosophila*, *Aplysia*, and perhaps *C. elegans* should contribute importantly to further gene discovery. Because we now know that the molecular mechanisms are at least in part shared across species and forms of memory, it also will be important to direct this molecular analysis to the simpler instances of nondeclarative memory in mice, using fear conditioning and the amygdala and eyeblink conditioning and the vestibulo-ocular reflex, whose modifications occur in the cerebellum and its deep nuclei.

The greatest challenges, however, lie in the *systems biology* of memory and in particular in the biology of declarative, conscious memory. We do not, as yet, understand the functions of the various subdivisions of the medial temporal-lobe system. Analyses of perception indicate that the visual image is deconstructed and processed in the cortex in a series of parallel processing streams. However, despite clear evidence for parallel anatomical pathways in the hippocampus, it has not been possible to delineate their functional significance. By analogy to the visual system, it would seem likely that different regions of the medial temporal-lobe system (the parahippocampal and perirhinal cortices, the entorhinal cortex, the dentate gyrus, the CA1 and CA3 regions of the hippocampus, and the subiculum) have specialized functional roles in memory storage: they might each mediate the store of different aspects of learned information (Figure 9). Alternatively, memory itself requires several operations such as encoding, storage, consolidation, and retrieval. Perhaps different regions carry out these different types of operations. To intervene in each of the critical regions and explore each of these component processes, we will need further improvements in genetic methods.

It also will be important to understand how acquired representations in the hippocampus act to support memory. For example, how does the spatial map, evident in the hippocampus, relate to spatial memory? How is this spatial map read out? How is the map of space in the hippocampus reflected in our conscious recollection of a space? That of course leads to a still larger question: how does declarative information become available to conscious introspection? How are nonspatial memories that are declarative represented in the hippocampus? How is it transformed from a hippocampal-dependent process to a hippocampal-independent and presumably neocortical-dependent process that is capable of being scanned by conscious attention in ways that we are still very far from understanding? Clearly one Decade of the Brain (and of *Neuron*) has not been enough. Will a millennium suffice?

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