I. INTRODUCTION

The following chapters review recent and current research on many important aspects of the neurobiology of learning and memory. This chapter gives some historical perspective to this active field. Having participated in this research for half a century, I am happy to share information, interpretations, and insights about this productive multidisciplinary area.

In antiquity, speculation about mechanisms of memory took the form of metaphors, and metaphors of memory continue to be proposed in the present day. By the last quarter of the nineteenth century, scientific hypotheses and investigations of memory and its mechanisms began to be made, and it appeared that progress would be rapid. Research on neurobiological mechanisms of memory appeared to stall, however, and by the middle of the twentieth century, some thinkers despaired about the possibility of progress in this apparently intractable field. But shortly after midcentury, research and theory took off again, and rapid progress has continued to this day.
II. METAPHORS OF MEMORY

Concern about memory and its mechanisms goes far back in recorded history. An ancient Egyptian legend, related by the Greek philosopher Plato (427–347 BCE) in his *Phaedrus*, told that Thoth, the god of knowledge, offered the gift of writing to King Thamus of Egypt. The king was reluctant to accept the gift, expressing the fear that writing would cause forgetfulness because people would no longer exercise their memories but tend to rely instead on external written characters.

Thinkers in antiquity speculated about the mechanisms of memory and suggested metaphors for them. A widespread metaphor for memory was writing on a tablet coated with wax. The god Thoth was often depicted writing on such a tablet. As Draaisma notes in his book *Metaphors of Memory: A History of Ideas About the Mind* (2000), the classic passage on the wax tablet as the metaphor for memory appears in Plato's (1987) *Theaetetus*. In this dialogue, Socrates suggests:

> [O]ur minds contain a wax block, which may vary in size, cleanliness and consistency in different individuals, but in some people is just right. . . . [W]henever we want to remember something we've seen or heard or conceived on our own, we subject the block to the perception or the idea and stamp the impression into it. . . . We remember and know anything imprinted, as long as the impression remains in the block; but we forget and do not know anything which is erased or cannot be imprinted (pp. 99-100).

This wax tablet, wrote Plato, was a gift of Mnemosyne, the goddess of memory in the Greek pantheon and mother of the muses. We still acknowledge this goddess when we speak of mnemonic devices.

The metaphor of the wax tablet returned at greater length and in greater detail in the work of Aristotle (384–322 BCE), the pupil of Plato. Aristotle suggested that in the case of illness that affected memory, the consistency of the wax would be too loose, so no clear image could be stamped on it, just as no impression would be formed if a seal were to impinge on running water. He proposed that this is also why young children and old people have poor memories. They are in a state of flux, the former because of their growth, the latter because of their decay (see ref. in Draaisma, 2000, p. 46). The close association of memory and writing appears from Latin through French to English. The Latin word *memoria* meant both “memory” and “memoir.” In French, *la memoire* means “memory” and *le memoire* means “memoir.” And English has the related words “memory” and “memoir,” derived from French.

Throughout the centuries, a series of metaphors was proposed for mechanisms of memory, each in keeping with current practices and technology. Here are some examples: The metaphor of a dovecote or aviary was long used; we still refer to this when we speak of placing a memory in a mental pigeonhole.
In the Middle Ages, books as well as libraries provided metaphors of memory. In the nineteenth century, the rapid progress of technologies for recording and transmitting information provided a series of metaphors for memory. Photography (from the 1830s) was one; think of the expression “a photographic memory.” Telegraphy, also from the 1830s, provided another metaphor. The telephone system, with its switchboard, offered a more flexible system in the 1870s. In 1877 came the phonograph, which provided a mechanical memory for sound. Early phonograph records inscribed the sound on wax-covered cylinders, thus updating the ancient technology of writing on wax tablets. Even in the late twentieth century, while research in the neurobiology of memory prospered, metaphors of memory based on recent technologies continued to be proposed, such as the digital computer and the hologram.

III. ADVANCES IN THE LAST QUARTER OF THE NINETEENTH CENTURY

By the last quarter of the nineteenth century, sufficient progress had been made in psychology and neurobiology for scientific research to begin in memory and its neural mechanisms. Psychology was becoming established as an independent academic discipline and as a laboratory science in Europe and North America. Wilhelm Wundt, a professor of philosophy with a doctorate in medicine, had founded the first formal laboratory of psychology at the University of Leipzig in 1879. William James, also a professor of philosophy with a medical degree, began teaching physiological psychology at Harvard University in 1875, and he had an informal laboratory of psychology.

The decade of the 1880s saw major advances in research on learning and memory. French psychologist Théodule Ribot published an important book, *The Diseases of Memory* (1881), in which he described and discussed impairments of memory as consequences of brain lesions and brain diseases. From his study of published reports, Ribot proposed that more recent memories were more likely to be impaired than were older memories. This formulation became known as "Ribot's law," and it was verified by experimental research a century later. In his book, Ribot wrote that he regretted that it was not possible to state impairments of memory in quantitative terms. Only a few years later, German psychologist Hermann Ebbinghaus showed how memory could be measured in his pathbreaking book *On Memory* (1885). This book inaugurated the experimental investigation of learning and memory, a field that soon expanded rapidly.

Contemporaries and immediate successors of Ebbinghaus soon enlarged the work he had started, emphasizing controlled research on memory in a laboratory setting. Although Ebbinghaus' research obviously encouraged others, they were ready to move in this direction, as was shown in a review by Postman:
Ebbinghaus' paradigm did not dominate or constrain the development of the field in its early years. Not only were many new methods of measurement and types of materials introduced in rapid succession, but the kinds of questions that were asked about memory soon began to move in different directions (Postman, 1985, p. 127).

An important monograph on studies of verbal memory was published by Müller and Pilzecker in 1900. In it they put forth the perseveration—consolidation hypothesis, which engendered much further research. This hypothesis held that neural activity initiated by a learning trial continues and recurs for some time after the original stimulation has ceased and that this perseveration aids the consolidation of a stable memory trace. In reviewing this book, William McDougall (1901) pointed out that the perseveration—consolidation hypothesis could be used to account for retrograde amnesia following head injury.

A. William James (1890) on the Physical Basis of Habit and Memory

In his major textbook Principles of Psychology (1890), James devoted separate chapters to habit, association, and memory. James asserted that habit, memory, and other aspects of behavior are based on physiological properties of the brain, even when he could not specify those properties very clearly. Thus James stated that the cerebral hemispheres seem to be the chief seat of memory (p. 98). James devoted Chapter 4 to habit and Chapter 16 to memory; a related chapter, 14, was devoted to association. The separation of the chapters on habit and on memory can be seen as a precursor to the distinction made in the 1980s between nondeclarative and declarative memories. Habits, according to James, reflected the “plasticity of the organic material [of the nervous system]” (p. 105). Neural activity could either “deepen old paths or . . . make new ones” (p. 107). James admitted that it was not yet possible to define in a detailed way what happens in the nervous system when habits are formed or changed, but he was confident that scientific research would find the answers (1890, p. 107):

[O]ur usual scientific custom of interpreting hidden molecular events after the analogy of visible massive ones enables us to frame easily an abstract and general scheme of processes which the physical changes in question may be like. And when once the possibility of some kind of mechanical interpretation is established, Mechanical Science, in her present mood, will not hesitate to set her brand of ownership upon the matter, feeling sure that it is only a question of time when the exact mechanical explanation of the case shall be found out.

James used mechanical here in the sense of mechanistic, that is, interpreting and explaining phenomena by referring to causally determined material forces.
James gave lessons on how to form habits effectively. And he drew an ethical lesson, with a molecular basis:

Could the young but realize how soon they will become mere walking bundles of habits, they would give more heed to their conduct while in the plastic state. . . . Every smallest stroke of virtue or vice leaves its never-so-little scar. The drunken Rip Van Winkle, in Jefferson's play, excuses himself for every fresh dereliction by saying, "I won't count this time!" Well! he may not count it, and a kind Heaven may not count it; but it is being counted none the less. Down among his nerve cells and fibres the molecules are counting it, registering and storing it up to be used against him when the next temptation comes (1890, p. 127).

James distinguished between what later came to be called short-term and long-term memories, referring to them as "primary" and "secondary" memories (1890, p. 670). Concerning the tendency of emotionally exciting experiences to be remembered well, James wrote, "An impression may be so exciting emotionally as to almost leave a scar on the cerebral tissues" (1890, p. 670).

James devoted three pages (pp. 676–678) to the experiments of Ebbinghaus (1885) under the heading "Exact Measurements of Memory." Considering Ebbinghaus' curve of forgetting, James commented, "The nature of this result might have been anticipated, but hardly its numerical proportions" (p. 677). James praised Ebbinghaus especially for his novel and successful attempt to test experimentally between two opposed hypotheses: This referred to Ebbinghaus' evidence that serial learning involves not only direct associations between adjacent items but also the formation of remote associations between nonadjacent items. James commented that the fact of these remote associations ought to make us careful, when we speak of nervous "paths," to use the word in no restricted sense. They add one more fact to the set of facts which prove that association is subtler than consciousness, and that a nerve-process may, without producing consciousness, be effective in the same way in which consciousness would have seemed to be effective if it had been there (p. 678).

As of 1890 there were few techniques available to study neural processes that might occur during learning and memory formation or ways of studying possible effects of memory on brain anatomy or neurochemistry. The development and use of such techniques characterized the research of the twentieth century, but speculation about neural junctions as sites of change in learning were already prevalent in the late nineteenth century, as we note next.

**B. Neural Junctions as Sites of Change in Learning**

In the 1890s, several scientists speculated that changes at neural junctions might account for memory. This was anticipated, as Finger (1994) points out, by associationist philosopher Alexander Bain (1872), who suggested that memory
formation involves growth of what we now call synaptic junctions: “For every act of memory, every exercise of bodily aptitude, every habit, recollection, train of ideas, there is a specific grouping or coordination of sensations and movements, by virtue of specific growths in the cell junctions” (p. 91).

Such speculations were put on a firmer basis when neuroanatomist Wilhelm von Waldeyer (Waldeyer-Hartz, 1891) enunciated the neuron doctrine, largely based on the research of Santiago Ramón y Cajal. Neurologist Eugenio Tanzi (1893) proposed the hypothesis that the plastic changes involved in learning probably take place at the junctions between neurons. He expressed confidence that investigators would soon be able to test by direct inspection the junctional changes he hypothesized to occur with development and training. About 80 years were to elapse, however, before the first results of this sort were announced.

Ramon y Cajal, apparently independent of Tanzi, went somewhat further in his Croonian lecture to the Royal Society of London (Cajal, 1894). He stated that the higher one looked in the vertebrate scale, the more the neural terminals and collaterals ramified. During development of the individual, neural branching increased, probably up to adulthood. And he held it likely that mental exercise also leads to greater growth of neural branches, as he stated with a colorful set of metaphors:

The theory of free arborization of cellular branches capable of growing seems not only to be very probable but also most encouraging. A continuous preestablished network — a sort of system of telegraphic wires with no possibility for new stations or new lines — is something rigid and unmodifiable that clashes with our impression that the organ of thought is, within certain limits, malleable and perfectible by well-directed mental exercise, especially during the developmental period. If we are not worried about putting forth analogies, we could say that the cerebral cortex is like a garden planted with innumerable trees — the pyramidal cells — which, thanks to intelligent cultivation, can multiply their branches and sink their roots deeper, producing fruits and flowers of ever greater variety and quality (Cajal, 1894, pp. 467-468).

But Ramón y Cajal then considered an obvious objection to his hypothesis:

You may well ask how the volume of the brain can remain constant if there is a greater branching and even formation of new terminals of the neurons. To meet this objection we may hypothesize either a reciprocal diminution of the cell bodies or a shrinkage of other areas of the brain whose function is not directly related to intelligence (p. 467).

We will return later to this assumption of constancy of brain volume and Ramón y Cajal’s hypotheses to permit constancy in the face of increased neuronal ramification.

The neural junctions didn’t have a specific name when Tanzi and Ramón y Cajal wrote early in the 1890s, but a few years later neurophysiologist Charles Sherrington (Foster and Sherrington, 1897) gave them the name synapse.
rington also stated that the synapse was likely to be strategic for learning, putting it in this picturesque way:

Shut off from all opportunities of reproducing itself and adding to its number by mitosis or otherwise, the nerve cell directs its pent-up energy towards amplifying its connections with its fellows, in response to the events which stir it up. Hence, it is capable of an education unknown to other tissues. (p. 1117).

During the first half of the twentieth century, psychologists and other scientists proposed memory hypotheses involving either the growth of neural fibrils toward one another to narrow the synaptic gap or more subtle chemical changes at synapses (see review in Finger, 1994). But the techniques then available allowed little progress on this issue.

C. Introduction of Research on Learning in Animal Subjects

Research on learning and memory was extended to animal subjects independently by psychologist Edward L. Thorndike and physiologist Ivan P. Pavlov. Thorndike demonstrated in his doctoral thesis (1898), conducted under the supervision of William James, how learning and memory can be measured in animal subjects, using cats, dogs, and chicks. This research led to the concept of trial-and-error learning and, later, to the "law of effect" (Thorndike, 1911). The field Thorndike opened with this research was quickly entered by others (Hilgard and Marquis, 1940, p. 6).

In 1902, American psychologist Shepard I. Franz opened a further line in animal research on learning and memory. He sought to determine the site of learning in the brain by combining Thorndike's methods of training and testing animals with the technique of localized brain lesions. Franz later recruited Karl S. Lashley, and through Lashley many others, to research on this topic.

In contrast to Thorndike's planned study of animal learning, Pavlov came upon the concept of conditioning from observations on salivary responses, made during his Nobel Prize–winning research on secretions of the alimentary tract. His initial contribution to the study of learning has been dated anywhere from 1897 to 1904 or even 1906. The American Psychologist [1997, 52(9)] and the European Psychologist [1997, 2(3)] published parallel sections in 1997 to commemorate the centenary of Pavlov's book, in Russian, Lectures on the Work of the Principal Digestive Glands (Pavlov, 1897). Pavlov's book included observations on psychic secretion, which foreshadowed his later research on conditioning. The first published use of the term conditioned reflex (actually conditional reflex) was in a report by I.F. Tolotschinoff (Tolochinov), one of Pavlov's associates, at the Congress of Natural Sciences in Helsinki in 1902. Pavlov discussed conditioning in his Nobel Prize lecture in 1904, although the main subject of
the lecture was the research on the digestive glands, for which the Nobel Prize was awarded. Pavlov's first paper in English on salivary conditioning was his 1906 Huxley lecture, "The scientific investigation of the psychical faculties or processes in the higher animals," which was published in both *The Lancet* and *Science*. Even this review did not, however, "lead to any immediate repetitions of Pavlov's work in America, so far as published records reveal" (Hilgard and Marquis, 1940, p. 10).

Conditioning is now such a widely used technique — including in the research reviewed in several chapters in this volume — that it is interesting to note that it did not gain acceptance rapidly. Only after the presidential address of John B. Watson to the American Psychological Association in 1915, "The place of the conditioned reflex in psychology" (Watson, 1916), did conditioning begin to gain a prominent place in textbooks, and its place in the laboratory lagged behind still further. The publication in 1927 and 1928 of translations of books by Pavlov, revealing the wealth of facts discovered by Pavlov and his colleagues during more than a quarter of a century of research on salivary conditioning in dogs, stimulated a series of replications and extensions to conditioning in other species.

1. *Earlier Observations of "Psychical Secretion"*

In evaluating Pavlov's contributions, it is important to note that Pavlov, as he stated in his 1904 Nobel Prize lecture, was not the first to observe that secretions of the salivary and gastric glands can be evoked by "psychic" (i.e., non-gustatory) stimuli. Although Pavlov did not feel it necessary to name his predecessors in this respect, several medical or physiological investigators recorded such observations in the eighteenth and nineteenth centuries, and many more must have seen this phenomenon. One of the earliest such reports I have seen is that of Robert Whytt in his book *An Essay on the Vital and Other Involuntary Motions of Animals* (1763, p. 280):

> We consider, that not only an irritation of the muscles of animals, or parts nearly connected with them, is followed by convulsive motions; but that the remembrance or idea of substances, formerly applied to different parts of the body, produces almost the same effect, as if these substances were really present. Thus the sight, or even the recalled idea of grateful food causes an uncommon flow of spittle into the mouth of a hungry person; and the seeing of a lemon cut produces the same effect in many people. . . . The sight of a medicine that has often provoked [sic] vomiting, nay, the very mention of its name, will in many delicate persons raise a nausea.

Note that in the last sentence, Whytt also anticipated Garcia's (1990) *bait-shyness* learning. Further descriptions of salivary responses presumably elicited by learned stimuli were made by Erasmus Darwin (the grandfather of Charles Darwin) in 1796, French physiologist C.-L. Dumas (1803), Claude Bernard...
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Pavlov's contribution was not to discover this phenomenon but to investigate it. He was the first to demonstrate that salivation could be evoked by a previously neutral stimulus after this had been paired with an effective stimulus. And he investigated carefully and skillfully both the conditions under which such acquisition occurs and conditions that do not lead to acquisition even though stimuli have been paired. This is one of many instances in the history of the field in which a casual observation has been exploited to lead to an important advance in knowledge.

2. **Pavlov's Physiological Theory**

The fundamental concepts in Pavlov's physiological theory, as summarized by Hilgard and Marquis (1940), were excitation and inhibition, conceived as states or processes located in the cerebral cortex. Afferent stimulation by an originally neutral stimulus caused an excitatory process to be initiated at a particular point $A$ on the cortex, from whence it spread or irradiated over the cortex. The irradiating excitation will be concentrated at any other focus of excitation, such as that aroused by an unconditioned stimulus. After a number of repetitions of the two stimuli, the excitation aroused by the neutral stimulus is drawn to the locus of the unconditioned stimulus in sufficient intensity to elicit the unconditioned response. The direction of the drainage of excitation is from the weaker to the stronger or more dominant focus of excitation (Hilgard and Marquis, 1940, p. 310).

These concepts were elaborated by Pavlov to account for such phenomena as conditioning, generalization, and extinction and also for sleep, hypnosis, and neurosis.

In spite of the tempting simplicity and scope of Pavlov's conception of cortical physiology, Hilgard and Marquis (1940) noted that it did not attract any wide degree of acceptance. Two of the primary objections they summarized are these:

1. Concepts of cortical physiology should be based on direct measures of cortical function, but Pavlov's "excitation" and "inhibition" were purely inferential concepts based on overt movements or amounts of saliva secreted (Hilgard and Marquis, 1940, p. 312).

2. Pavlov's physiological conceptions are explicitly based on the premise that conditioning is exclusively a cortical function. Recent experimentation ... demonstrates, however, that conditioning is possible at a subcortical level. ... The two-dimensional character of Pavlov's irradiation concept does not easily permit extension of the theory to embrace the integrated functioning of cortical and subcortical centers" (p. 313).
IV. PESSIONISM IN MIDCENTURY, THEN RAPID GAINS

By 1950, the search for neural mechanisms of learning and memory seemed to have reached an impasse. During graduate studies at Harvard in the late 1940s, I heard mainly pessimistic opinions about prospects for the field. For example, when Karl S. Lashley surveyed the literature on possible synaptic changes as a result of training, he concluded that there was no solid evidence to support any of the “growth” theories. Specifically, Lashley offered these criticisms: (a) Neural cell growth appears to be too slow to account for the rapidity with which some learning can take place. (We return to this point later.) (b) Because he was unable to localize the memory trace, Lashley held there was no warrant to look for localized changes.

Edwin G. Boring, the historian of psychology, also testified in 1950 to the lack of progress in this area: “Where or how does the brain store its memories? That is the great mystery. . . . The physiology of memory has been so baffling a problem that most psychologists in facing it have gone positivistic, being content with hypothesized intervening variables or with empty correlations” (1950, p. 670).

In other words, we were still at the level of the ancient Egyptians and Greeks, having metaphors but no neurobiological mechanisms for memory. At the end of his chapter on the history of research on brain functions, Boring gave his view about what was needed for further progress:

In general it seems safe to say that progress in this field is held back, not by lack of interest, ability, or industry, but by the absence of some one of the other essentials for scientific progress. Knowledge of the nature of the nerve impulse waited upon the discovery of electric currents and galvanometers of several kinds. Knowledge in psychoacoustics seemed to get nowhere until electronics developed. The truth about how the brain functions may eventually yield to a technique that comes from some new field remote from either physiology or psychology. Genius waits on insight, but insight may wait on the discovery of new concrete factual knowledge (1950, p. 688).

A few years later, Hans-Lukas Teuber stated, in an Annual Review of Psychology chapter on physiological psychology, that

the absence of any convincing physiological correlate of learning is the greatest gap in physiological psychology. Apparently, the best we can do with learning is to prevent it from occurring, by intercurrent stimulation through implanted electrodes, . . . by cerebral ablation, . . . or by depriving otherwise intact organisms, early in life, of normal sensory influx (Teuber, 1955, p. 267).

In fact, some major advances were beginning to occur in research on the neural mechanisms of learning and memory. Some of these resulted from the application of recently developed techniques, such as single-cell electrophysiological recording and electron microscopy, and the use of new neurochemical methods, as we review shortly. Another major influence encouraging research
on neural mechanisms of learning and memory was Donald O. Hebb’s 1949 monograph, *The Organization of Behavior*. I had the good fortune to be exposed to Hebb’s optimistic perspective in a seminar he gave at Harvard in the summer of 1947, using as a text a mimeographed version of his book that was published in 1949. Hebb (1949) was more positive about possible synaptic changes in learning than his colleague Lashley. Hebb noted some evidence for neural changes and did not let the absence of conclusive evidence deter him from reviving hypotheses about the conditions that could lead to the formation of new synaptic junctions and that underlie memory. In essence, Hebb’s hypothesis of synaptic change underlying learning resembled James’ formulation: “When two elementary brain processes have been active together or in immediate succession, one of them, on recurring, tends to propagate its excitement into the other” (James, 1890, p. 566). Hebb’s dual-trace hypothesis also resembled the consolidation-perseveration hypothesis of Müller and Piéron. Much current neuroscience research concerns properties of what are now known as Hebbian synapses. Hebb was wryly amused that his name was connected to this resurrected hypothesis rather than to concepts he considered original (Milner, 1993, p. 127). The emphasis on the synaptic hypothesis reflects the fact, noted by Gallistel (1990, p. 570), that most neuroscientists have been more concerned with how synaptic changes can store information than with how neural networks can compute memories. By the 1990s the idea that correlated activity could lead to new neural connections was so well accepted that it could be epitomized in six words: Neurons that fire together wire together (Löwel and Singer, 1992, p. 211).

V. NEUROCHEMICAL AND NEUROANATOMICAL EFFECTS OF TRAINING AND EXPERIENCE

Ten years after Hebb’s 1949 book was published, his postulate of use-dependent neural plasticity had still not been demonstrated experimentally. It seemed to many that it would not be possible, with available techniques, to find changes in the brain induced by training or experience. In fact, some neurobiologists spoke of a catch-22 in trying to find neurochemical changes as a result of training in an extract of whole brain: If a change is detected, it can probably be ruled out as being a result of training — any changes observed can more reasonably be attributed to grosser and less specific concomitants of learning such as stress, attentiveness, and so on (Agranoff, Burrell, Dokas, and Springer, 1978, p. 628). At a symposium in 1957 my colleagues and I proposed that an approach to this problem would be to make neurochemical analyses of specific regions of trained and untrained brains. This might be able to integrate and permit measurement of small changes taking place over many thousands of neural units. If such changes were found within a region, then subsequent
analyses might be able to focus down more closely (Rosenzweig, Krech, and Bennett, 1958, p. 338).

In the early 1960s, two experimental programs announced findings demonstrating that the brain can be altered measurably by training or differential experience. First was the demonstration by our group at Berkeley that both formal training and informal experience in varied environments led to measurable changes in neurochemistry and neuroanatomy of the rodent brain (Krech, Rosenzweig, and Bennett, 1960; Rosenzweig, Krech, and Bennett, 1961; Rosenzweig, Krech, Bennett, and Diamond, 1962). Soon after came the report of Hubel and Wiesel that occluding one eye of a kitten led to reduction in the number of cortical cells responding to that eye (Wiesel and Hubel, 1963; Hubel and Wiesel, 1965; Wiesel and Hubel, 1965).

The original clues for the discovery of the Berkeley group came from data on rats given formal training in a variety of problems in order to examine possible relations between individual differences in brain chemistry and problem-solving ability. We did obtain significant correlations between levels of activity of the enzyme acetylcholinesterase (AChE) in the cerebral cortex and the ability to solve spatial problems (e.g., Krech, Rosenzweig, and Bennett, 1956; Rosenzweig, Krech, and Bennett, 1958). When we tested the generality of this finding over six different behavioral tests, we found a surprise: As we reported at a 1959 symposium, total AChE activity was higher in the cerebral cortex of groups that had been trained and tested on more difficult problems than in those given easier problems, and all the tested groups measured higher in total cortical AChE activity than groups given no training and testing (Rosenzweig, Krech, and Bennett, 1961, p. 102 and Fig. 4). It appeared that training could alter the AChE activity of the cortex! To test this further, we conducted an experiment in which littermates were either trained on a difficult problem or left untrained; the trained rats developed significantly higher total cortical AChE activity than their untrained littermates (Rosenzweig, Krech, and Bennett, 1961, p. 103). Control experiments showed that the results could not be attributed to the fact that the trained rats were underfed to increase their motivation or were handled.

Instead of continuing to train rats in problem-solving tests, a time-consuming and expensive procedure, we decided to house the animals in different environments that provided differential opportunities for informal learning. Measures made at the end of the experiment showed that informal enriched experience led to increased cortical AChE activity (Krech, Rosenzweig, and Bennett, 1960). The discovery that formal training or differential experience caused changes in cortical chemistry was soon followed by the even more surprising finding that enriched experience increased the weights of regions of the neocortex (Rosenzweig et al., 1962).

Work by students of Hebb (e.g., Forgays and Forgays, 1952) provided the models for the environments used in these experiments. Typically, we assigned
littermates of the same sex by a random procedure among various laboratory environments, the three most common being these: (a) a large cage containing a group of 10–12 animals and a variety of stimulus objects, which were changed daily, called the enriched condition (EC) because it provided greater opportunities for informal learning than did the other conditions; (b) the standard colony or social condition (SC), with three animals in a standard laboratory cage; (c) SC-size cages housing single animals, called the impoverished condition or isolated condition (IC). All three conditions provided food and water ad libitum.

Over the next several years, replications and extensions by us (e.g., Bennett, Diamond, Krech, and Rosenzweig, 1964a) and by others (e.g., Altman and Das, 1964; Geller, Yuweiler, and Zolman, 1965; Greenough and Volkmar, 1973) added to the evidence that training or differential experience could produce measurable changes in the brain. Control experiments demonstrated that the cerebral differences could not be attributed to differential handling, locomotor activity, or diet. The brain-weight differences caused by differential experience were extremely reliable, although small in percentage terms. Moreover, these differences were not uniformly distributed throughout the cerebral cortex. They were almost invariably largest in the occipital cortex and smallest in the adjacent somesthetic cortex; the rest of the brain outside the cerebral cortex tended to show very little effect (Bennett et al., 1964a; Bennett, Krech, and Rosenzweig, 1964b). Thus the experience caused changes in specific cortical regions and not undifferentiated growth of brain. Later work also showed effects of differential experience in other parts of the brain that have been implicated in learning and formation of memory — the cerebellar cortex (Pysh and Weiss, 1979) and the hippocampal dentate gyrus (Juraska, Fitch, Henderson, and Rivers, 1985; Juraska, Fitch, and Washburne, 1989).

Further early studies revealed experience-induced changes in other measures, especially in the occipital cortex. These measures included not only cortical thickness (Diamond, Krech, and Rosenzweig, 1964) but also detailed cellular measures: sizes of neuronal cell bodies and nuclei (Diamond, 1967), size of synaptic contact areas (West and Greenough, 1972), an increase of 10% in the numbers of dendritic spines per unit of length of basal dendrites (Globus et al., 1973), an increase in the extent and branching of dendrites (Holloway, 1966) amounting to 25% or more (Greenough and Volkmar, 1973), and a parallel increase in the numbers of synapses per neuron (Turner and Greenough, 1985). Mainly because of the increase in dendritic branching, the neuronal cell bodies were spaced farther apart in the cortex of EC rats than in IC rats. These effects indicated substantial increases in cortical volume and intracortical connections; they suggested greater processing capacity of the cortical region concerned. They contradicted the speculation of Ramón y Cajal (Cajal, 1894), noted earlier, that with training, neural cell bodies would shrink in order to allow neural arborizations to grow, thus allowing brain volume to remain constant.
Instead, increased arborization requires larger cell bodies to maintain them, and the volume of the cortex increases as cell bodies and dendrites grow.

These experimental reports indicated growth of number and/or size of synaptic connections as results of training or enriched experience. Some workers declared for one or the other of these possibilities, as when neurophysiologist John C. Eccles (1965, p. 97) stated his belief that learning and memory storage involve "growth just of bigger and better synapses that are already there, not growth of new connections." But Rosenzweig et al. (1972) reviewed findings and theoretical discussions suggesting that negative as well as positive synaptic changes may store memory. Depending on where one measures and the kind of training or differential experience, one may find an increase in the number of synapses, an increase in their size, a decrease in the number, or decrease in the size.

Did the discovery of neurochemical and neuroanatomical effects of training or experience require novel experimental techniques? Yes and no. Accurate measurement of AChE activity in large numbers of tissue samples became practical only in the early 1950s, when we began our research. We first used a newly devised "pHstat" to titrate automatically the rate of hydrolysis and liberation of acid catalyzed by AChE. Then, when the Beckman UV spectrophotometer became available, we used it. On the other hand, most of the neuroanatomical effects of training or experience could have been discovered decades earlier, if anyone had had a reason to look for them; these were not findings that required technical advances for their discovery.

Skepticism or frank disbelief was the initial reaction to our reports that significant changes in the brain were caused by relatively simple and benign exposure of animals to differential experience. By the early 1970s, some neurobiologists began to accept these results. Thus neurobiologist B.G. Cragg (1972, p. 42) wrote,

> Initial incredulity that such differences in social and psychological conditions could give rise to significant differences in brain weight, cortical thickness, and glial numbers seems to have been overcome by the continued series of papers from Berkeley reporting consistent results. Some independent confirmation by workers elsewhere has also been obtained.

Soon after the early publications of neurochemical and anatomical plasticity came another kind of evidence of cortical plasticity — the announcement by Hubel and Wiesel that depriving one eye of light in a young animal, starting at the age at which the eyes open, reduced the number of cortical cells responding to stimulation of that eye (Wiesel and Hubel 1963; Hubel and Wiesel 1965; Wiesel and Hubel 1965). Depriving an eye of light is a rather severe and pathological condition. In contrast, giving animals different amounts of experience without depriving them of any sensory modality is a rather mild and natural treatment, yet it leads to measurable changes of neurochemistry.
and neuroanatomy, and it has significant effects on problem-solving ability. The report of Wiesel and Hubel (1965) that changes can be induced in the visual system only during a critical period early in the life of the kitten served to solidify the belief of many neurobiologists that neural connections in the adult brain are fixed and do not vary as a result of training.

Greenough (see Chapter 2 of this volume and Black and Greenough, 1998) follows Piaget (1980) in distinguishing between two kinds of information acquired from the environment: (1) general information acquired by all members of a species from common features of their environments (i.e., "expected" information), and (2) idiosyncratic information that the individual uses to adapt to its unique environment (i.e., "unexpected" information). As Black and Greenough (1998) point out, exposure to light and visual pattern stimulation provides general, "expected" information, whereas exposure to a complex environment provides idiosyncratic information. Exposure to "expected" stimulation usually occurs early in development, and it may be important in preparing the animal to respond adequately to idiosyncratic information.

A. Differential Experience Produces Cerebral Changes Throughout the Life Span and Rather Rapidly

Further experiments revealed that significant cerebral effects of enriched versus impoverished experience could be induced at any part of the life span and with relatively short periods of exposure. In contrast, Hubel and Wiesel had reported that depriving an eye of light altered cortical responses only if the eye was occluded during a critical period early in life. Later, however, investigators found that modifying sensory experience in adult animals — especially in the modalities of touch and hearing — could alter both receptive fields of cells and cortical maps, as reviewed by Kaas (1991) and Weinberger (1995).

Initially we supposed that cerebral plasticity might be restricted to the early part of the life span, so we assigned animals to differential environments at weaning (about 25 days of age) and kept them there for 80 days. Later, members of our group obtained similar effects in rats assigned to the differential environments for 30 days as juveniles at 50 days of age (Zolman and Morimoto, 1962) and as young adults at 105 days of age (Rosenzweig, Bennett, and Krech, 1964; Bennett, Diamond, Krech, and Rosenzweig, 1964a). Riege (1971), in our laboratory, found that similar effects occurred in rats assigned to the differential environments at 285 days of age and kept there for periods of 30, 60, or 90 days. Two hours a day in the differential environments for a period of 30 or 54 days produced similar cerebral effects to 24-hr exposure for the same periods (Rosenzweig, Love, and Bennett, 1968). Four days of differential housing produced clear effects on cortical weights (Bennett, Rosenzweig, and Diamond, 1970) and on dendritic branching (Kilman
et al., 1988); Ferchmin and Eterovic (1986) reported that four 10-min daily sessions in EC significantly altered cortical RNA concentrations. The fact that differential experience can cause cerebral changes throughout the life span and relatively rapidly was consistent with our interpretation of these effects as due to learning. Recall also that our original observation of differences in cortical neurochemistry came from experiments on formal training. Later, Chang and Greenough (1982) reported that formal visual training confined to one eye of rats caused increased dendritic branching in the visual cortex contralateral to the open eye. Also, single-trial peck-avoidance training in chicks was found to result in changes in density of dendritic spines (Lowndes and Stewart, 1994).

Although the capacity for these plastic changes of the nervous system and for learning remains in older subjects, the cerebral effects of differential environmental experience develop somewhat more rapidly in younger than in older animals, and the magnitude of the effects is often larger in the younger animals. Also, continuing plasticity does not hold for all brain systems and types of experience. As noted earlier, changes in responses of cortical cells to an occluded eye are normally restricted to early development, as Wiesel and Hubel (1963) found. But this restriction may itself be modifiable. Baer and Singer (1986) reported that plasticity of the adult visual cortex could be restored by infusing acetylcholine and noradrenaline. Further work showed that the plastic response of the young kitten brain to occlusion of one eye also depends on glutamate transmission, because treating the striate cortex with an inhibitor of the glutamate NMDA receptor prevented the changes (Kleinschmidt, Baer, and Singer, 1987). Thus, the extent to which the brain shows plastic changes in response to a particular kind of experience depends on the age of the subject, the brain region, and the kind of experience and also on special circumstances or treatments that enhance or impair plasticity. The factor of age is reviewed in Chapter 15 of this volume, by Barnes and Penner.

B. Enriched Experience Improves Ability to Learn and Solve Problems

Hebb (1949, pp. 298–299) reported briefly that when he allowed laboratory rats to explore his home for some weeks as pets of his children and then returned the rats to the laboratory, they showed better problem-solving ability than rats that had remained in the laboratory throughout. Furthermore, they maintained their superiority or even increased it during a series of tests. (Whether Hebb's children showed better problem-solving ability after having rats as household pets was apparently not investigated.) Hebb concluded that "the richer experience of the pet group during development made them better able to
profit by new experience at maturity — one of the characteristics of the ‘intelligent’ human being” (pp. 298–299, italics in the original). Moreover the results seemed to show a permanent effect of early experience on problem solving at maturity.

We and others have found that experience in an enriched laboratory environment improves learning and problem-solving ability on a wide variety of tests, although such differences have not been found invariably. One general finding is that the more complex the task, the more likely it is that animals with EC experience will perform better than animals from SC or IC groups (see review and different explanations offered for this effect: Renner and Rosenzweig, 1987, pp. 46–48).

We were unable, however, to replicate an important aspect of Hebb’s report — that over a series of tests, EC rats maintain or increase their superiority over IC rats. On the contrary, we found that IC rats tend to catch up with EC rats over a series of trials; this occurred with each of three different tests, including the Hebb–Williams mazes (Rosenzweig, 1971, p. 321). Thus we did not find that early deprivation of experience caused a permanent deficit, at least for rats tested on spatial problems. Also, decreases in cortical weights induced by 300 days in the IC (versus the EC) environment could be overcome by a few weeks of training and testing in the Hebb–Williams mazes (Cummins, Walsh, Budtz-Olsen, Konstantinos, and Horsfal, 1973). Later we will see a similar effect in birds.

C. Similar Neuroanatomical Effects of Training and Experience Occur in All Species Tested to Date

Experiments with several strains of rats showed similar effects of EC versus IC experience on both brain values and problem-solving behavior, as reviewed by Renner and Rosenzweig (1987, pp. 53–54). Similar effects on brain measures have been found in several species of mammals — mice, gerbils, ground squirrels, cats, and monkeys (reviewed by Renner and Rosenzweig, 1987, pp. 54–59); and effects of training on brain values of birds have also been found. Thus the cerebral effects of experience that were surprising when first found in rats have now been generalized to several mammalian and avian species. Anatomical effects of training or differential experience have been measured in specific brain regions of Drosophila (R. Davis, 1993; Heisenberg et al., 1995). Synaptic changes with training have also been found in the nervous systems of the molluscs Aplysia and Hermisenda, as reviewed by Krasne and Glanzman (1995). In Aplysia, long-term habituation led to decreased numbers of synaptic sites, whereas long-term sensitization led to an increase (Bailey and Chen, 1983); this is a case where either a decrease or an increase in synaptic numbers stores memory. Thus, as noted by Greenough, Withers, and Wallace (1990, p. 164),
“experience-dependent synaptic plasticity is more widely reported, in terms of species, than any other putative memory mechanisms.” Thus the cerebral effects of experience that were surprising when first reported for rats in the early 1960s are now seen to occur widely in the animal kingdom, “from flies to philosophers” (Mohammed, 2001).

D. Experience May Be Necessary for Full Growth of Brain and of Behavioral Potential

Sufficiently rich experience may be necessary for full growth of species-specific brain characteristics and behavioral potential. This was seen in research on differential experience conducted with different species of the crow family. Species that cache food in a variety of locations for future use are found to have significantly larger hippocampal formations than related species that do not cache food (Krebs et al., 1989; Sherry et al., 1989). But the difference in hippocampal size is not found in young birds who are still in the nest; it appears only after food storing has started, a few weeks after the birds have left the nest (Healy and Krebs, 1993). Even more interesting is the finding that this species-typical difference in hippocampal size depends on experience; it does not appear in birds that have not had the opportunity to cache food (Clayton and Krebs, 1994). Different groups of hand-raised birds were given experience in storing food at three different ages: either 35–59 days posthatch, 60–83 days, or 115–138 days. Experience at each of these periods led to increased hippocampal size, much as we had found for measures of occipital cortex in the rat. Thus, both birds and rats appear to retain considerable potential for experience-induced brain growth if it does not occur at the usual early age.

VI. GENETIC STUDIES OF LEARNING ABILITY: FROM SELECTION TO MOLECULAR BIOLOGY

A major advance at midcentury was the discovery of the structure of DNA by Francis Crick and James D. Watson in 1953. This soon led to understanding the genetic code and major advances in molecular biology, including progress in the neurobiology of learning and memory, as reviewed in Chapter 8. Because of the importance of their discovery, Crick and Watson were awarded the Nobel Prize in Physiology or Medicine in 1962. As a key to their discovery, Crick and Watson relied on X-ray photographs of DNA made by chemist Rosalind Franklin; some scientists believe that if she had not died prematurely in 1958, Franklin might have shared the Nobel Prize (Maddox, 2002, 2003). In fact, genetic research on learning ability began long before the work of Crick and Watson, as we review next.
In the late nineteenth century, Francis Galton was convinced that intelligence and learning ability are inherited, and he had thought of breeding dogs for intelligence, as he reported later: “[I]t would be a most interesting occupation... to pick the cleverest dogs [one] could hear of, and mate them together, generation after generation — breeding purely for intellectual power” (Galton, 1909, p. 319). Although he believed that the costs of such an experiment could largely be covered by selling the superior animals that would result, Galton never undertook this project, nor was he able to persuade others to do so. I believe the first experiment to breed animals for learning ability was that conducted by Edward C. Tolman (1924) with rats. This successful preliminary work was then extended by Tolman’s former student Robert C. Tryon (1940, 1942), using a 17-unit automatic maze developed by Tolman, Tryon, and Jeffress (1929). Tryon started by testing a large number of male and female rats of heterogeneous stocks. Males and females with low error scores were then bred together, and so were males and females with high error scores. Among the offspring of the low-error parents, those who themselves made few errors were kept for breeding. Similarly, in the other group, those who made many errors were mated. By the seventh generation, there was very little overlap of scores between the “bright” and “dull” lines. Further selective breeding did not increase the separation.

Why did experimental selection for learning ability wait for the 1920s when Galton had conceived of such an experiment by the end of the nineteenth century? Factors that made the experiment feasible by the 1920s but not when Galton originally conceived of it include the following:

1. Choice of the laboratory rat, rather than the dog, as the main subject for experiments on learning made such selection experiments economically feasible. This was especially the case for an experiment of selective breeding, since the generation time for rats is considerably shorter than for dogs.

2. By the 1920s there were animal laboratories in university departments of psychology supported by academic budgets. Galton would have had to undertake such an experiment with his own means, and even though he thought that eventually some of the costs could be recouped by sale of intelligent dogs, there would have been important start-up costs.

3. There was also the conceptual question of measuring intelligence. Galton did not indicate how he would measure the intelligence of dogs other than by observation and rating. By the 1920s there was a considerable background of experience and theory for testing the learning ability of animals. Tolman and his students improved the feasibility of testing large numbers of animals
over several successive generations by devising a multiple-unit automatically recording maze (Tolman, Tryon, and Jeffress, 1929).

Beginning in midcentury, however, behavior geneticists began to believe that Tryon's strains differed mainly in motivation rather than in learning per se. This came about when Tryon's student Lloyd V. Searle (1941, 1949) attempted to determine whether Tryon's maze-bright rats were generally superior in learning to the maze-dulls or whether their superiority was confined to the test employed in the selection program. Searle used 10 maze-bright, 10 maze-dull, and 15 animals of a crossed line, giving them a variety of tests of learning, activity, and emotional behavior. He (1949, p. 323) concluded:

> No evidence was found that a difference exists between the Brights and Dulls in the learning capacity per se. A detailed study of the behavior profiles indicated that the Brights are characteristically food-driven, economical of distance, . . . and timid in response to open space. Dulls are disinterested in food . . . and timid of mechanical apparatus features. It is concluded that brightness and dullness in the original Tryon Maze may be accounted for in large part by such motivational and emotional patterns. Although indications exist that the two strains may be differentiated with reference to certain basic "cognitive" tendencies, the procedures followed in this experiment were not sufficiently analytical to indicate their nature.

In fact, Tryon had investigated the same question, earlier than Searle and with a more complete experiment. About 1940, Tryon sought to test the possibility that motivational differences between the strains might account for the difference in their error scores in the maze. To do this, he ran an experiment with animals of the 22nd generation, using the following groups: (a) 71 maze-bright rats with "normal" hunger motivation, i.e., given the standard ration throughout the experiment; (b) 43 maze-bright rats that had been satiated with extra rations; (c) 71 maze-dulls with "normal" hunger motivation; and (d) 57 maze-dulls whose motivation was heightened by reduced rations. The results showed that the level of hunger motivation affected running speed but did not affect mean error scores of the groups. Whether normally hungry or satiated, the maze-brights made only about a third as many errors as the normally hungry or strongly hungry maze-dulls. Tryon never published these results, but about 20 years later he gave them to me to include in a paper on the effects of heredity and environment on brain measures and learning ability in the rat (Rosenzweig, 1964).

Tryon concluded, unlike Searle, that error scores were practically independent of food motivation in both strains. But Searle's results, having been published, convinced many readers that Tryon had selected for motivation and emotion rather than for learning ability. In the 1960s, my colleagues and I found that descendants of the maze-bright rats made significantly fewer errors than descendants of the maze-dulls on the Hebb-Williams maze, the Dashiell checkerboard maze, and the Lashley III maze (Rosenzweig, 1964), thus indicating some generality for Tryon's conclusions.
B. Effects of Mutations on Learning Ability

Geneticists have employed the fruit fly, *Drosophila melanogaster*, as a favorite subject since the early 1900s. Modern neurogenetic dissection of *Drosophila* behavior was pioneered by Seymour Benzer (1967, 1973). The application of this approach to the study of mechanisms of learning and memory became possible only after Benzer and his colleagues demonstrated that *Drosophila* can learn (Quinn et al., 1979). As reviewed by Dudai (1989), two methods were then employed to isolate mutations that affect learning or memory specifically without affecting other factors, such as perception and motivation. In the first, mutants previously isolated by a variety of criteria—morphological, developmental, biochemical, or physiological—are subjected to tests of learning and memory. Because these mutants have salient abnormalities, the specificity of any defect in learning or memory must be tested with special care. Several previously identified mutants have been reported to show relatively specific impairments in learning (e.g., Tempel, Livingstone, and Quinn, 1984; Heisenberg et al., 1985; Cowan and Siegel, 1986).

The second method is more straightforward. Here one treats flies with a mutagen and screens the progeny for defects in learning and/or memory. If such effects are found, the mutants must also be tested for defects in factors, such as perception and motivation, that might account for impaired performance on tests of learning or memory. Several mutants for learning have been isolated in this way (e.g., Dudai et al., 1976; Quinn, Sziber, and Booker, 1979; Aceves-Pina, Booker, Duerr, Livingstone, et al., 1983).

C. Molecular Biological Studies of Learning and Memory Formation

In addition to studying mutations, methods of molecular biology have made it possible to affect genes in a number of ways that have been applied to research on the mechanisms of learning and memory. Chapter 3 in this book, by Wang, Dubnau, Tully, and Zhong, discusses in detail the main methods and the results obtained to date with them. Mutations and gene knockouts are genetic lesions, and the results of such treatments are subject to the problems and criticisms that beset lesion techniques in general. Moreover, at first genetic techniques were rather blunt instruments with which to perform lesions. That is, they were not restricted in time or location—they affected animals throughout their development and throughout the body. Fortunately, techniques were later developed to restrict the changes to specific times and to certain brain regions. The importance of the gene-modification techniques for research in learning and memory is reflected by the fact that Martinez, Thompson, and Sikorski (Chapter 4 of this volume) devote substantial sections of their chapter...
to gene expression in learning and memory, and Wang, Dubnau, Tully, and Zhong (Chapter 3 of this volume) discuss the role of genetic manipulation in *Drosophila* on learning and memory.

**VII. CHANGING CONCEPTS OF LEARNING AND MEMORY FORMATION**

Research on the neurobiology of learning and memory has been influenced in the second half of the twentieth century by changing ideas about learning and memory formation. One important idea has been the variety of forms of learning and of memory mechanisms. Another has been the distinction between direct and modulatory processes in memory formation.

**A. Variety of Forms of Learning and of Memory Mechanisms**

Most theorists of learning in the first half of the twentieth century, such as Clark Hull (1943), E. R. Guthrie (1935), and B. F. Skinner (1938), attempted to explain learning by means of a single set of rules. Edward C. Tolman (1949), however, was convinced that there is more than one kind of learning and that different kinds might follow different laws. Findings in the second half of the twentieth century bore out Tolman's insight as a variety of kinds of learning were described and as different neural mechanisms were discovered. Some of the different kinds of learning and memory stores were these: (a) short-term versus long-term memory, a distinction that had been anticipated by William James in 1890; (b) declarative versus procedural (or nondeclarative) memory, distinguished in 1980 (Cohen and Squire, 1980); (c) storage of different attributes of memory, distinguished by Underwood (1969) and Spear (1976), as processed by different regions of the brain (Kesner, 1980 and Chapter 8 of this volume; McDonald and White, 1993); (d) processing of spatial memory as accomplished by certain cells in the hippocampus (O'Keefe and Dostrovsky, 1971; see Chapter 5 of this volume, by Mizumori, Smith, and Puryear); circuits in the cerebellum suffice for formation of so-called delay eye-blink conditioning when little or no time elapses between the end of the conditioned stimulus (CS) and the onset of the unconditioned stimulus (US), but the hippocampus is required for formation of so-called trace conditioning, when time intervals between the US and CS are longer (see Chapter 13 of this volume, by Ohyama and Mauk).

The discovery of different forms of learning and memory was dependent on elaboration of more specific behavioral tests that were capable of discriminating among the varieties of learning and memory. Lashley's pessimistic
evaluation of theories of memory formation, mentioned earlier, in Section IV, came from his failure to discriminate between short-term and long-term memory stores and his use of spatial mazes that could be solved with inputs from any of several sensory modalities.

An important concept in testing types of memory and their neural substrates is double dissociation. Lashley (1952) had criticized ablation studies that purported to show localization even of sensory function. He pointed out that failure of an animal to continue to make a trained visual object discrimination after lesion of a temporal cortical area might instead mean impairment of comparison behavior or of comprehension of the training situation. This might be overcome, he suggested, by testing whether the lesion left intact the ability to discriminate in another modality, such as somesthesis. In an important review, Hans-Lukas Teuber (1955, p. 283) countered that more was needed to resolve this question than simply to show that the lesion did not impair discrimination in another modality. Such a “simple dissociation” might only mean that visual discrimination was more vulnerable to temporal cortical lesions than was tactile discrimination. What was needed for conclusive proof, Teuber argued, was double dissociation, that is, evidence that lesion of one cortical area impaired visual object discriminations without loss on comparable tactile tasks, while lesion of another cortical area impaired tactile discriminations without loss on the visual tasks, and that the impairments in the two tasks be comparable in severity. Subsequent investigators took up the challenge of finding double dissociations and also extended it to studies of the localization of brain regions involved in learning and memory. At the same time, investigators devised learning tasks intended to involve rather specific processes, and they abandoned earlier, rather nonspecific learning paradigms, such as Thorndike’s puzzle boxes and Lashley’s mazes, that could be solved in a variety of ways.

Knowlton, Mangels, and Squire (1996) started a review article by stating, “Students of brain and behavior have long recognized that double dissociations [references to Teuber (1955) and later authors] provide the strongest evidence for separating the functions of brain systems” (p. 1399). They presented evidence for a double dissociation between human brain regions and kinds of memory: Amnesic patients, with damage to the limbic-diencephalic regions, show impaired formation of declarative but not of nondeclarative memories, whereas patients with Parkinson’s disease, who have damage to the neostriatum (caudate nucleus and putamen), show impaired formation of habits but not of declarative memories. Kesner, in a series of studies summarized in his chapter in this volume (Chapter 8), has found evidence of double and triple dissociations between brain regions involved in working memory for different attributes of the learning situation. A similar research project, by McDonald and White (1993), also found a triple dissociation, using three different problems, all run in the radial maze: (1) A neural system that includes the hippocampus
acquires information about relationships among stimuli and events (declarative memories); (2) a different system that includes the dorsal striatum (mainly the caudate nucleus) mediates the formation of reinforced stimulus–response associations (habits, or nondeclarative memories); (3) a third system that includes the amygdala mediates rapid acquisition of behaviors based on biologically significant events with affective properties.

**B. Distinction Between Direct and Modulatory Processes in the Formation of Memory**

Beginning in the 1970s, a distinction began to be drawn between systems that might provide the substrates for memory and systems whose manipulations could modulate memory formation (e.g., Gold and McGaugh, 1975). This distinction became widely used, as shown by a 1984 symposium on the neurobiology of learning and memory (Lynch, McGaugh, and Weinberger, 1984) that included a section on modulation of memory, with four papers and five commentaries. The first of these papers stated: “Formation of the memory trace involves both necessary or basic processes (sometime called intrinsic) and also modulatory (or extrinsic) influences that affect the rate or level of the direct processes” (Rosenzweig and Bennett, 1984, p. 265). As examples, Rosenzweig and Bennett noted that synthesis of proteins is required for formation of long-term memory, and this direct process can be modulated by several sorts of processes and treatments, including the level of arousal, excitant or depressant agents, and drugs that affect the cholinergic system. Making this distinction between direct and modulatory processes helps to clarify the roles of the many factors that affect memory formation.

In the present book, direct processes are considered in Chapters 8, 9, 10, and 13, by Kesner, Preston and Wagner, Miller and Buschman, and Ohyama and Mauk, respectively, among others. Modulatory processes are considered in Chapters 7 and 16, by Korol and Gold and by Wenk, respectively, among others.

**VIII. NEUROCHEMICAL MECHANISMS OF LEARNING AND MEMORY**

Research on neurochemical mechanisms of learning and memory has become a prominent line of investigation since the 1960s. In part this was encouraged by the findings of the effects of enriched experience and formal training on brain chemistry. Some investigators suggested that memory might be encoded in one or another brain chemical. Another source of this research was interest in mechanisms of consolidation of memory.
A. Tests of the Hypothesis That Protein Synthesis Is Required for Memory Storage

By what processes does enriched experience or formal training lead to plastic changes in cerebral neurochemistry and neuroanatomy? We found early on that enriched experience causes increased rates of protein synthesis and increased amounts of protein in the cortex (Bennett et al., 1964a). Later, training (imprinting) was reported to increase the rates of incorporation of precursors into RNA and protein in the forebrain of the chick (Haywood, Rose, and Bateson, 1970), and enriched experience in rats was found to lead to increased amounts of RNA (Ferchmin, Eterovic, and Caputto, 1970; Bennett, 1976) and increased expression of RNA in rat brain (Grouse, Schrier, Bennett, Rosenzweig, and Nelson, 1978). Maze training led to increased ratios of RNA to DNA in rat cortex (Bennett, Rosenzweig, Morimoto, and Hebert, 1979).

We viewed these findings in the light of the hypothesis, perhaps first enunciated by Katz and Halstead (1950), that protein synthesis is required for memory storage.

Tests of the protein-synthesis hypothesis of memory formation were initiated by Flexner and associates in the early 1960s (e.g., Flexner, Flexner, Stellar, de la Haba, and Roberts, 1962; Flexner, Flexner, de la Haba, and Roberts, 1965), and much research followed their design: (1) giving animal subjects brief training that, without further treatment, would yield evidence of retention at a test a few days later; (2) administering to experimental subjects an inhibitor of protein synthesis at various times close to training while control subjects received an inactive substance; and (3) comparing test performance of experimental and control subjects. By the early 1970s, considerable evidence indicated that protein synthesis during or soon after training is necessary for the formation of long-term memory (LTM), but the interpretation of the findings was clouded by serious problems, including the following. (1) The inhibitors of protein synthesis then available for research (such as puromycin and cycloheximide) were rather toxic, which impeded experiments and complicated interpretation. (2) It appeared that inhibition of protein synthesis could prevent memory formation after weak training but not after strong training (e.g., Barondes, 1970).

A newly discovered protein-synthesis inhibitor, anisomycin (ANI), helped to overcome these problems. Schwartz, Castellucci, and Kandel (1971) reported that ANI did not prevent an electrophysiological correlate of short-term habituation or sensitization in an isolated ganglion of *Aplysia*, but they did not investigate whether ANI can prevent long-term effects. The discovery by Bennett, Orme, and Hebert (1972) that ANI is an effective amnestic agent in rodents opened the way to resolving the main challenges to the protein-synthesis hypothesis of the formation of LTM. ANI is much less toxic than other protein-synthesis inhibitors, and giving doses repeatedly at 2-hr intervals
can prolong the duration of cerebral inhibition at amnestic levels. By varying the duration of amnestic levels of inhibition in this way, we found that the stronger the training, the longer protein synthesis had to be inhibited to prevent formation of LTM (Flood, Bennett, Orme, and Rosenzweig, 1975; Flood, Bennett, Rosenzweig, and Orme, 1973). We also found that protein must be synthesized in the cortex soon after training if LTM is to be formed; short-term memory (STM) and intermediate-term memory (ITM) do not require protein synthesis (e.g., Bennett, Orme, and Hebert, 1972; Mizumori, Rosenzweig, and Bennett, 1985; Mizumori, Sakai, Rosenzweig, Bennett, and Wittreich, 1987).

B. Neurochemistry of Short-Term and Intermediate-Term Memories

Further studies were then designed to find the neurochemical processes that underlie formation of STM and ITM. Lashley’s concern, mentioned earlier, that some kinds of memory appear to be formed too quickly to allow growth of neural connections, ignored the distinction between STM and LTM, even though William James (1890) had already distinguished between these stores (although under different names). Observing this distinction was necessary if one was to look for different mechanisms of the two kinds of memory traces that Hebb distinguished: transient, labile memory traces, on the one hand, and stable, structural traces, on the other.

Much research on the neurochemistry of STM and ITM has been done with chicks, which have several advantages for this research: Chicks can be trained rapidly in a one-trial peck-avoidance paradigm and can be tested within seconds after training or hours or days later. Large numbers of chicks can be studied in a single run, so one can compare different agents, doses, and times of administration within the same batch of subjects. Unlike invertebrate preparations, the chick system can be used to study the roles of different vertebrate brain structures and to investigate questions of cerebral asymmetry in learning and memory. The chick system permits study of learning and memory in the intact animal. The successive neurochemical stages occur more slowly in the chick than in the rat, thus allowing them to be separated more clearly. Further advantages have been stated elsewhere (e.g., Rosenzweig, 1990; Rosenzweig, Bennett, Martinez, Colombo, et al., 1992).

Although some amnestic agents, such as ANI, diffuse readily throughout the brain, others affect only a restricted volume of tissue at amnestic concentrations (Patterson, Alvarado, Warner, Rosenzweig, and Bennett, 1986). Such agents can be used to reveal the roles of different brain structures in different stages of memory formation (e.g., Patterson, Alvarado, et al., 1986; Serrano, Rodriguez, Bennett, and Rosenzweig, 1995).
C. Both Enriched Experience and Formal Training Evoke Similar Neurochemical Cascades

Using the chick system, several investigators have traced parts of a cascade of neurochemical events from initial stimulation to synthesis of protein and structural changes (e.g., Gibbs & Ng, 1977; Ng and Gibbs, 1991; Rose 1992a, 1992b; Rosenzweig, Bennett, Martinez, Colombo, et al., 1992). At some, if not all, stages, parallel processes occur. Briefly, here are some of the stages. The cascade is initiated when sensory stimulation activates receptor organs, which stimulate afferent neurons by using various synaptic transmitters, such as acetylcholine (ACh) and glutamate. Inhibitors of ACh synaptic activity, such as scopolamine and pirenzepine, can prevent STM. So can inhibitors of glutamate receptors, including both the NMDA and AMPA receptors. Alteration of regulation of ion channels in the neuronal membrane can inhibit STM formation, as seen in effects of lanthanum chloride on calcium channels and of ouabain on sodium and potassium channels. Inhibition of second messengers is also amnestic, for example, inhibition of adenylate cyclase by forskolin or of diacylglycerol by bradykinin. These second messengers can activate protein kinases — enzymes that catalyze the addition of phosphate molecules to proteins. We found that two kinds of protein kinases are important in the formation, respectively, of ITM and LTM. Agents that inhibit calcium-calmodulin protein kinases (CaM kinases) prevent formation of ITM, whereas agents that do not inhibit CaM kinases but do inhibit protein kinase A (PKA) or protein kinase C (PKC) prevent formation of LTM (Rosenzweig, Bennett, Martinez, Colombo, et al., 1992; Serrano, Beniston, Oxonian, Rodriguez, Rosenzweig, and Bennett, 1994). From this research, Serrano et al. (1995) were able to predict for a newly available inhibitor of PKC its effective amnestic dose and how long after training it would cause memory to decline. One-trial training leads to an increase of immediate early gene messenger RNA in the chick forebrain (Anokhin and Rose, 1991) and to an increase in the density of dendritic spines (Lowndes and Stewart, 1994). Many of these effects occur only in the left hemisphere of the chick or are more prominent in the left than in the right hemisphere. Thus, learning in the chick system permits study of many steps that lead from sensory stimulation to formation of neuronal structures involved in memory.

The neurochemical cascade involved in formation of memory in the chick is similar to the cascades found in long-term potentiation (LTP) in the mammalian brain (e.g., Colley and Routtenberg, 1993) and in the nervous systems of invertebrates (e.g., Krasne and Glanzman, 1995).

Many of the steps in the formation of memory in the chick can also be modulated by opioids and other substances. Opioid agonists tend to impair, and opioid antagonists to enhance, memory formation. Different opioids appear to modulate formation of different stages of memory (e.g., Colombo, Martinez, Bennett, and Rosenzweig, 1992; Colombo, Thompson, Martinez, Bennett,
and Rosenzweig, 1993; Patterson, Schulteis, Alvarado, Martinez, Bennett, Rosenzweig, and Hruby, 1989; Rosenzweig et al., 1992).

Several groups of investigators have sought to determine which proteins must be synthesized to hold LTP or LTM; only a few examples will be mentioned here. Routtenberg and his colleagues produced evidence that synthesis of protein F1 (GAP-43) is involved in LTP (Meberg, Valcourt, and Routtenberg, 1995). Studies with Aplysia suggested that different proteins are involved in LTM (e.g., Kennedy et al., 1992; Kuhl et al., 1992).

D. Can Parts of the Neurochemical Cascade Be Related to Different Stages of Memory Formation?

Some of the difficulty in attempting to relate parts of the neurochemical cascade to different stages of memory formation have come from problems of defining stages of memory, as discussed more fully elsewhere (Rosenzweig, Bennett, Colombo, Lee, and Serrano, 1993). Consider, for example, some very different attempts to state the duration of STM. Early investigators of human STM (Brown, 1958; Peterson and Peterson, 1959) reported that it lasts only about 30 sec if rehearsal is prevented. Agranoff, Davis, and Brink (1966) reported that in goldfish, if formation of LTM is prevented by an inhibitor of protein synthesis, STM can last up to three days, although normally LTM forms within an hour after training. Kandel et al. (1987) wrote that in Aplysia, “A single training trial produces short-term sensitization that lasts from minutes to hours” (p. 17) and that long-term memory is “memory that lasts more than one day” (p. 35). Rose (1995) suggested that, in the chick, memories that persist only a few hours involve a first wave of glycoprotein synthesis, whereas “true long-term memory” requires a second wave of glycoprotein synthesis, occurring about 6 hr after training.

Instead of considering that STM can last several hours or even a day or more, others posited one or more intermediate-term memory (ITM) stages occurring between STM and LTM (e.g., McGaugh 1966, 1968). Thus, Gibbs and Ng (1977) referred to a “labile” stage occurring between STM and LTM and later called this intermediate-term memory (e.g., Gibbs and Ng, 1984; Ng and Gibbs, 1991). My coworkers and I have discussed mechanisms of STM, ITM, and LTM in a series of papers (e.g., Rosenzweig and Bennett, 1984; Rosenzweig, Bennett, Martinez, and Colombo, 1992, 1993; Mizumori, Sakai, et al., 1987; Patterson, Alvarado, Rosenzweig, and Bennett, 1988). In investigating effects of protein kinase inhibitors (PKIs) on memory formation in chicks, we reported that those agents that inhibit CaM kinase activity disrupted formation of what some workers with chicks identified as ITM (lasting from about 15 min to about 60 min posttraining); those agents that inhibit PKC, PKA, or PKG (protein kinase G) but do not inhibit CaM kinase disrupted the
formation of LTM (Rosenzweig, Bennett, Martinez, Colombo, et al., 1992; Serrano et al., 1994). Other investigators preferred to refer to different phases or stages of LTM rather than use the term ITM. Thus, studying the LTP analog to memory in slices of rat hippocampus, Huang and Kandel (1994) reported findings similar to those of Rosenzweig et al. (1992) and Serrano et al. (1994) with regard to the roles of two classes of protein kinases: Inhibitors of CaM kinase activity disrupted what Huang and Kandel called a transient, early phase of LTP (E-LTP), evoked by moderately strong stimuli and lasting from 1 hr to less than 3 hr after induction of LTP. Agents that inhibit PKA but do not inhibit CaM kinase disrupt the formation of what they called a later, more enduring phase of LTP (L-LTP), evoked by strong stimulation and lasting at least 6–10 hr. Weak stimuli evoke only short-term potentiation (STP), lasting only 20–30 min. As already mentioned, Rose (1995) suggested that, in the chick, a kind of LTM that lasts a few hours involves a first wave of glycoprotein synthesis, whereas “true long-term memory” requires a second wave of glycoprotein synthesis, occurring about 6 hr after training. Rather than call the memory associated with Rose’s first 6-hr-long wave a form of LTM, it may be better to think of it as ITM and to note that there is an earlier STM, lasting only a few minutes, as has been shown in many experiments with the chick.

These and other findings supported the hypothesis of at least three sequentially dependent stages of memory formation, each dependent on different neurochemical processes. These results are important, not only for investigators of the neurochemistry of memory, but also for neuropsychologists and others who work with patients who suffer from memory disorders. A review by Kopelman (1992, pp. 136–138) found mixed results in attempts to distinguish losses of ITM and LTM in Korsakoff’s and Alzheimer’s patients. If it becomes possible to distinguish patients with disorders of ITM from those with impairment of STM or LTM, then perhaps their deficits can be traced to different disorders of the nervous system. If we can identify the neurochemical processes underlying each stage of memory formation, this could lead to attempts at rational pharmacological treatments. If investigators could then understand the genetics involved, they might eventually find genetic treatments for some memory defects.

### E. Is Memory Encoded in Brain Chemicals?

Beginning early in the 1960s, several investigators proposed that memory is encoded in one or another brain chemical. Among the chemicals proposed as repositories of memory were RNA, glucocorticoids, and peptides such as “scotophobin.” Later, investigators attempted to set up guidelines to evaluate such proposals.
1. Proposed "Memory Molecules"

Reports of "memory molecules" were prominent for a time, not only in scientific circles but also in the popular press. A book by Louis Irwin (2006) presents a lively account of this topic and offers biographical sketches of several of the contributors. One of the first investigators in this field was Swedish neurochemist Holger Hyden. In 1959 Hyden hypothesized that any particular pattern of neuronal activation would alter the sequence of bases in molecules of RNA. Then in 1962, Hyden and Egyházi sampled RNA from the vestibular nucleus of rats taught to climb a wire to obtain food reward. They reported that the training caused changes in concentration of RNA and in the base composition of RNA. This report set off a flurry of speculation that memory could be encoded in RNA.

Hyden's reports suggested to psychologist James McConnell a mechanism for results he had been obtaining with planaria. He had been training planaria to turn left or right at a choice point and claimed that if a naive planarian cannibalized a trained planarian, the cannibal was then likely to choose the direction to which its prey had been trained. When these results were published, training planaria soon became a popular topic for high school science fairs, where positive results were often reported, although university laboratories had difficulty replicating them. A witticism of the time was that students might gain knowledge more efficiently by cannibalizing their professors than by studying. Biochemists at Berkeley were impressed enough by the possibility of finding a chemical code for memory that they decided to try to replicate the work with planaria. Edward Bennett was in charge of this project, but he could not obtain clear evidence of learning by planarians.

Attention then moved to "transfer of memory" in rats, when psychologist Allan Jacobson and coworkers reported that when they extracted RNA from the brains of trained rats and injected it into naive rats, the naive rats displayed operant-training responses acquired by the trained rats (Babich, Jacobson, et al., 1965). Many laboratories sought to replicate or to extend this exciting report. Our laboratory attempted to replicate the study exactly, even ordering rats from the small supplier Jacobson used and getting his technician to extract RNA by the method used in their report. We were unable to obtain positive results, and other labs also reported failure to replicate Jacobson's transfer of training. In 1966, Science published a report by 23 authors from eight different laboratories, including ours, announcing failure to obtain transfer of a variety of tasks with RNA (Byrne, Samuel, et al., 1966).

Meanwhile biochemist Georges Ungar had reported that trained responses in rats could be transferred to naive animals by peptides extracted from the brain (Ungar and Oceguera-Navarro, 1965). The best known of these peptides became one that Ungar called scotophobia because it appeared to encode learned fear of the dark; it was extracted from brains of rats who learned to avoid the
dark side of an enclosure because they were given foot shock there (Ungar et al., 1972). As with Jacobson's report of transfer, most laboratories that tried to replicate Ungar's report failed to do so. Later, David Malin (1976), who had earlier collaborated with Ungar, reported that when scotophobin was injected into mice forced to remain in a black box, the mice developed elevated blood corticosteroid level, while mice in a lighted box did not. Thus scotophobin was apparently interacting with a particular environmental stimulus to elevate stress and cause the animal to flee the stress-inducing situation. In other words, scotophobin may have been a modulatory agent, a concept discussed earlier, in Section VII-B, rather than encoding memory.

2. Criteria for Neurochemistry of Memory

As evidence accumulated that learning and experience induce chemical changes in the brain and that inhibiting some chemical processes around the time of learning blocks formation of memory, some investigators tried to devise guidelines and criteria to judge whether such changes and processes are necessary and sufficient for formation of memory. Of course, reports of many studies stated one or more criteria against which to test their findings, but Entingh, Dunn, Wilson, Glassman, and Hogan (1975) and Rose (1981) tried to list several guidelines or criteria that would be applicable to a variety of studies. Some of these criteria are the following. (a) There must be changes in the quantity of the system or substance or its rate of production or turnover in some localized region of the brain during memory formation. (b) The amount of change should be related to the strength or amount of training, up to a limit. (c) Stress, motor activity, or other processes that accompany learning must not, in the absence of memory formation, result in the structural or biochemical changes. (d) If the cellular or biochemical changes are inhibited during the period over which memory formation would normally occur, then memory formation should be prevented and the animal should be amnesic. (However, Flood, Bennett, Rosenzweig, and Orme, 1973, found cases in which the protein synthesis required for LTM formation was only postponed by inhibition of protein synthesis and occurred later than usual, after the inhibition wore off.) Research on learning and memory, chiefly with chicks, showed that some neurochemical processes appeared to fulfill all the stated criteria, as I have discussed elsewhere (Rosenzweig, 1996, pp. 18–19).

In Chapter 4 of this volume, Martinez, Thompson, and Sikorski discuss whether LTP and long-term depression (LTD) — which involve neurochemical, electrophysiological, and neuroanatomical changes — are memory mechanisms. Most of the research on LTP has been done on rodent hippocampal preparations. It is generally believed that the hippocampus does not store memories for the long term, because ablation of the hippocampus does not destroy long-term memories; rather, the hippocampus appears to help to
process information for long-term storage elsewhere in the brain. Experiments in which hippocampal lesions were made at different numbers of days after training in rodents showed that memory was not impaired if the lesions were made more than two or three days after training (Kim and Fanselow, 1992; Winocur, 1990). Because of such findings, it was not clear what purpose would be served by a hippocampal mechanism for holding memory more than a few days in the rodent. Thus, some theorists considered the hippocampus to be a "temporary memory store" (Rawlins, 1985) or an "intermediate-term buffer store" (Treves and Rolls, 1994).

While conceding that convincing proof does not exist that LTP and LTD are involved in learning and memory, Martinez, Thompson, and Sikorski (Chapter 4 of this volume) believe that after many years of research, dating back to the initial discovery of LTP by Bliss and Lomo (1973), LTP and LTD remain the best candidates for a cellular process of synaptic change that underlies learning and memory in the vertebrate brain. They review findings of a cascade of neurochemical events underlying LTP that is similar to those found in research on memory formation.

IX. ELECTROPHYSIOLOGICAL STUDIES OF LEARNING AND MEMORY

A lucky accident led to the first electrophysiological observations of training with a human subject. The French neurophysiologists Gustav Durup and Alfred Fessard (1935) were studying how the alpha rhythm is blocked when a person's field of vision is illuminated. One day, after switching on the light several times and seeing the subject's alpha rhythm disappear from the record each time, the experimenter again threw the light switch, but the bulb failed and the room remained dark—nevertheless the alpha rhythm again disappeared! Seeking to explain this puzzling occurrence, the investigators hypothesized that the sound of the switch became a conditioned stimulus predicting the appearance of light and thus caused the EEG to respond as if light were present. Tests with other subjects soon demonstrated that the sound of the switch did not block the alpha rhythm in naive subjects but came to do so after pairings of sound and light.

This research became more widely known after the end of World War II, and many investigators took up studies of EEG correlates of conditioning in the late 1940s and the 1950s. But precise localization of EEG activity in the human cortex is difficult because of the overlying skull and tissue. Besides, the critical events might not be occurring in the cortex but in deeper brain structures. So the focus of research shifted to recording from the brains of alert, behaving animal subjects, often with indwelling electrodes. With the invention of microelectrodes around 1950 it became possible to record the activity of single neurons during training. We will see this technique applied to investi-
gating cellular activity during conditioning of a variety of animals, including relatively simple mollusks.

A. Sites of Synaptic Plasticity in the Nervous System of *Aplysia*

Observations on nonassociative learning in relatively simple invertebrates date back at least to the first decade of the twentieth century (e.g., Jennings, 1906). The relative simplicity of the central nervous systems of some invertebrates led several investigators to try to find in them the neural circuits necessary and sufficient for learning, with the goal of studying plastic synaptic changes in these circuits. Invertebrate preparations, such as the large sea slug *Aplysia californica*, appeared to offer the following advantages for this research, although we will see that some of these were overestimated.

1. The number of nerve cells in an *Aplysia* ganglion is relatively small compared to that in a mammalian brain or even a brain region, although the number in an *Aplysia* ganglion is still of the order of 1,000.

2. In the ganglia of mollusks such as *Aplysia*, the cell bodies form the outside and the dendritic processes are on the inside. This arrangement, the opposite of that in the mammal, made it easy to identify and record from cells of such invertebrates.

3. Many individual cells in molluskan ganglia can be recognized both because of their shapes and sizes and because the cellular structure of the ganglion is uniform from individual to individual. Thus it was possible to identify certain cells and to trace their sensory and motor connections. The neurotransmitters in some of the large identifiable cells were also known.

Because of such advantages, J.W. Davis (1986, p. 268) stated, in the first edition of this book, that “invertebrates offer the promise of immediate and comprehensive understanding of the physiological processes underlying associative learning, which may in turn provide insights into mammalian learning.” But research in the decades since 1986 showed Davis’ prediction to have been overoptimistic.

A well-known example of such research is the program initiated by Eric Kandel that has investigated sites and mechanisms of plasticity for both nonassociative and associative learning in *Aplysia* (e.g., Kandel, Schacher, Castellucci, and Goelet, 1987; Kandel, Schwartz, and Jessell, 1995). Kandel's research indicated that conditioning of the gill-withdrawal reflex took place within a straight-through sensory-motor chain that controls the behavior being studied. Many interesting results have been reported from this program, although some investigators have voiced reservations about the some of the methods and findings.

Although Kandel concluded the gill-withdrawal response is a simple unitary reflex and is controlled only by cells in the abdominal ganglion, other
investigators have challenged both of these conclusions. In fact, the gill-withdrawal response was found to occur even when the central nervous system of *Aplysia* has been inactivated or removed.

Colebrook and Lukowiak (1988) further pointed out that in experiments on conditioning *Aplysia* no one had recorded both the electrical activity of the motor neurons and the gill responses in the *same* animals. When they carried out such an experiment, they found that although both the neural responses and the gill-withdrawal amplitudes to the CS showed mean increases as a result of conditioning, over one-third of the animals showed an increase in one but not in the other measure! That is, the behavioral response and its supposed neural cause did not necessarily act in the same way. Colebrook and Lukowiak (1988) concluded that many loci and neural mechanisms are likely to be involved in conditioning of the gill-withdrawal response, with both the ganglia and peripheral sites combining their effects.

Kandel and his associates then began to move in this direction, using a reduced preparation for simultaneous behavioral and cellular studies of plasticity of the gill-withdrawal response. They published preliminary reports on nonassociative learning with this preparation (Cohen, Henzi, Kandel, and Hawkins, 1991; Hawkins, Cohen, and Kandel, 1992). To investigate the role of different motor neurons in the ganglion, they inactivated one or another neuron by hyperpolarization; they reported that one motor neuron is responsible for about 70% of the gill-withdrawal response. They then recorded responses of this neuron during habituation, dishabituation, and sensitization. The “results suggest that habituation in this preparation is largely due to depression at central synapses, whereas dishabituation and sensitization are due to central and peripheral facilitation with different time courses” (Hawkins, Cohen, and Kandel, 1992, p. 360).

Further work on the sites involved in conditioning has not yet appeared, but it is likely that conditioning as well as sensitization involves the peripheral as well as the central nervous system of *Aplysia*. Thus the results of more recent research challenged the earlier conclusions that plasticity is located exclusively in the ganglia of *Aplysia*. However, there seems to be no reason to question that synaptic mechanisms of plasticity occur at some large neurons in the abdominal ganglion. Support for Kandel’s neurochemical hypotheses came from research with *Drosophila* mutants that were impaired in learning and memory, as Kandel and associates pointed out in a review (Kandel, Schacter, et al., 1987, p. 26). These mutants were found to have deficiencies in some of the neurochemical steps identified by Kandel and his associates as being important for learning, and this provided independent support for the generality of their hypotheses.

But even in the ganglion, the story was far from complete, because a single touch to the siphon was found to activate electrical responses in about 150 different neurons (Zecevic, Wu, Cohen, London, Hopp, and Falk, 1989), and
many of these probably played roles in the complex gill movements. Other investigators reported that approximately 200 abdominal ganglion neurons are involved in the gill-withdrawal response, and most of them were also involved in respiratory movements (Wu, Cohen, and Falk, 1994). Study of the different kinds of responses mediated by these neurons suggested that the different behaviors are generated by altered activities of a single, large distributed network rather than by separate small networks, each dedicated to a particular response. Wu, Cohen, and Falk (1994) reported that the large motor neurons probably contributed less than 10% to the gill-withdrawal response. Beyond these problems at the central sites, the mechanisms of plasticity at peripheral neural sites in *Aplysia* have not yet been studied, so there is still much to learn about the mechanisms of learning, even in what some investigators hoped would be a “simple” kind of learning in a “simple” organism.

**B. Conditioning in *Hermissenda***

Another marine mollusk, *Hermissenda crassicornia*, has been the subject of extensive research on mechanisms of conditioning, by Daniel Alkon and his colleagues (e.g., Alkon, 1975, 1989, 1992; Farley and Alkon, 1985). In the laboratory, pairing light with rotation on a turntable caused conditioned suppression of the tendency to approach the light. The plasticity in this system occurred in the eyes of *Hermissenda*, which contain only five photoreceptor cells.

The work with *Hermissenda*, which found the important changes with training to occur in the neuronal membrane, afforded quite a different picture of basic mechanisms of conditioning from that furnished by the research with *Aplysia*, which focused on changes that occur at the presynaptic side of the synaptic junction. Similarities as well as differences of the neurochemical mechanisms of learning in *Aplysia* and *Hermissenda* emerged from a comparison by Clark and Schuman (1992). After noting important similarities, they pointed out some distinctions:

> Compared with plasticity in *Aplysia* siphon sensory cells, plasticity in *Hermissenda* Type B photoreceptors involves a different sensory modality (light rather than touch), different types of potassium conductances (I<sub>K</sub> and I<sub>K-calc</sub>, rather than I<sub>K</sub>), primarily a different second-messenger system (protein kinase C, rather than CAMP-dependent kinase), and an inhibitory rather than an excitatory synaptic potential, among other differences. These are meaningful distinctions, and their existence suggests that each preparation will provide unique insights into cellular mechanisms of learning (Clark and Schuman, 1992, p. 598).

It was proposed that similar research with other species of invertebrates and vertebrates might show whether these are only two of a wide variety of possible mechanisms of learning or whether either would prove to be general over a number of species.
C. The Mammalian Cerebellum Houses the Brain Circuit for a Simple Conditioned Reflex

While many investigators studied learning in the apparently simpler nervous systems of invertebrates, others tried to define a circuit for learning in intact mammals. Thus, psychologist Richard F. Thompson and his colleagues have been studying the neural circuitry of eyelid conditioning since the 1970s (Thompson, 1990; Lavond, Kim, and Thompson, 1993). Prior behavioral research had produced a great deal of knowledge about how the eye-blink reflex of the rabbit became conditioned when an air puff to the cornea (US) follows an acoustic tone (CS). A stable conditioned response (CR) developed rather rapidly, and this is similar to eyelid conditioning in humans. The basic circuit of the eye-blink reflex is simple, involving two cranial nerves and some interneurons that connect their nuclei.

Early in their work, Thompson and his colleagues found that during conditioning the hippocampus developed neural responses whose temporal patterns resembled closely those of the eyelid responses. Although the hippocampal activity closely paralleled the course of conditioning and did so better than the activity of other limbic structures, this result did not prove that the hippocampus is required for conditioning to occur. In fact, destruction of the hippocampus had little effect on acquisition or retention of the conditioned eyelid response in rabbits (Lockhart and Moore, 1975). Therefore the hippocampus is not required for this conditioning. It may, however, participate in the conditioning, as indicated by the finding that abnormal hippocampal activity can disrupt the acquisition of conditioning.

Thompson and his coworkers then searched further, mapping in detail the brain structures where neurons were active electrically during conditioning. They found that learning-related increases in activity of individual neurons were prominent in the cerebellum, both in its cortex and deep nuclei and in certain nuclei in the pons.

In the cerebellum, there were only negligible responses to CS and US before the stimuli were paired, but a neuronal replica of the learned behavioral response emerged during conditioning. These responses, which preceded the behavioral eye-blink responses by 50 milliseconds or more, were found in the deep cerebellar nuclei ipsilateral to the eye that was trained. The interpositus nucleus appeared to be particularly involved (McCormick and Thompson, 1984). Lesion experiments were then undertaken to find whether the cerebellar responses were required for conditioning or whether, like the hippocampal responses, they only correlated with the CRs. In an animal that had already been conditioned, destruction of the ipsilateral interpositus nucleus abolished the CR. The CR could not be relearned on the ipsilateral side, but the contralateral eye could still be conditioned normally. In a naive animal, prior destruction of the interpositus nucleus on one side prevented conditioning on that side. The effect of the cerebellar lesions could not be attributed to inter-
ference with sensory or motor tracts because the animal still showed a normal unconditioned blink when an air puff was delivered to its eye.

The circuit of the conditioned reflex was then mapped in further detail using a combination of methods: electrophysiological recording, localized lesions, localized stimulation of neurons, localized infusion of small amounts of drugs, and tracing of fiber pathways (Krupa, Thompson, and Thompson, 1993). Based on these experiments, Thompson proposed a schematic circuit for the conditioned eye-blink response (Thompson and Krupa, 1994, Fig. 1, p. 536).

Since the main input to the deep cerebellar nuclei comes from the cerebellar cortex, lesions of the cortex would be expected to abolish the eyelid CR, just as lesions of the deep nuclei do. Such a finding was reported by a group working in England (Yeo, Hardiman, and Glickstein, 1985). Thompson and his associates, however, did not find lesions of the cerebellar cortex to interfere with the CR unless the lesions were very large. Perrett, Ruiz, and Mauk (1993) reported that lesions of the anterior cerebellar cortex prevented rabbits from acquiring accurate timing of the CR. They proposed that motor learning involves two sites of plasticity in the cerebellum; the CS-US association occurs at synapses between the mossy fibers and the deep cerebellar nuclei, whereas temporal discrimination is mediated by synapses between granule cells and Purkinje cells in the cerebellar cortex. More recent work on this question is reviewed in Chapter 13 of this volume, by Ohyama and Mauk.

The role of the cerebellum in conditioning is not restricted to eye-blink conditioning. The cerebellum is also needed for conditioning of leg flexion; in this task, an animal learns to withdraw its leg when a tone sounds in order to avoid a shock to the paw (Donegan, Foy, and Thompson, 1983; Voneida, 1990). On the other hand, the cerebellum is not required for all forms of conditioning of skeletal muscular responses. Thompson and his colleagues found that cerebellar lesions did not prevent operant conditioning of a treadle-press response in the rabbit (Holt, Mauk, and Thompson, unpublished, cited in Lavond, Kim, and Thompson, 1993, p. 328).

Studies with human subjects were consistent with the animal research. Patients with unilateral cerebellar lesions (usually caused by a stroke) show normal eyeblink reflexes with both eyes, but they can acquire a conditioned eyeblink responses only on the side where the cerebellum is intact (Papka, Ivry, and Woodruff-Pak, 1994). A PET study found that when humans received paired tone-airpuff training, several regions of the cerebellum and other brain structures showed increased glucose metabolism (Logan and Grafton, 1995). During the first, control session of the experiment, PET scans were taken while subjects received unpaired tone and right-eye airpuff stimuli. In the second session, one to six days later, subjects were given paired tone-airpuff trials. In the third session, two to seven days after the first, PET scans were made while the subjects received paired trials. Comparison of the scans showed increased session-three activity in several regions of the cerebellum and also in other
brain regions: right inferior thalamus/red nucleus, right hippocampal formation, right and left ventral striatum, right cortical middle temporal gyrus, left cortex occipitotemporal fissure. Thus the neural network involved in human eyelid conditioning includes not only the cerebellar and brainstem regions found by Thompson and his colleagues but also the hippocampus, the ventral striatum, and regions of the cerebral cortex.

D. Sleep and Memory Consolidation

After electrical recording helped to define the stages of sleep, beginning with the report of Aserinsky and Kleitman (1953), some investigators began to study the relation of stages of sleep to memory consolidation. Leconte and Bloch (1970) found that depriving rats of rapid-eye-movement (REM) sleep in the hours after learning impaired retention for avoidance conditioning. In a related experiment, the percentage of REM sleep to total sleep increased after a session of avoidance conditioning (Leconte, Hennevin, and Bloch, 1973). These results suggested that processing of newly acquired information continues during sleep as well as during waking. Bloch (1976) referred in this regard to the perseveration-consolidation hypothesis of Müller and Pilzecker (1900), which we cited earlier (Section IV-B). Some of Müller and Pilzecker’s subjects in verbal learning experiments reported that, although instructed not to rehearse material between experimental sessions scheduled days apart, they found the material coming back to mind without their trying to recall it.

Attempts to relate learning in humans to REM sleep have usually yielded negative results, according to a review by sleep researcher Peretz Lavie (1996, p. 140). He did, however, note some positive findings: During intensive learning of a new language, young people show increases in REM sleep, and so do people who recover language after becoming aphasic because of brain damage. Most significant, however, was the discovery by Lavie and colleagues of a young man who showed virtually no REM sleep — some nights showed no REM and overall only 2–5% of his total sleep was spent in REM, whereas healthy people of his age have 20–25% REM sleep (Lavie et al., 1984). The patient had been injured when fragments of a shell entered his brain; one splinter was lodged in the pons in the region believed to control activation of REM sleep. After recovery from the main effects of his injuries, this man had completed high school and then law school, so the great reduction in REM sleep had not impaired his learning or memory.

Indications that brain activity during sleep is related to memory formation continued to appear. By monitoring the electrical activity of neurons in the hippocampus during sleep, Bruce McNaughton and colleagues may have observed such consolidation in process. The neurons in question appeared to be “place cells”: that is, while a rat learns its way in a maze, certain hippocampal
cells come to favor firing when the rat is in a particular place in the maze, some cells firing when the rat is in one place, other cells firing more commonly when it is in another place. In such studies, large, prominent landmarks are placed around the room containing the maze so that the rat can use them to keep track of its position. The firing of a particular hippocampal cell indicates not where the rat actually is, but the rat's perceived location in the maze.

Wilson and McNaughton (1994) simultaneously recorded the activity of many (>50) hippocampal cells before, during, and after rats learned a new maze. The activity of two cells that developed very different place preferences in the course of learning the maze was uncorrelated throughout the experiment. But hippocampal cells that developed place preferences for neighboring portions of the maze came to fire together. So while the activity of these cells was uncorrelated before learning the maze, their firing was positively correlated by the end of the task. When the scientists examined the records made before the maze was learned, they found, as expected, that the activity of the cell pairs that was uncorrelated before maze learning during waking was also uncorrelated during prior sleep episodes. But after the maze was learned and the hippocampal neurons developed a correlation in their discharge, that correlated discharge was also seen during slow-wave sleep (SWS). They were able to find such neuron pairs (with uncorrelated discharge before learning and correlated discharge after learning) only by monitoring so many neurons at once. Although these results were intriguing, it was not clear how to integrate them with results of other studies, most of which related memory formation to rapid-eye-movement (REM) sleep rather than to SWS. But the facts that hippocampal neurons were active during sleep in these rats and that the postlearning sleep activity reflected the modified discharge learned in the maze indicated that some active process was going on. It was almost as if the postlearning sleep reinforced the new relationships between the cells in their firing. A further study reported that even the order in which various hippocampal cells fired during the training session was reflected in the order in which they fired during sleep afterwards (Skaggs and McNaughton, 1996). Whether this electrical activity during the posttraining sleep period in fact helped the rats remember the maze in subsequent trials remained to be seen. Because posttraining sleep has been shown to improve memory retention and because the hippocampus seems to be important for at least some kinds of memory formation, these observations of neuronal activity seemed to resemble tantalizingly sleep-consolidation of memory.

X. MEMORY DURING AGING

As we have noted, thinkers in antiquity (e.g., Aristotle) commented on the decline of memory with old age and tried to account for it, and this decline
was mentioned over the ages. But when scientists began in the second half of the twentieth century to measure memory as a function of age and to test possible mechanisms for its changes, the subject became more complicated. Some kinds of memory appeared to start their decline at relatively early ages, whereas others remained relatively intact over age. The capacity of older people to learn and remember has become a topic of heightened interest, in part because of the growing proportion of elderly people in the populations of developed countries and concern for reductions in performance that accompany normal aging. It also reflects attention to pathological forms of cognitive impairment that are more likely to affect older people, such as Alzheimer's disease.

Accurate comparisons of learning and memory in people of different ages were impeded by confounding factors. For example, apparent differences in learning ability and memory formation may be influenced by such factors as educational level, how recently subjects had experienced formal training, and motivation. One way to avoid such confounds was to employ animal subjects. And work with animals showed declines with age in some forms of learning and memory (e.g., Kubanis and Zornetzer, 1981). After experimenters allowed for confounding factors with human subjects, differences related to age were found to occur with some tasks but not with all (e.g., Anderson and Craik, 2000; Balota et al., 2000).

What kinds of tasks were found to be more likely than others to show decrements with aging? Elderly people in normal health were found to show some memory impairment in tasks of conscious recollection that required effort (Hasher and Zacks, 1979) and that relied primarily on internal generation of the memory rather than on external cues (Craik, 1985). Giving elderly subjects easily organized task structures, or cues, often raised their performance to the level of the young. Thus the type of task helped to determine whether impairment was observed. A number of different mechanisms appeared to be involved in the changes in learning and memory with age, as reviewed by Barnes and Penner in Chapter 15 of this volume.

XI. HOW TO IMPROVE MEMORY

As far back as classical antiquity, thinkers were concerned with how to improve memory. Thus, to be able to remember the successive parts of a speech in correct order, the Roman orator Cicero (106–43 BCE) advocated use of the method of loci, which he attributed to the Greek poet Simonides. In this mnemonic method, one imagines the successive parts as being located at distinctive points along a path through a familiar house or along a familiar street. What has research contributed to this question?
In the first experimental study of memory, Ebbinghaus (1885) investigated the effects of repetition in improving memory for lists of words. In some earlier sections of this chapter, we noted methods that improved learning and memory. Thus, in Section V-B we saw that enriched experience improves ability to learn. In Section VI we reviewed genetic methods of improving learning ability. In Section X we noted that providing easily organized task structures can improve memory. Heinrichs brings this topic up to date in Chapter 17 of this volume.

Research on neurochemical processes in memory formation in the last quarter of the twentieth century led to efforts to find drugs that could improve learning and memory. Many experiments with animals showed beneficial effects of certain drugs on learning and/or on memory performance, but they were far from finding a drug that met three major criteria: (1) The drug should be able to improve several of the many kinds of learning and memory. (2) The drug should have a broad effective dose range so that determining the correct dose for an individual would not be a time-consuming and expensive procedure. (3) Use of the drug over long periods of time should produce no important negative side effects. In 1992 I listened to a debate on this topic between two experts on the effects of drugs on memory. The optimist (if that is the right term in this case) made the provocative prediction that within 18 months a drug would be found that could improve memory for large numbers of people. The pessimist predicted that it would be 10 years before such a drug would be discovered. In fact, even the 10-year span was too short; no such drug has yet been discovered.

In this area, as in many others, people tend to be gullible and look for "quick fixes." Not only individuals, but large businesses and government agencies, including the armed forces, have spent enormous amounts of money on unproved and dubious systems to improve learning and memory. In the 1980s the National Research Council of the United States appointed a committee to evaluate several popular systems alleged to improve learning and memory. Their report, a book entitled *Enhancing Human Performance* (Druckman and Swets, 1988), found that none of those systems had proved merit, and most of them had no plausible scientific basis. The committee also spelled out procedures and criteria that a business or organization should use to evaluate a system claimed to improve learning and memory before deciding whether to adopt it.

Although it will take time, more effective systems of training and effective memory drugs are likely to be discovered, so it is important to make adequate preparations for a drug that could significantly improve the ability of large numbers of people to learn better and to remember more surely. Such a "smart pill" would effectively increase intelligence by making more information immediately available to individuals and by cutting the time needed to learn and remember. But would we be ready for the social effects of a drug...
that would significantly increase memory and intelligence? For one thing, who would benefit first from such a drug, both within nations and among nations? Would not such a drug be likely to increase the disparities between the wealthier and poorer social groups, both nationally and internationally? What would be the effects on society if students could complete the curriculum significantly more rapidly and enter the labor market earlier? Although such a drug would promise many benefits in the long run, it would cause a host of difficult problems during the transition to the new era of generally higher intelligence.

These problems were raised in the 1960s by Prof. René Cassin, one of the authors of the International Declaration of Human Rights. He was awarded the Nobel Peace Prize in 1968 for his work in promoting binding international treaties on human rights. In his 80s, Cassin toured the world speaking to groups of biological scientists, educators, and jurists and warning them of this problem as well as of others that could arise from modern biological research. I have raised these problems at three International Congresses of Psychology (Rosenzweig, 1981, 1989, 1994) and I do so again here, because I believe that psychologists and neurobiologists should be aware of the problems as well as the benefits that could arise from this research. We have a responsibility, along with others, to plan for the beneficial use of this research and to anticipate and try to prevent negative effects. We must help to decide how to set up adequate planning for the time when our research can lead to significant improvements in learning and memory.

XII. CONCLUSIONS

1. Concern about memory and its mechanisms goes far back in recorded history. In antiquity, speculation about mechanisms of memory took the form of metaphors, and metaphors of memory continue to be proposed in the present.

2. Formal research on learning and memory extends back to the late nineteenth century. Early work has often been neglected, and older ideas have sometimes later been presented as new.

3. Hermann Ebbinghaus’ 1885 book initiated empirical research on human learning and memory. Psychology was ready for this, as shown by the fact that other investigators soon joined this field, using a variety of methods and materials.

4. Laboratory research on learning in animal subjects was begun independently by Edward L. Thorndike (1898), on the basis of planned experiments, and Ivan P. Pavlov (1897–1906), on the basis of unexpected observations during research on alimentary secretions. Thorndike’s research was followed up rapidly by other investigators. Although Pavlov conducted an extensive program of
research on conditioning, few other laboratories took up this research until the late 1920s.

5. Training and differential experience were demonstrated by the early 1960s to cause measurable changes in neurochemistry and neuroanatomy of weanling rats (e.g., Krech, Rosenzweig, and Bennett, 1960; Rosenzweig, Krech, Bennett, and Diamond, 1962). Similar results were later shown in adult animals. Later research found a variety of neurochemical and neuroanatomical changes.

6. Depriving one eye of visual experience in young kittens was found to cause changes in the electrical responses of cells in the visual cortex (Wiesel and Hubel, 1963). Wiesel and Hubel reported that such effects could not be induced in kittens more than a few months of age, but later workers found changes in cortical responses of adult animals as a result of experience.

7. Formation of long-term memory was demonstrated in the early 1970s to require synthesis of proteins in the hours following training (e.g., Bennett, Orme, and Hebert, 1972; Flood, Bennett, Rosenzweig, and Orme, 1973).

8. Formation of short-term, intermediate-term, and long-term memories has been found to require a cascade of neurochemical events, and rather similar sequences have been found in birds, mammals, and invertebrates.

9. Different kinds of learning and memory follow different rules, involve different brain sites, and require somewhat different cascades of neurochemical events. Because of these differences, generalizing about learning and memory and their biological mechanisms must be done with great care.

10. Newer biomedical and behavioral techniques are adding to knowledge of the neural mechanisms of learning and memory. The biomedical techniques include noninvasive brain imaging and molecular biological approaches. The behavioral techniques include tests for implicit as well as explicit memory, and tests for various kinds of declarative and nondeclarative memories. Biomedical as well as behavioral investigators use computational models.

11. Double dissociation of brain regions and cognitive processes is a strong method of localizing regions particularly involved in specific cognitive processes.

12. Although it may be simpler to conduct behavioral tests on one set of subjects and neurobiological tests on others, more powerful experimental designs perform both behavioral and neurobiological measures on the same set of subjects.

13. Increasingly, investigators are using multiple tests of hypotheses and seeking converging evidence to establish conclusions.

14. Changes in learning and memory formation with aging are diverse and have been hard to characterize. A number of neurobiological changes in the brain with aging have been found to correlate with changes in performance.

15. Many methods have been found to improve learning and memory, but discovery of a “smart pill” has proved elusive. Critics have pointed out that
discovery of such a drug would pose social problems and that investigators should share responsibility in preparing for this eventuality.

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