Chapter 55

Learning and Memory: Basic Mechanisms

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During the last quarter of this century, we have witnessed remarkable progress in understanding how the nervous system encodes and retrieves information. Current research is focused at the cellular level, where the encoding process entails activity-dependent changes in the strength of synaptic connections among neurons.

Antecedents to modern thinking can be traced back more than a century. The American psychologist William James was among the first to discuss the physiological basis of the manner in which associations become formed. His "law of neural habit" states:

When two elementary brain processes have been active together or in immediate succession, one of them, on reoccurring, tends to propagate its excitement into the other. (James, p. 256)

According to the law of neural habit, the condition that drives the formation of associations is the coactivity of elementary brain processes (contiguity). Although James did not specifically identify the locus of the physiological modifications, others were quick to do so. The Italian anatomist Tanzi1 appears to be the first to have advanced the hypothesis that the synapse was the locus of the change that encodes experience. The work of the great Spanish neuroanatomist Ramón y Cajal suggested that the nervous system was not a synécum, but rather a collection of physically separate neurons that signal information to one another only at specialized synaptic points of interaction. If signaling between neurons takes place at synapses, it follows that changes in the signal strength could alter the flow of activity within the brain and, consequently, the way an organism responds to experiences. According to this view, then, learning is the product of synaptic changes. Donald Hebb argued that learning involves coincident synaptic activation of neurons, the "Hebb synapse" (discussed later).

Some general principles have emerged from research on several vertebrate and invertebrate model systems. A list of these principles might include the following:

1. Multiple memory systems are present in the brain.
2. Short-term forms of learning and memory require changes in existing neural circuits.
3. These changes may involve multiple cellular mechanisms within individual neurons.
4. Second-messenger systems appear to play a role in mediating cellular changes.
5. Changes in the properties of membrane channels are often correlated with learning and memory.

In this chapter we describe several selected memory systems and neural and molecular mechanisms implicated in learning. Before doing so, however, we first present an overview of some of the methods that have been used to study learning.

Definitions and Types of Learning

There Are Multiple Memory Systems

Current views recognize a number of different forms of learning and memory involving different neural systems (Fig. 55.1). Many workers distinguish between "declarative" and "nondeclarative" (or "proce-
declarative memory. Declarative memory generally refers to explicit memories of "what"—that is, one's own previous experiences, recognition of familiar scenes and objects, and so on; some workers have even equated it with the information one can be aware of. Declarative memory is a major topic of the next chapter.

Here we focus on nondeclarative, implicit, or procedural memory—memory of "how to." The vast majority of memory processes in nonhuman animals, and many aspects of memory in humans, are of this sort. Consider all your likes and dislikes, all the skilled movements you perform (tennis, golf, swimming, bicycle riding, not to mention walking and talking), and so on. "Nondeclarative" is really a gray bag category: by some terminologies it can include the phenomenon of priming, defined as an increased ability to identify or detect a stimulus as a result of prior exposure. In this chapter we consider certain aspects of implicit memory, particularly nonassociative learning (sensitization), basic associative learning, and mechanisms of memory storage.

The categories of memory shown in Fig. 55.1 are somewhat arbitrary and by no means mutually exclusive. When an organism learns something important, several of these memory systems can become engaged.

At a more general level, all aspects of learning share a common thrust. As Rescorla has stressed, basic associative learning is the way organisms, including humans, learn about causal relationships in the world. It results from exposure to relations among events in the world. In both modern Pavlovian and cognitive views of learning and memory, the individual constructs a representation of the causal structure of the world and adjusts this representation through experience to bring it in tune with the real causal structure of the world, striving to reduce any discrepancies or errors between internal representation and external reality.

We will first describe in some detail the paradigms that have been used to study "procedural" or nondeclarative learning and provide examples of mechanistic analyses that have been performed in several selected invertebrate and vertebrate model systems. At the end of the chapter, we focus on possible mechanisms of a phenomenon known as long-term potentiation (LTP). LTP is viewed by many as a mechanism of memory storage in the nervous system, particularly in forebrain structures. The process of long-term depression (LTD) also occurs in forebrain structures. In addition, LTD is thought to be the mechanism for memory storage in the cerebellum.
Paradigms Have Been Developed to Study Nondeclarative (Procedural) Learning

Nonassociative Learning

Associative learning, involves the establishment of a relationship between two events (see later). Nonassociative learning, one of the more basic learning processes, involves the effect of a single event on response probability. The three examples of nonassociative learning that have received the most attention are habituation, dishabituation, and sensitization. Habituation is defined as a reduction in responding to a repeatedly delivered stimulus. Dishabituation refers to the restoration or recovery of a habituated response due to the presentation of another, typically strong, stimulus to the animal. Sensitization is an enhancement or augmentation of a response produced by the presentation of a strong stimulus. In vertebrate systems, at least, dishabituation appears to be an instance of sensitization.\(^{17}\)

Associative Learning

Interest in associative learning has a rich philosophical and experimental tradition, starting with the rules of association—similarity, contrast, and contiguity—formulated by Aristotle around 350 B.C. British associationism of the 17th and 18th centuries provided a more formal statement of the laws of association, emphasizing both simultaneous and successive associations. Associative learning is a very broad category that includes much of the learning we do: learning to be afraid, learning to talk, learning a foreign language, learning to play the piano. In essence, associative learning involves the formation of associations among stimuli and/or responses. It is generally subdivided into classical versus instrumental conditioning or learning. Classical or Pavlovian conditioning is the procedure in which a neutral stimulus, termed a conditioned stimulus (CS), is paired with a stimulus that elicits a response, termed an unconditioned stimulus (US), for example, food that elicits salivation; or a shock to the foot that elicits limb withdrawal.

Ivan Pavlov, a Russian physiologist who had been studying digestion in dogs, discovered classical conditioning by accident—a celebrated case of serendipity. Pavlov received the Nobel Prize, incidentally, for his work on digestion. He noticed that the mere sight of the food dish caused the dogs to salivate and decided to continue the experiments to see if dogs would also salivate in response to a bell at feeding time. Pavlov-trained dogs to stand in a harness and, after the sound of a bell, fed them meat powder (Fig. 55.2).\(^{18}\) He recorded the salivary responses of the dogs. At first, the bell did not elicit any response; the meat powder, of course, elicited reflex salivation, termed the unconditioned response (UR). He noticed that after a few pairings of the bell and meat powder the dogs began to salivate when the bell rang before they received the meat powder. This is termed the conditioned response (CR). This type of conditioning came to be called reward or appetitive classical conditioning. If the bell or another stimulus was followed by an unpleasant event, such as a strong electric shock, then a variety of aversive responses became conditioned. This type of conditioning is often termed a form of fear conditioning. Sartorial muscle movements appropriate to dealing with the US (e.g., leg flexion with a paw shock US) are also learned in aversive classical conditioning. A key aspect of Pavlovian conditioning is that the animal or human subject cannot control the occurrence of the CS and the US; they are determined by the experimenter. Instrumental learning or operant conditioning describes a situation in which the animal or person must perform some response in order to obtain reward or avoid punishment. That is, the subject can control the occurrence of the US. Aspects of instrumental learning are treated in Chapter 56.

Classical (Pavlovian) conditioning According to the traditional view, classical or Pavlovian conditioning is an operation that pairs one stimulus, the conditioned stimulus or CS, with a second stimulus, the unconditioned stimulus or US, as noted earlier. The US reliably elicits a response termed the unconditioned response or UR. Repeated pairings of the CS and US result in the CS eliciting a response, defined as the conditioned response or CR. Critically important variables are:

1. Order: the CS precedes the US,
2. Timing: the interval between CS and US is critical for most examples of conditioning,
3. Contiguity: the pairing or contiguity of the CS and US is necessary for conditioning.

Conditioning procedures in which the CS and US overlap in time are called delay conditioning, whereas trace conditioning consists of a procedure in which a time interval of no stimulation exists between the CS and the US. These temporal relations for delay and trace conditioning are depicted in Fig. 55.3. Often, the CR is similar to the US (e.g., in Pavlov's experiments both were salivation).

The contemporary view of Pavlovian conditioning The traditional view of Pavlovian conditioning emphasized the contiguity of the CS and US. A more general and contemporary view of Pavlovian conditioning emphasizes the relationship between the CS and
the US. That is, the information that the CS provides about the occurrence of the US is the critical feature for learning. This perspective on Pavlovian conditioning is consistent with current cognitive views of learning and memory, as noted previously. Indeed, in some situations the CR is quite different from the UR: footshock causes an increase in activity (UR) in the rat;ivor learned to a tone paired with this same footshock is expressed as freezing (CR). Note, however, that both responses are adaptive.

As noted earlier, conditioning involves learning about the relations between events in the organism's
environment. In this view, contingency is a key factor in organizing the organism's environment. Consider the following experiment. A group of rats is given a series of paired stimuli in which tone (CS) and footshock (US) are paired. The animals learn very well to freeze (CR) when the CS occurs. Another group of rats is given the same number of paired CS–US trials but is also given a number of presentations with the US alone. Animals in this group do not learn to freeze to the CS at all. Both groups had the same number of contiguous pairings of CS and US, but the contingency, the probability that the US is predicted by the CS, was very much lower in the group that was also given trials with the US alone.¹

Summary

Memory systems can be divided into two broad categories: declarative and nondeclarative, with each system being observed by distinct brain structures. Nondeclarative memory can be further divided into several categories including nonassociative and asso-

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**INVERTEBRATE STUDIES: KEY INSIGHTS INTO BASIC MECHANISMS OF PROCEDURAL LEARNING**

The Nervous Systems of Invertebrates: Have Simple Circuits, Relatively Large Cells, and Express Both Simple and Complex Forms of Learning

Invertebrates are useful for analyzing the cellular and molecular events that underlie learning. The nervous systems of many invertebrates contain only several thousand neurons compared to the billions of cells in vertebrate nervous systems, yet these neurons have biochemical and biophysical properties similar to those of vertebrates. In some circumstances, a given behavior may be mediated by 100 neurons or less, allowing determination of the entire neural circuit generating the behavior. The neurons of many invertebrates are relatively large and can be repeatedly identified as unique individuals, permitting examination of the functional properties of individual cells. Molecular and biophysical events underlying the changes in cellular properties can then be determined.

For many years, the general belief was that the small number of neurons found in most invertebrates limits their behavioral capabilities to only the simplest forms of behavioral modifications such as habituation and sensitization. However, it has become clear that even invertebrates exhibit more complex behavioral modifications, such as classical conditioning, operant conditioning, and higher-order forms of classical conditioning. Here we review the extensive analyses on *Aplysia* and *Hermissenda*, two gastropod mollusks. These studies have contributed in a major way to our understanding of the neural and molecular mechanisms of simple forms of learning. See Box 55.1" for a brief description of other invertebrate model systems.

The Marine Mollusk *Aplysia* Is Well Suited to Studying Mechanisms of Learning and Memory

A number of characteristics make *Aplysia* well suited for the examination of the molecular, cellular, morphological, and network mechanisms underlying neuronal...
Gastropod Mollusks

*Aplysia* and *Hermisenda* (see text).

**Placiebranchus** The opsiphanbranch Placiebranchus is a venomous marine carnivore. When exposed to food, the animal exhibits a characteristic bite-strike response. After pairing of a food stimulus (CS) with a strong electric shock to the oral veil (US), the CS, instead of eliciting a bite-strike response, elicits a withdrawal and suppression of feeding responses (conditioned response, CR). The CR is acquired within a few trials and is retained for up to 4 weeks. Neural correlates of associative learning have been analyzed by examining responses of various identified neurons in the circuit to chemosensory inputs in animals that have been conditioned. One correlate is an enhanced inhibition of command neurons for feeding.62

**Tritonia** To escape a noxious stimulus, the opsiphanbranch Tritonia differens initiates stereotypical rhythmic swimming. This response exhibits both habituation and sensitization and involves changes in many different components of swim behavior in each case.63 The neural circuit consists of sensory neurons, precentral pattern-generating (CPG) neurons, and motor neurons. Habituation appears to involve plasticity at multiple loci, including decrement at the first afferent synapse. Sensitization appears to involve enhanced excitability and synaptic strength in one of the CPG interneurons.

**Pond snail** (*Lymnaea stagnalis*) The pulmonate Lymnaea stagnalis exhibits fairly rapid nonaversive conditioning of feeding behavior. A neutral chemical or mechanical stimulus (CS) applied to the lips is paired with a strong stimulant of feeding such as sucrose (US).64 Greater levels of pairing, a component of the feeding behavior, can be produced by a single trial, and this response can persist for at least 14 days. The circuit consists of a network of three types of CPG neurons, 10 types of motor neurons, and a variety of modulatory interneurons. An analog of the behavioral response occurs in the isolated central nervous system. The enhancement of the feeding motor program appears to be due to an increased activation of the CPG cells by chemosensory inputs from the lips.

**Land snail** (*Helix*) Land snails can be conditioned to avoid food using procedures similar to those used with Placiebranchus. A food stimulus such as a piece of carrot (CS) is paired with an electric shock to the dorsal surface of the snail (US). After 5–15 pairings, the carrot, instead of eliciting a feeding response, elicits withdrawal and suppression of feeding responses. The transmitter serotonin appears to have a critical role in learning. Animals injected with a toxin that destroys serotonergic neurons exhibit normal responses to the food and the shocks alone, but are incapable of learning. Helix also exhibit habituation and sensitization of avoidance responses elicited by tactile stimuli.65

**Limax** The pulmonate Limax is an herbivore that locomotes toward desirable food odors. This behavior makes it well suited to food-avoidance conditioning. The slug’s normal attraction to a preferred food odor (CS) is significantly reduced when the preferred odor is paired with a bitter taste (US). In addition to this example of classical conditioning, food avoidance in Limax exhibits higher-order features of classical conditioning, such as blocking and second-order conditioning. An analog of taste-aversion learning occurs in the isolated central nervous system, facilitating subsequent cellular analyses of learning in Limax. The procerebral (PC) lobes in the central ganglion processes olfactory information and is a likely site for the plasticity.66

**Arthropods**

**Cockroach** (*Periplaneta americana*) and locust (*Schistocerca gregaria*) Learned modifications of leg positioning in the cockroach and locust may be useful in the cellular analysis of operant conditioning. When the animal is suspended over a dish containing a fluid, initially it makes many movements, including those that cause the leg to come in contact with the liquid surface. When contact with the fluid is paired with an electric shock, the insect rapidly learns to hold its legs away from the fluid. Neural correlates of the conditioning have been observed in somata of the leg motor neurons. These correlates include changes in intrinsic firing rate and membrane conductance.
Crayfish (Procambarus clarkii) - The crayfish tail-flip response exhibits habituation and sensitization. A key component of the circuit is a pair of large neurons called the lateral giants (LGs), which run the length of the animal's nerve cord. The LGs are the decision and command cells for the tailflip. Learning is related to changes in the strength of synaptic input driving the LGs.

Honeybee (Apis mellifera) - Honeybees, like other insects, have simple brains. For example, the antennal reflex of Apis mellifera is produced as a result of presenting gustatory stimuli to the antennae. Classical conditioned of feeding behavior can be produced by pairing visual or olfactory CS with sugar solutions (US) to the antennae. The small size of bee neurites is an obstacle in pursuing detailed cellular analyses of these behavioral modifications. Nevertheless, regions of the brain necessary for associative learning have been identified, and some neural correlates have been described. In particular, intracellular recordings have revealed that one identified cell, the ventral unpaired median (VUM) neuron, is sufficient to mediate the reinforcing effects of the US.

Drosophila - Because the neural circuitry in the fruit fly is both complex and inaccessible, the fly might seem to be an unpromising subject for studying the neural basis of learning. However, the ease with which genetic studies are performed compensates for the difficulty in performing electrophysiological studies. A frequently used protocol employs a two-stage differential odor-shock avoidance procedure, which is performed on large groups of animals simultaneously rather than on individual animals. Animals learn to avoid odors paired (CS+) with shock but not one explicitly unpaired (CS-). This learning is typically retained for 4-6 h, but retention for 24 h to 1 week can be produced by a spaced training procedure. Several mutants deficient in learning have been identified. Many of these mutants elements of the cAMP signaling pathway are affected. Experiments using inducible genes demonstrate a role for cAMP-responsive transcription factors in the induction of long-term memory. These transcription factors are also important for long-term memory in Aplysia and in vertebrates.

Annelids - Leech - Defensive reflexes in the leech (Hirudo medicinalis) exhibit habituation, dishabituation, sensitization, and classical conditioning. For example, the shortening response is enhanced following pairing of a light touch to the head (CS) with electric shock to the tail (US). The identified 5 neurons appear critical for sensitization, as their ablation disrupts sensitization. Interestingly, ablation of the S cells only partly disrupts dishabituation, indicating that separate processes contribute to dishabituation and sensitization. Separate processes also contribute to dishabituation and sensitization in Aplysia. The transmitter serotonin (5-HT) appears to mediate at least part of the reinforcing effects of sensitizing stimuli and the US. Serotonin appears to play similar roles in Aplysia, Helix, Hermissenda, and Tritonia.

Nematoda - Caenorhabditis elegans - Although analyses in C. elegans are just beginning, this animal promises to be a valuable vehicle for cellular and molecular studies of learning. Its principal advantages are threefold. First, its nervous system is extremely simple. It has a total of 302 neurons, all of which have been described in terms of their locations and synaptic connections. Second, the developmental lineage of each neuron is completely specified. Third, it is amenable to genetic and molecular manipulations. Recently, the animal has been shown to exhibit several forms of learning. When a vibratory stimulus is applied to the medium in which they locomote, adult C. elegans will swim backward. This reaction, known as the tap withdrawal reflex, exhibits habituation, dishabituation, sensitization, and long-term (24 h) retention of habituation training, and context conditioning. Although the neurons are small and it is difficult to record their electrical activity, aspects of the neural circuit have been described. The particular role of individual neurons is being elucidated using laser ablation to remove specific neurons from the circuit.

Modifications (plasticity) and learning and memory. The animal has a relatively simple nervous system with large, identifiable neurons that are accessible for detailed anatomical, biophysical, and biochemical studies. Neurons and neural circuits that mediate many behaviors in Aplysia have been identified. In several cases, these behaviors have been shown to be modified by learning. Moreover, specific loci within neural circuits at which modifications occur during learning have been identified, and aspects of the cellular mechanisms underlying these modifications have been analyzed and modeled.

17. RESEARCH IN COGNITIVE NEUROSCIENCE
The Siphon–Gill and Tail–Siphon Withdrawal Reflexes of *Aplysia*

Within the mantle cavity of *Aplysia* is the respiratory organ of the animal, the gill, and protruding from the mantle cavity is the siphon (Fig. 55.4). The siphon–gill withdrawal reflex is elicited when a tactile or electrical stimulus is delivered to the siphon, the stimulus causes withdrawal of the siphon and gill (Fig. 55.4A). A second behavior that has been examined extensively is the tail–siphon withdrawal reflex. Tactile or electrical stimulation of the tail elicits a coordinated set of defensive responses, two components of which are a reflex withdrawal of the tail and the siphon (Fig. 55.4B).

These two defensive reflexes in *Aplysia* exhibit three forms of nonassociative learning: habituation, dishabitation, and sensitization. A single sensitizing stimulus can produce a reflex enhancement that lasts minutes (short-term sensitization), whereas prolonged training (e.g., multiple stimuli) produces an enhancement that lasts days to weeks (long-term sensitization). *Aplysia* also exhibits several forms of associative learning, including classical conditioning and operant conditioning.

A prerequisite for the analysis of the neural and molecular basis of these different forms of learning is an understanding of the neural circuit that controls the behavior. The afferent limb of the siphon–gill withdrawal reflex consists of sensory neurons with somata in the abdominal ganglion. The siphon sensory neurons (SN) monosynaptically excite gill and siphon motor neurons (MN) that are also located in the abdominal ganglion (Fig. 55.5A). Activation of the gill and siphon motor neurons leads to contraction of the gill and siphon. Excitatory, inhibitory, and modulatory interneurons (IN) in the withdrawal circuit have also been identified, although only excitatory interneurons are illustrated in Fig. 55.5. The afferent limb of the
FIGURE 55.5 Simplified circuit diagrams of the siphon-gill (A) and tail-siphon (B) withdrawal reflexes. Stimuli activate the afferent terminals of mechanoreceptor sensory neurons (SN) whose somata are located in central ganglia. The sensory neurons make excitatory synaptic connections (triangles) with interneurons (IN) and motor neurons (MN). The excitatory interneurons provide a parallel pathway for excitation of the motor neurons. Action potentials elicited in the motor neurons, triggered by the combined input from the SNs and INs, propagate out peripheral nerves to activate muscle cells and produce the subsequent reflex withdrawal of the organs. Modulatory neurons (not shown here but see Fig. 55.6A1), such as those containing serotonin (5-HT), regulate the properties of the circuit elements and, consequently, the strength of the behavioral responses.

tail—siphon withdrawal reflex consists of a bilaterally symmetric cluster of sensory neurons located in the left and right pleural ganglia. These sensory neurons make monosynaptic excitatory connections with motor neurons in the adjacent pedal ganglion, which produce withdrawal of the tail (Fig. 55.5B). In addition, the tail sensory neurons form synapses with various identified excitatory and inhibitory interneurons. Some of these interneurons activate motor neurons in the abdominal ganglion that control reflex withdrawal of the siphon. Moreover, several additional neurons modulate the tail—siphon withdrawal reflexes (see Fig. 55.6A1).

The sensory neurons for both the siphon—gill and tail—siphon withdrawal reflexes are similar and appear to be important plastic elements in the neural circuits. Changes in their membrane properties and the strength of their synaptic connections (synaptic efficacy) are associated with sensitization. Moreover, the properties of these neurons are modulated by procedures that mimic short- and long-term sensitization training.

Multiple Cellular Processes and Short- and Long-Term Sensitization in Aplysia

Short-term sensitization. Short-term sensitization is induced when a single brief train of shocks to the body wall results in the release of modulatory transmitters, such as serotonin (5-HT), from a separate class of interneurons referred to as facilitatory neurons (Fig. 55.6A1). These facilitatory neurons regulate the properties of the sensory neurons and the strength of their connections with postsynaptic interneurons and motor neurons, a process called heterosynaptic facilitation (Figs. 55.6A2 and 55.6A3). The molecular mechanisms
contributing to heterosynaptic facilitation are illustrated in Fig. 5.5a. The first step is the binding of 5-HT to one class of receptors on the outer surface of the membrane of the sensory neurons. This leads to the activation of adenylate cyclase, which in turn leads to an elevation of the intracellular level of the second messenger cyclic adenosine 3',5'-monophosphate (cyclic AMP, or cAMP) in sensory neurons. When cAMP binds to the regulatory subunit of cAMP-dependent protein kinase (protein kinase A, or PKA), the catalytic subunit is freed and can now add phosphate groups to specific substrate proteins and, hence, alter their functional properties. One consequence of this protein phosphorylation is an alteration in the properties of membrane channels. Specifically, the increased levels of cAMP lead to a decrease in the serotonin-sensitive potassium current $I_{S-K}$, the delayed $K^+$ current $(I_{s-K})$, and the delayed $K^+$ current $(I_{s-K})$. See Chapter 5. These changes in membrane currents lead to depolarization of the membrane potential, enhanced excitability, and an increase in the duration of the action potential.

Cyclic AMP also activates a facilitation process that is independent of membrane potential and spike duration. This process is represented in Fig. 5.5b (large open arrow) as the translocation or mobilization of transmitter vesicles from a storage pool to a releasable pool. The translocation makes more transmitter-containing vesicles available for release, with subsequent...
action potentials in the sensory neuron. The overall effect is a short-term AMP-dependent enhancement of transmitter release.

Serotonin also acts through another class of receptors to increase the level of the second messenger diacyl-glycerol (DAG). DAG activates protein kinase C (PKC), which, like PKA, contributes to facilitation that is independent of spike duration (i.e., mobilization of vesicles). In addition, PKC regulates a nifedipine-sensitive Ca²⁺ channel (I₃,₆), and the delayed K⁺ channel (Iₖ,A). Thus, the delayed K⁺ channel (Iₖ,A) is dually regulated by PKC and PKA. The modulation of Iₖ,A contributes importantly to the increase in duration of the action potential (Fig. 55.6A). Because of its small magnitude, the modulation of Iₖ,A appears to play a minor role in the facilitatory process.

The consequences of activating these multiple second-messenger systems and modulating these various cellular processes are demonstrated when test stimuli elicit action potentials in the sensory neuron at various times after the presentation of the stimulating stimuli (Fig. 55.6A). More transmitter than normal is available for release as a result of the mobilization process and each action potential is broader, allowing a greater influx of Ca²⁺ to trigger release of the available transmitter. The combined effects of mobilization and spike broadening lead to the release of more transmitter from the sensory neuron and consequently a longer postsynaptic potential in the motor neuron. Longer postsynaptic potentials lead to enhanced activation of interneurons and motor neurons and thus an enhanced behavioral response (i.e., sensitization).

Researchers have mathematically modeled and simulated aspects of the modulation of membrane channels and the dynamics of second-messenger systems, namely, calcium regulation and transmitter storage and release. 2-3

Long-term sensitization. Repeated sensitizing stimuli (shocks over a period of 1-6 h) induce long-term sensitization, the memory of which can persist for days to weeks. Repeated training leads to more prolonged phosphorylation and activation of nuclear regulatory proteins by PKA. These proteins affect the regulatory regions of DNA, lead to increased transcription of RNA, and hence increase synthesis of specific proteins. Some of the resulting proteins may be transcription factors, which can activate other genes, and some of these genes may be able to maintain their own activation. One of the newly synthesized proteins initiates the internalization and degradation of neuronal cell adhesion molecules (NCAMs), allowing the restructur- ing of the axon arbor. 2 The sensory neuron can now form additional connections with the same postsynaptic target or make new connections with other cells.

Other newly synthesized proteins, such as intermedi- ate filament proteins (IFP) and Apo-1, an activator of TGFβ, are also likely to contribute to the growth of new processes. 2-3 Increased synthesis of calmodulin (CaM) also occurs, but the functional significance of this effect has not been determined.

Prolonged stimulation and increased levels of AMP also activate a process that decreases the level of PKA regulatory subunits, further prolonging PKA activation. 2 With fewer regulatory subunits of PKA bound to catalytic subunits, the catalytic units are persistently active and may contribute to long-term facilitation of transmitter release through the same Ca²⁺-dependent processes seen in the short term. Some of these changes induced by AMP and PKA include a decrease in I₃,₆ and enhanced excitability, as well as a possible prolongation or amplification of their effects on nuclear regulatory proteins (see previous discussion). As with short-term sensitization, the long-term enhanced responses of the animal to test stimuli are based on the enhanced release of transmitter from existing contacts between sensory neurons and motor neurons or between sensory neurons and interneurons. However, increases in axonal arborization (Fig. 55.6B) and synaptic contacts are unique to long-term sensitization, and these developments may contribute to the enhanced activation of interneurons and motor neurons that receive connections from the sensory neurons (e.g., Fig. 55.5).

Mechanisms Underlying Associative Learning in Aplysia

The withdrawal reflexes of Aplysia are subject to classical conditioning. 2-3 The short-term classical conditioning observed at the behavioral level reflects a cellular mechanism called activity-dependent neuromodulation. Delivering a US, such as an electric shock to the tail or a peripheral nerve, releases a modulatory neurotransmitter, such as 5-HT, that nonspecifically enhances transmitter release from the sensory neurons. This nonspecific enhancement contributes to short-term sensitization (see prior discussion). The associative learning results from the pairing of a CS (e.g., spike activity in one sensory neuron) with the US; an interaction that causes a selective amplification of the modulatory effects of the US in that specific sensory neuron. Unpaired activity does not amplify the effects of the US. The amplification of the modulatory effects in the paired sensory neuron leads to a pairing-specific enhancement of transmitter release from the sensory neuron.

In this proposed mechanism, increased Ca²⁺ levels resulting from spike activity in the sensory neuron alter adenylyl cyclase levels via calmodulin and increase the CaM²⁺ level produced by 5-HT. Thus, Ca²⁺ and CaM appear to play a role in the activity-
dependent neuromodulation underlying associative conditioning of the tail and gill withdrawal reflexes. In addition, activity changes in the intracellular levels of Ca\(^{2+}\) in the postganglionic neuron (i.e., motor neuron) may also contribute to associative changes in synaptic strength at the sensory–motor neuron synapse.\(^{107}\)

An important conclusion is that short-term associative learning operates via a mechanism that is an elaboration of the cAMP-dependent mechanisms contributing to a simpler form of learning—sensitization. This finding raises the interesting possibility that even more complex forms of learning may be achieved by using these simpler forms as building blocks. Indeed, theoretical studies have shown that a mathematical model of the learning rule (activity-dependent neuromodulation) for simple classical conditioning, when incorporated into simple neural circuits, has the capability to simulate higher-order features of classical conditioning such as second-order conditioning and blocking, as well as features of operant conditioning.\(^{76}\)

The Pacific Nudibranch *Hermissonida* Is Useful for Studying Mechanisms of Pavlovian Conditioning

One organism that has contributed to the development of a cellular and molecular understanding of associative learning and memory is the Pacific nudibranch *Hermissonida*. *Hermissonida* exhibits a robust example of Pavlovian conditioning. The relative simplicity of the pathways supporting the conditioned stimulus and unconditioned stimulus has made it possible to identify neurons and their convergence within the central nervous system. Because the sensory systems activated by the CS and US are central, interactions within and between both pathways can be studied in an isolated nervous system, and, in fact, mechanisms underlying Pavlovian conditioning have been identified in sensory neurons of the CS pathway.\(^{111}\)

**Visually Guided Locomotion and Foot Contraction of *Hermissonida***

Pavlovian conditioning of *Hermissonida* involves changes in locomotion and foot length produced by stimulation of the visual and vestibular systems.\(^{28,50}\) *Hermissonida* normally exhibits a positive phototaxis when stimulated with light. Light-elicited locomotion, orientation and movement toward a light source, and reduced locomotion in the brightest part of a light gradient can all be observed in response to light. In addition to the effects of illumination on locomotion, light elicits a lengthening of the foot. Stimulation of the gravity receptors (hair cells) by rotating the animals on a turntable with rotation produces both an inhibition of locomotion and foot shortening, the unconditioned response (UR). The Pavlovian conditioning procedure consists of pairing light, the CS, with high-speed rotation, the US. After conditioning, the CS suppresses the normal positive phototactic response and elicits foot shortening (CR). The learning lasts for days to weeks, depending on the number of conditioning trials. A diagram of the conditioning procedure used with *Hermissonida* is shown in Fig. 35.7. The change in behavior elicited by the CS depends on the association of the two sensory stimuli. In addition, unpaired presentations of the US prior to conditioning degrade subsequent conditioning produced by CS-US pairings.\(^{28}\)
Examples of nonassociative learning, such as habitua-
tion and sensitization, have not been a primary focus of
investigations in *Hermisenda*. However, short-term
nonassociative effects have been identified. These
effects are typically expressed in the initial trials of
a conditioning session and decrease rapidly after
multifetal training is terminated. Because the associa-
tive and nonassociative contributions to behavior
follow different time courses and are of different
magnitudes, the mechanism of classical conditioning
in *Hermisenda* is not likely to be an amplification
or potentiation of the mechanism of short-term sensiti-
zation.

Cellular and subcellular analyses of one-trial condi-
tioning in *Hermisenda* have provided an opportunity
to examine time-dependent mechanisms of memory
consolidation. In a one-trial conditioning procedure
shown to modify photocytosis, serotonin appears to be
the neurotransmitter or neuromodulator in the US
pathway. Light (CS) paired with direct application of
5-HT (to mimic the US) to the exposed nervous system
of otherwise intact *Hermisenda* significantly sup-
presses photocytotic behavior when the animals are
tested 1 day after one-trial conditioning. One-trial conditioning also produces cellular change in identi-
fied type B photoreceptors, the cells of cellular plastic-
ity produced by multirtrial Pavlovian conditioning (see
next section).

Mechanisms of Conditioning in *Hermisenda*

An essential step in the physiological analysis of
conditioning is identifying the loci in the animal's ner-
vous system where the memory of the associative ex-
xperience is stored. In the *Hermisenda* nervous system
one site of memory storage is the primary sensory neu-
rons (type A and type B photoreceptors) of the path-
way mediating the CS. Each non-image-forming eye
of *Hermisenda* is relatively simple, consisting of
three type B photoreceptors and two type A photo-
receptors.

In type B photoreceptors, conditioning is accompa-
nied by a number of modifications that alter the re-
ponse of the cells to the CS. These include a significant
increase in CS-elicited spike activity, enhanced excit-
ability to extrinsic current, an increase in the input
resistance, both increased and decreased amplitudes of
light-elicited generator potentials, a decrease in spike
frequency accommodation, and reductions in the peak
amplitudes of several diverse K currents. Cellu-
lar modifications in conditioned animals are also ob-
served in the type A photoreceptors. Lateral type A
photoreceptors of conditioned animals exhibit a sig-
nificant increase in CS-elicited spike activity, a decrease
in generator potential amplitude, and enhanced excit-
ability and decreased spike frequency accommodation
to extrinsic current. The enhanced excitability of iden-
tified sensory neurons of conditioned animals, ex-
pressed by significant increases in both the amplitude
of CS-elicited generator potentials and spike activity
elicited by the CS, may be a major contributor to
changes in the duration and amplitude of complex
postsynaptic potentials and enhanced spike activity
recorded in postsynaptic targets after conditioning.
In addition to the enhanced excitability of type A
and B photoreceptors found after conditioning, there are
changes in the strength of synaptic connections be-
tween identified photoreceptors. Taken collectively,
these experiments show that in the US pathway of
conditioned animals cellular changes are found at mul-
tiple sites involving changes in both excitability and
synaptic strength.

Voltage-clamp studies of type B photoreceptors have
identified two K currents, I_\text{K,B} and I_\text{K,A}, that are
reduced after conditioning. Reductions in several
diverse K conductances could account for both the
enhanced excitability and the enhancement of IPSNs
observed in conditioned animals.

Several second-messenger systems appear to be re-
sponsible for the reduction in the K currents of type
B photoreceptors. Evidence suggests that the phospho-
inositide system may contribute to reductions in K
currents observed in conditioned *Hermisenda*. Ac-
tivation of PKC by phorbol esters and diacylglycerol
analogs and intracellular injection of PKC into type B
photoreceptors reduced both I_\text{K,B} and I_\text{K,A}. Activation
of PKC may be initiated by the actions of an agonist
released by stimulation of the US pathway and 5-HT
and/or \(\gamma\)-aminobutyric acid (GABA) may be released
by stimulation of statecyst hair cells by rotation (US).
Several 5-HT immunoreactive neurons may provide
polyynaptic input to the visual system from stimula-
tion of the US pathway. In addition, the monosynaptic
inhibitory input to photoreceptors from statecyst hair
cells is presumed to be GABAergic and may be part of
the US pathway. The mitogen-activated protein kinase
(MAPK) pathway is also activated in Pavlovian condi-
tioning of *Hermisenda*.

One-Trial Conditioning of *Hermisenda*

One-trial conditioning in *Hermisenda* has provided
insights into the mechanisms of time-dependent mem-
ory consolidation. The one-trial conditioning proce-
dure described earlier produces CS-elicited short- and
long-term enhancement (STE and LTI) of generator
potentials recorded from identified type B photorecep-
tors, as well as enhanced excitability to the CS and
extrinsic current. LTI depends on protein and mes-
senger RNA (mRNA) synthesis, is expressed only in
lateral B photoreceptors, and is dependent on the contiguity of the CS and US. Figure 55.8 illustrates a cellular model for associative memory in the type B photoreceptors. Changes in these neurons by one-trial conditioning are the result of activation of PKC and MAPK by stimulation of the US pathway and elevated intracellular Ca²⁺ levels produced by the presentation of the CS. This activation of PKC due to elevated intracellular Ca²⁺ may result in phosphorylation of channel proteins.

The induction of short-term enhancement by one-trial conditioning is caused by activation of PKC because pretreatment with broad-spectrum protein kinase inhibitors and downregulation of PKC activity block the induction of short-term enhancement. Moreover, evidence indicates that short- and long-term enhancement may be parallel processes. The conditions that are sufficient to block short-term enhancement—downregulation of PKC and kinase inhibition—do not block long-term enhancement. We conclude from these results that short- and long-term enhancement are not sequential processes, but are parallel processes involving independent mechanisms.

Researchers have developed an in vitro conditioning procedure to examine biochemical mechanisms underlying one-trial conditioning. In this procedure, stimulating the isolated eyes and proximal optic nerve of *Hermesia* with light (CS) paired with 5-HT administration produced an increase in protein phosphorylation detected at different times following in vitro conditioning. In addition, the increase in protein phosphorylation detected 2 h after conditioning is dependent on CS-US pairing.
Summary

Invertebrates have an enormous capacity for learning and offer experimental advantages for analyzing the cellular and molecular mechanisms of learning. Each of the animals described has its own unique strengths. Behaviors in the gastropod mollusks and the leech are mediated by relatively simple neural circuits, which can be elucidated with conventional electrophysiological approaches. Once the circuit is specified, the neural loci for the particular example of learning can be found, and biophysical, biochemical, and molecular approaches can be used to identify mechanisms underlying the change. The relatively large size of some of these cells allows those analyses to take place at the level of individual identified neurons. Individual neurons can be surgically removed and assayed for changes in the levels of second messengers, protein phosphorylation, and RNA and protein syntheses. Moreover, peptides and nucleotides can be injected into individual neurons. Invertebrates such as Drosophila and Caenorhabditis elegans have small neurons, but offer tremendous advantages for obtaining insights into mechanisms of learning and memory through genetic approaches.

Despite the differences in the levels of analyses and the examples of learning that have been analyzed, we can make some general observations based on the studies described in this section. First, learning is associated with changes in the properties of individual neurons and synapses. Support for this assertion comes from studies on Aplysia, Hermissenda, Pleurobrachia, Lymnaea stagnalis, Tritonia, cockroach, locust, crayfish, honeybee, and leech. Second, modulatory transmitters appear to be important for inducing cellular changes associated with many examples of learning; serotonin appears to be particularly important in Aplysia, Hermissenda, Hela, Tritonia, crayfish, and leech, whereas other amines are important in the honeybee and Drosophila. Third, work in Aplysia, Hermissenda, honeybee, and Drosophila has shown that second-messenger systems are engaged during learning and that these messengers affect multiple cellular processes. Fourth, changes in the properties of membrane channels are associated with learning. This assertion is supported by findings in Aplysia, Hermissenda, Pleurobrachia, and Tritonia. Fifth, studies in Aplysia, Hermissenda, and Drosophila have shown that long-term memory is associated with changes in protein synthesis. Aplysia and Drosophila provide evidence that changes in protein synthesis are induced by activation of CREB, a CAMK-dependent transcription factor. The findings from invertebrates are consistent with those now emerging from vertebrates. A universal mechanism for learning, however, does not appear to be present. Rather, different animals and, indeed, different neural circuits within any one animal, draw on a palette of multiple mechanisms supporting plasticity to mediate specific types of learning.

CLASSICAL CONDITIONING IN VERTEBRATES: DISCRETE RESPONSES AND FEAR AS MODELS OF ASSOCIATIVE LEARNING

When animals, including humans, are faced with an aversive or threatening situation, at least two complementary processes of learning occur. Learned fear or arousal develops very rapidly, often in one trial. Subsequently, the organism learns to make the most adaptive behavioral motor responses to deal with the situation. These observations led to so-called two-process theories of learning development of an initial learned fear or arousal, followed by slower learning of discrete behavioral responses. As the latter learning develops, fear subsides. We now think that, at least in mammals, a third process typically takes place in which declarative memory for the events and their relations develops (see Fig. 55.3 and later discussion). In this section, we focus on the learning of discrete responses, using eyelink conditioning as the model system, and on conditioned fear.

Eyelink Is a Model System for Studying the Conditioning of Discrete Behavioral Responses in Vertebrates

A vast amount of research has used Pavlovian conditioning of the eyelink response in humans and other mammals. The eyelink response exhibits all the basic laws and properties of Pavlovian conditioning equally in humans and other mammals. The basic procedure is to present a neutral CS, such as a tone or a light, followed a quarter of a second or so later by a puff of air to the eye or by a peripheral (around the eye) shock (US). This is an example of what is known as a delay procedure, e.g., Fig. 55.3A. Initially, there is no response to the CS and a reflex eyelink to the US. After a number of such trials, the eyelid begins to close in response to the CS before the US occurs. In a well-trained subject, the eyelid closure (CR) becomes very precisely timed so that it is maximally closed about
FIGURE 55.9 The adaptive nature of classical conditioning. This example shows the development of the conditioned eyelid response over the trials of training. The CS is typically a "neutral" light or tone; the US here is a puff of air to the cornea. The eyelid closure response is induced by upward movement of the tracing. The first marker is tone CS onset; the second is puff US onset. In Trial 1 the eyelid does not move to the CS but classes (blinks) following onset of the US. The conditional response (CR) is any measurable degree of eyelid closure prior to the onset of the US. Note that after learning, the CR peaks at the onset of the US, i.e., maximum eyelid closure at puff onset. If the CS-US onset interval were longer (e.g., 500 ms), the CR would now peak at the onset of the US, 500 ms after CS onset. The conditional response is adaptive for this type of learning, a period (88) of about 250 ms between CS onset and US onset (shown here) yields the best learning. This best learning time varies widely depending on the type of response (e.g., for fear learning, several seconds is best).

the time that the air puff or shock (US) onset occurs (see Fig. 55.9). This very adaptive timing of the eyeblink CR develops over the range of CS-US onset intervals (i.e., 38, Fig. 55.3) in which learning occurs, about 100 ms to 1 s. Thus, the conditioned eyelid response is a very precisely timed elementary learned motor skill. The same is true of other discrete behavioral responses learned to deal with aversive stimuli (e.g., the forelimb or hindlimb flexion response and the head turn).

As noted previously, there are two basic procedures in classical conditioning: delay and trace (Fig. 55.3). Pavlov first described trace classical conditioning. He stressed that the organism must maintain a "trace" of the CS in the brain in order for the CS and the US to become associated. In eyeblink conditioning in animals, a typical trace interval between CS offset and US onset is 500 ms. The trace eyeblink procedure is much more difficult to learn than the delay procedure.

Two brain systems, the hippocampus and the cerebellum, become massively engaged in eyeblink conditioning. When the US is sufficiently aversive to elicit learned fear, the amygdala also plays a role. In an experimental design using a click CS and glabellar (forehead) tap US in restrained cats, a very short latency (<20 ms) eyeblink muscle EMG (electrical response recorded from muscles around the eye) CR involving the motor cortex develops. However, bilateral removal of the motor cortex does not appear to affect either learning or expression of the standard longer latency adaptive delay or trace CRs. This short latency EMG response may be a component of the startle response elicited by a sudden acoustic stimulus.

Hippocampus and Classical Conditioning

In eyeblink conditioning, neuronal unit activity in hippocampal fields CA1 and CA3 increases very rapidly in paired (tone CS–corneal air puff US) training trials, shifts forward in time as learning develops, and forms a predictive "temporal model" of the learned behavioral response both within trials and over the trials of training (Fig. 55.10). To summarize a large body of research, the growth of the hippocampal unit response, under normal conditions, invariability and strongly predicts subsequent behavioral learning. This increase in neuronal activity in the hippocampus becomes significant by the second or third trial of training, long before behavioral signs of learning develop. Neurons in the hippocampus become engaged in many other types of learning as well (see Chapter 59).

In eyeblink conditioning, many neurons identified as pyramidal neurons in fields CA1 and CA3 show learning-related increases in discharge frequency during the trial period (Fig. 55.10). Typically, a given neuron models only some limited time period of the trial, although some pyramidal neurons model the entire learned behavioral response, as in Figs. 55.10A and 55.10B. Thus, the pyramidal neuron representation of the behavioral learned response is distributed over both space and time in the hippocampus.

The results just described were obtained using the basic delay procedure, in which hippocampal lesions did not impair simple response acquisition in rabbits, similarly, humans with hippocampal–temporal lobe anterograde amnesia (Chapter 39) are able to learn simple acquisition of the eyeblink conditioned response, but cannot recall the learning experience. The
involvement of the hippocampus depends on the difficulty of the task. For example, such amnesic humans are massively impaired on conditional discriminations in eyelink conditioning (e.g., blink to tone only if preceded by light) but not on simple discriminations. Bilateral hippocampal lesions in rabbits markedly impair subsequent acquisition of the trace CR. Interestingly, when the hippocampal lesion is made immedi-

FIGURE 55.10 Engagement of hippocampal neurons in eyelink conditioning. Responses of identified pyramidal neurons during paired (A and B) and unpaired (C-F) presentations of tone and control sippet. The upper traces show the averaged nictitating membrane (NM), a component of the eyelink response for all trials during which a given cell was recorded. The bottom traces show the response of the recorded neuron in the form of a peristimulus time histogram. The total length of all NM responses was 750 ms. Arrows occurring early in the trial period indicate tone onset; arrows occurring late in the trial indicate sippet onset. (H) Hippocampus. In this particular figure, A and B show examples of responses of two pyramidal neurons recorded from two different animals during delay conditioning. The results in C and D show the response of a pyramidal neuron recorded from an animal given unpaired tone-alone (E) and sippet-alone (F) presentations. (G and H) Same for a different pyramidal cell recorded from a different control animal. From Berger et al. [7].
ality after trace learning in rabbits, the CR is abolished; when the lesion is made a month after training, the CR is not impaired (Fig. 5.51). These results are striking in light of reports of declarative memory deficit following damage to the hippocampal system in humans and monkeys (see Chapter 59). These deficits have two key temporal characteristics: (1) profound and permanent anterograde amnesia and (2) profound but clearly time-limited retrograde amnesia. Subjects have great difficulty learning new declarative tasks and/or information (anterograde amnesia) and have substantial memory loss for events some period preceding brain damage (retrograde amnesia), but relatively intact memory for earlier events. So even in a simple procedural learning task such as eyelid conditioning, hippocampal-dependent "declarative" memory processes develop.

What are the mechanisms of the changes in the hippocampus? The process of long-term potentiation is widely considered to be the most likely mechanism of memory storage in the hippocampal system (see later discussion). In the case of classical conditioning, there are a number of parallels between the properties of LTP and the properties of the learning-induced increase in neuronal activity in the hippocampus. Both LTP and the learning-induced increase in hippocampal neuron activity are associated with pyramidal neurons, both begin to develop after very brief periods (e.g., 100 Hz for 1 s for LTP; 1-3 trials of training in eyelid conditioning), both approach a limit asymptotically over a period of many minutes, both show the same magnitude of increase, and both are developed only with very specific parameters of stimulation. Further, there is a persistent increase in the extracellularly recorded monosynaptic population spike in the dentate gyrus in response to stimulation of the perforant path as a result of eyelid conditioning, just as occurs when LTP is induced by tetanus of the perforant path.

There are strikingly parallel and persisting increases in glutamate AMPA (α-amino-3-methylisoxazole-4-propionic acid) receptor binding on hippocampal membranes in both eyelid conditioning (well-trained animals) and in vivo expression of LTP by stimulation of the perforant path projection to the hippocampal dentate gyrus. The pattern of increased binding is similar in both. Glutamate NMDA (N-methyl-D-aspartate) receptors play a critical role in induction of LTP (at least in the dentate gyrus and CA1) and also appear to be involved in acquisition of the trace ey-
bink CR. Mechanisms of LTP are discussed at length in the following section.

The Cerebellum System and Classical Conditioning of Discrete Responses

Since publication of the classic papers of Marr and Albus, the cerebellum has been favored as a structure for modeling mammalian learning. Figure 55.12 is a highly simplified diagram of a current qualitative working model of the role of the cerebellum in the basic classical (delay) conditioning of eyeblink and other discrete responses. Laterality is not addressed in Fig. 55.12; the critical region of the cerebellum is ipsilateral to the trained eye (or limb), whereas the critical regions of the pontine nuclei, red nucleus, and inferior olive are contralateral (see Fig. 55.13 for a more realistic representation). In this section, the data refer to the basic eyewink CR, unless otherwise noted. In brief, the reflex eyeblink response pathways activated by corneal airpuff (or periorbital shock) include the trigeminal nucleus, direct projections to the relevant motor nuclei (mostly the seventh and accessory sixth), and indirect projections to the motor nuclei via the brainstem reticular formation (Fig. 55.12). Analyses of response latencies rule out any direct role of the cerebellum in the reflex response. The tone (and light) CS pathways project to the cerebellum as mossy fibers, mostly relaying through the pontine nuclei. The mossy fibers, in turn, activate granule cells and these granule cells project to Purkinje cells via parallel fibers. The US pathway projects from the trigeminal nuclei to the inferior olive and from there to the cerebellum as
climbing fibers. The CS-activated mossy fiber–parallel fiber pathway and the US-activated climbing fiber pathway converge and make synaptic connections on Purkinje neurons in the cerebellar cortex (parallel fiber–climbing fiber) and on neurons in the interpositus nucleus (mossy fiber–climbing fiber). The CR pathway projects from the interpositus nucleus of the cerebellum via the superior cerebellar peduncle to the red nucleus, and from there to the premotor and motor nuclei (middle seventh and accessory sixth) controlling the eyelid response.

This circuitry has been identified using a number of methods, including lesion studies, electrophysiological recordings, electrical microstimulation, and anatomical characterization of projection pathways. For example, in animals, neurons in the cerebellar cortex and interpositus nucleus respond to the CS and US before training and develop amplitude–time–course models (e.g., Fig. 55.13) of the learned behavioral response. These models predict and predict the occurrence and form of the CR within trials and over the trials of training (see Fig. 55.13). By inference from PET analysis, the same process occurs in humans (see Fig. 55.14). In animals, appropriate lesions of the anterior interpositus nucleus completely and permanently prevent learning. Similar lesions inflicted after training permanently abolish the CR but are without effect on the UR. To the same way, in humans, cerebellar lesions can completely prevent learning of the CR, but are again without effect on the UR. Interestingly, when the interpositus lesion (in rabbits) is incomplete, resulting in a marked impairment in the CR but not complete abolition, the attenuated CR does not recover with further training.

Appropriate lesions of the pontine nuclei (the CS pathway) can selectively abolish the CR to one modality of CS, and stimulation of the pontine nuclei serves as a supraspinal CS yielding faster learning than peripheral CSs. Finally, lesions of the appropriate region of the inferior olive completely prevent learning if they are made before training. Lesions made after training, at the same location, result in extinction and abolition of the CR. Electrical microstimulation of this same region elicits discrete movements, and the exact movements so elicited can be trained to occur to any neutral stimulus. The inferior olive–climbing fiber system, incidentally, is the only system in the brain other than reflex afferents where this occurs. These results constitute a verification of the theories developed initially in the classic papers of Mace and

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**Figure 55.13** Engagement of neurons in the cerebellar interpositus nucleus in eyelid conditioning. Histograms of a unit cluster recording from the interpositus nucleus over the course of training are shown. The eyelid response (neuronal membrane extension) is shown on the tracing above each histogram. (A) Results of a day of unpaired CS and US presentations. There is weak activity to the US. However, when paired training (B) is given (days 1 and 2), as behavioral learning develops, loyal decline prior to US onset occurs; there is a massive increase in neuronal discharges in the CS period that precedes and correlates with performance of the conditioned eyelid response. Total trace duration: 750 ms, CS–US onset interval: 250 ms. Each trace and histogram is the average or summation of 1 day of training (120 trials). From McCormick and Thompson.6
Albus et al. These theories proposed that the cerebellum was a neuronal learning system in which there was a convergence of mossy-parallel fibers that conveyed information about stimuli and movement contexts (CSs here) and the climbing fibers that conveyed information about specific movement errors and aversive events (USs here). This convergence might occur on Purkinje neurons in the cerebellar cortex to alter the synaptic efficacy of the parallel fiber synapses on Purkinje dendrites. There is a similar convergence of mossy and climbing fibers on neurons in the interpositus nucleus (see Figs. 55.12 and 55.13).

The Cerebellum: The Locus of the Long-Term Memory Trace

Overall, the results described to this point demonstrate that the cerebellum is necessary for learning, retention, and expression of classical conditioning of the eyelid and other discrete responses. The next and more critical issue concerns the locus of the memory traces. We will next consider evidence that points to the cerebellum as the location where long-term memory traces for this type of learning are formed and stored.

Reversible inactivation has proved to be a powerful tool to localize sites of memory storage in systems where the essential circuitry is known, as in eyelid conditioning (Figs. 55.12 and 55.13). In brief, if inactivation of a structure abolishes the learned response, the structure is considered to be part of the circuitry necessary for expression of the learned response. If the structure is inactivated during training and the animal immediately shows complete learning when the inactivation is subsequently removed, then the structure is not involved in acquiring the learned response but lies on the efferent path from the memory trace.

However, if the animal shows no evidence of having learned following inactivation training, then either the memory trace is normally located in the structure or the structure is a necessary afferent to the trace. Reversible inactivation can be produced by local cooling using a cold probe or by infusion of a drug. A variety of drugs can produce reversible inactivation, including muscimol, a GABA agonist that inactivates only neuron somas and not axons, and TTX, a sodium channel blocker that blocks both neuron somas and axons (see Chapter 6).

Several parts of the circuit shown in Fig. 55.12 have been reversibly inactivated during training in naive animals. In brief, inactivation of the motor nuclei (Fig. 55.12a), red nucleus (Fig. 55.12b), superior cerebellar peduncle (Figs. 55.12d and 55.12e), and a localized region of the anterior interpositus nucleus and overlying cerebellar cortex (Fig. 55.12c) each prevent expression of the CR (only motor nuclei inactivation also prevents expression of the UR). After training carried over during reversible inactivation of motor nuclei, the red nucleus, or the superior cerebellar peduncle, the CR is found to be fully learned as soon as the inactivation has ceased (Figs. 55.12a, 55.12b, 55.12d, and 55.12e). However, localized cerebellar inactivation (Fig. 55.12c) completely prevents learning; animals must learn from scratch as if completely untrained. These results argue strongly for cerebellar localization of the memory trace. This hypothesis is supported by the observation that inhibition of protein synthesis in the cerebellar interpositus nucleus appears to prevent long-term retention of the conditioned eyelid response.
Experimentally, it has proved extremely difficult to determine the relative roles of the cerebellar cortex and interpositus nucleus in eyeblink conditioning using the lesion method. These difficulties were overcome by making use of the mutant Purkinje cell degeneration (pcd) mouse strain. In this mutant, Purkinje neurons (and all other neurons studied) are normal throughout pre- and perinatal development. At about 2–4 weeks after birth, the Purkinje neurons in the cerebellar cortex degenerate and disappear. For about 2 months after this time, other neuronal structures appear relatively normal. Thus, during this period of young adulthood,
the animals have a complete, selective functional de- 
cortication of the cerebellum.7,8
The pdf mice learned very slowly, very poorly, and to a 
much lower level than wild-type controls and 
showed extinction with subsequent training to the CS 
alone. Thus, the cerebellar cortex plays a critically 
important role in normal learning (of discrete behavioral 
response); but some degree of learning is possible with- 
out the cerebellar cortex.
Putative Mechanisms of Memory Storage 
in the Cerebellum
Classic theories of the cerebellum as a learning ma- 
chine (see earlier) proposed that conjoint activation of 
 Purkinje neurons by parallel fibers and climbing fibers 
would lead to alterations in synaptic strength of the 
parallel fiber synapses. An early20 discovered that such con-
joint activation leads to a long-lasting depression of the 
efficacy of parallel fiber synapses to Purkinje neuron 
dendrites. This process is known as cerebellar long-
term depression, LTP and associates showed that such a 
process plays a key role in adaptation of the vestibulo-
oculur reflex.20,7,5,6,12
In eyelid conditioning, many of the Purkinje neu-
rons that exhibit learning-related changes show de-
creases in simple spike responses in the CS period14 
that are consistent with LTD. The current view at the 
molecular level is that LTD is due to a persisting de-
crease in AMPA receptor function at parallel fiber syn-
apses on Purkinje neuron dendrites.15,17,18,12 This de-
crease in AMPA receptor function is, in turn, the result 
of glutamate activation of AMPA and metabotropic 
receptors on Purkinje neuron dendrites, together with 
increased intracellular calcium (normally by climbing 
fiber activation).
Classical conditioning studies using "gene knock-
out" mice have strengthened the argument that LTD is 
a key mechanism of memory storage in the cerebellar 
cortex.27 Thus, mice that lack metabotropic glutamate 
receptors (mGluR1) critical for LTD show marked im-
pairments in cerebellar cortical LTD as expected, but 
also in eyelid conditioning.27 They also show gener-
alized motor impairments, that is, some degree of 
ataxia, as do the pfd mice mentioned previously.
Current studies present evidence supporting the 
view that LTD is more important for learning (e.g., 
eyelid conditioning) than for motor coordination. 
Thus, the PKCy knockout mutant mouse maintains to 
adulthood the perinatal condition of more than one 
climbing fiber per Purkinje neuron (wild-type adults 
have only one climbing fiber per Purkinje neuron).
This mutant exhibits normal LTD but impaired motor 
coordination (also, primarily, to the multiple climbing 
fiber innervation of Purkinje neurons). In striking con-
trast, these animals learn the conditioned eyelid re-
sponse more rapidly than do wild-type controls.27 This 
is consistent with the view that the climbing fiber sys-
tem is the reinforcing or teaching pathway.
In striking contrast, a quite different mutant, the 
GFAP (glial fibrillary acidic protein) knockout 
mouse,21,2 shows marked deficiency in cerebellar corti-
cal LTD and in eyelid conditioning. The performance of 
such mutants is very similar to that of pdf mice. Unlike 
the PKC mutants, these animals do not show 
any obvious impairments in motor coordination or 
general motor behavior. GFAP, which is expressed fol-
lowing neuronal injury is not present in neurons, only 
in glial cells. In the cerebellum it is normally present 
in substantial amounts in the Bergmann glia that sur-
round the parallel fiber and the synapses between the 
climbing fibers and the Purkinje neuron dendrites. Al-
though the Bergmann glia appear morphologically 
normal in GFAP knockout mice, they have no GFAP.
The point here is that an abnormality limited to glial 
cells markedly impairs a form of synaptic plasticity 
(LTD) and a form of basic associative learning and 
memory, suggesting a key role for glia in processes of 
learning and memory.
We have focused on the essential role of the cerebel-
 lum in the classical conditioning of discrete behavioral 
responses, a basic form of associative learning and 
memory. To date, this is perhaps the clearest and most 
decisive example of evidence for the localization of a 
memory trace to a particular brain region in mammals 
(cerebellum). The cerebellum has also been pinpointed as 
the location where complex, multijoint movements 
are learned and stored27 (see Chapter 35 on the cere-
bellum).
Actually, growing evidence suggests that the cere-
bellum is critically involved in many other forms of 
learning and memory, including cardiovascular condi-
tioning,27 discrete response instrumental avoidance 
learning,27 motor learning,27 spatial learning and mem-
ory,27,27 and adaptive timing.27 There is even a growing 
literature implicating the cerebellum in complex 
cognitive processes.27
The type of learning exemplified by the cerebellar 
circuitry underlying classical conditioning of discrete 
responses has been termed "supervised learning."27 
Information from one network of neurons acts as an 
instructive signal (a US) to influence the pattern of 
connectivity in another network (e.g., CS); other exam-
les include adaptation of the VOR and calibration of 
the auditory space map in the barn owl (Chapter 22).
In eyelid conditioning, the neutral CSs (e.g., tone or 
light) influence the activity of neurons in the cerebel-
um only weakly and do not yield the behavioral re-
sponse. As a result of training, the strong connections
established between networks of neurons are not functionally coupled prior to learning. That is, diffuse cerebellar mossy and/or parallel fibers activated by the CS develop sufficient strength of their synaptic connections to successfully signal the specific circuit initially formed by the highly localized climbing fiber projections to the cerebellum activated by the corneal air puff US. In sum, weak and ineffective anatomical connections become powerful and effective through learning. Note, however, that the connections do exist before training. This may be a general principle in all aspects of learning and memory.

**Fear Conditioning Is a Model System for Investigating the Neural Substrates of Emotional Memory**

Significant progress has been made in identifying the neural substrates of emotional memory processing, and many of the advances have relied on studies using fear conditioning in animals as a model system. Classical fear conditioning, also known as aversive classical conditioning or Pavlovian defensive conditioning, consists of repeated temporal pairings of an affectively neutral stimulus (CS) with an aversive event (US; see Fig. 55.16). The US elicits a multitude of physiological and behavioral responses (URs), and over several conditioning trials, conditioned responses (CRs) develop in response to the CS itself. In a typical experiment, a rat is presented with a tone followed by a brief electric shock to the foot. After several tone–shock pairings, the tone acquires aversive properties and begins to elicit a set of responses (the CRs) characteristic of a state of fear: freezing; autonomic responses, such as changes in skin conductance, heart rate, blood pressure, or pupillary dilation; and endocrine responses, such as conditioned hormone release. The CRs thus form a set of observable indices that can be used to gauge emotional learning and memory as the organism acquires the association between the CS and the US. This emotional stimulus learning becomes extinguished if the subject receives subsequent CS-alone presentations, as evidenced by a decrease in the number of CRs produced (Fig. 55.16).

**Conditioned Fear Involves Specific Neural Circuits**

The role of the amygdala. Across various paradigms, species, and response measures, the amygdala has consistently emerged as a brain structure essential to the acquisition and expression of conditioned fear. Pretreatment lesions of the amygdala prevent the development of a CR, whereas posttraining lesions of the amygdala disrupt the expression of a CR that has already been learned, even after extensive over-

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**FIGURE 55.16.** A hypothetical illustration of fear conditioning. (A) Typical paradigm arrangement of stimuli during the acquisition and extinction phases of a simple delay conditioning task. CS, conditioned stimulus; US, unconditioned stimulus. (B) The corresponding acquisition and extinction learning curves as exemplified by conditioned freezing responses. The dependent measure is the percentage of time the animal spends freezing during the CS presentation. Hypothetical data from the first three trials of acquisition (A1–A3) and the first three trials of extinction (E1–E3) are shown. From LeDoux. Training. Single and multiple unit recordings from neurons in the central nucleus of the amygdala reveal changes in neural activity that parallel the emergence of a CR, and responses similar to CRs can be elicited by electrical stimulation of the amygdala.

**Anatomical connections of the amygdala.** In combination with lesion data, neuroanatomical tract-tracing studies have begun to elucidate the afferent and efferent connections of the amygdala to sensory and motor areas involved in transmitting information about the CS and US and generating emotional responses. Most of the work on CS pathways has involved auditory stimuli, although visual CS pathways...
have also been described. Information regarding an auditory CS reaches the amygdala by way of two neural routes: a direct thalamo–amygdala pathway from the auditory thalamus (medial portion of the medial geniculate nucleus and posterior intralaminar nucleus) to the lateral nucleus of the amygdala and an indirect thalamo–cortico–amygdala pathway linking the auditory thalamus with the lateral nucleus of the amygdala by way of connections within the auditory cortex (Fig. 55.17). The direct thalamo–amygdala pathway is more rapid but provides a cruder representation of the incoming sensory stimulus than the indirect thalamo–cortico–amygdala pathway. The direct pathway is thought to function in two ways: first, as a quick route for simple stimulus features to evoke defensive emotional responses; and second, as a method of priming the amygdala to set up appropriate emotional responses to incoming stimuli that are more highly processed by the indirect pathway. Lesion studies have shown that either of these routes in isolation is sufficient to mediate responding in simple conditioning protocols involving one CS. However, the indirect pathway via the cortex appears to be critical for performance on discrimination tasks involving two CSs, where one CS is paired with the US and the other is not. On these tasks, more complex analysis of the
Evidence shows that lesions of the medial prefrontal cortex in rats selectively interfere with the extinction of conditioned fear responses. If extended to human populations, this finding is potentially important for clinical applications in which the suppression of acquired fear responses is impaired. Other brain areas appear to selectively influence the acquisition of some CS-US associations but not others. For example, lesions of the cerebellar vermis attenuate acquisition of conditioned bradykinesia, but the cerebellum does not appear to have an effect on fear conditioning. This contrasts with the essential role of cerebellar structures in eyeblink conditioning.

**Mechanisms of Fear Conditioning**

One way to observe changes in CS processing at the cellular level is through changes in the receptive field properties of neurons encoding sensory information. Electrophysiological recordings of single neurons in the medial division of the medial geniculate nucleus of the thalamus, in the auditory cortex, and in the lateral amygdala show receptive field plasticity during fear conditioning, that is, changes in the properties of neurons in the receptive field.[1] Because the cells in the medial division of the medial geniculate are very broadly tuned, it is likely that the changes observed in the auditory cortex and lateral amygdala are simply consequences of the thalamic source—rather, there appears to be active neural tuning at each of these stations. Moreover, the plasticity seen in different regions may reflect different adaptive functions: plasticity in the medial geniculate may allow very rapid evaluation of changes in CS significance; plasticity in the auditory cortex may provide more detailed fine-tuning in the auditory frequency domain; and plasticity in the lateral amygdala may serve as a substrate for central CS-US associative learning. This evidence for receptive field plasticity during fear conditioning is another example of experience-dependent changes observed in sensory areas of the adult brain during learning and memory tasks.

**Human Fear and Anxiety**

The role of the amygdala Fear conditioning has been readily demonstrated in human subjects, and many experimental preparations for measuring fear in animals can be adapted for use with human populations. Fear conditioning in brain-damaged human subjects, however, has not been well studied, although patients with amygdala damage show deficits in fear-conditioning tasks. Selective amygdala damage in humans also leads to deficits in the recognition of facial expressions of fear. Furthermore, in patients with...
undergoing surgical treatment, electrical stimulation of the amygdala typically evokes feelings of fear and anxiety. Thus, in humans as well as other species, the amygdala appears to be centrally involved in regulating mechanisms of fear.

Human anxiety disorders The marked similarities in the clinical symptomatology of anxiety in humans and measures of conditioned fear in animals have led researchers to propose using fear conditioning as a model for studying human anxiety disorders, such as posttraumatic stress disorder, phobia, and panic. One clinical marker for posttraumatic stress disorder is an increase in startles. Conditioning studies have shown enhanced startle responses in this population in comparison to controls.

In addition, fear conditioning using phobic stimuli generally shows greater resistance to extinction than fear conditioning using nonphobic stimuli, although some acquisition effects have also been reported. Behavioral therapies based on classical conditioning procedures have been relatively successful in the treatment of patients with phobic disorders. These therapies have evolved to incorporate contemporary theories of conditioning, instrumental learning, and cognition.

Summary Fear conditioning has become extremely useful as a model for understanding the neural basis of emotional learning and memory. Significant progress has been made in revealing the anatomical structures involved in fear conditioning, and studies have demonstrated a critical role for the amygdala in the acquisition and expression of conditioned fear. Other structures, such as the medial prefrontal cortex and the hippocampus, appear to contribute to other aspects of emotional memory, such as learning more intricate emotional associations among stimuli and extinction. Physiological research has revealed corresponding cellular plasticity in the sensory cortex, thalamus, and amygdala during the acquisition of fear conditioning. The neural structures regulating conditioned fear associations may also play a role in other forms of emotional learning, such as instrumental learning or retard. Furthermore, research on fear conditioning in animals has been extended to study anxiety in human popula-
LONG-TERM POTENTIATION

Since the early 1970s we have witnessed stunning progress in understanding how the nervous system encodes and retrieves information. At the cellular level, the usual assumption is that the encoding process entails activity-dependent changes in the strength of synaptic connections among neurons. An extensively studied candidate mechanism is the synaptic phenomenon called long-term potentiation, a persistent increase in synaptic strength (as measured by the amplitude of the EPSP) that can be rapidly induced by brief neural activity.

The intense current experimental interest in LTP is driven by the working hypothesis that this form of synaptic plasticity may participate in the information encoding and/or retrieval process in several brain regions. In this section, we will describe the properties of LTP and how it is studied, review its underlying mechanisms, and conclude with remarks about linkages between LTP and behavior.

Long-Term Potentiation Occurs in a Variety of Neural Synapses

Since the start of the 20th century, scientists from numerous disciplines have hypothesized that learning and memory could be encoded via activity-dependent changes in the strength of the synaptic connections between neurons; yet the evidence that mammalian synapses could undergo an appropriate type of modification began to appear only in the last quarter of the century. The first interesting data appeared in 1975, when Timothy Bliss and Terje Lomo demonstrated LTP in the anaesthetized rabbit. In this classic paper, Bliss and Lomo reported that brief, high-frequency stimulation of the perforant pathway input to the dentate gyrus produced a long-lasting enhancement of the extracellularly recorded field potential. Studies of non-anesthetized animals showed that LTP can last for weeks or months.

Since this discovery, countless LTP studies have been done in numerous laboratories throughout the world. These subsequent studies have been done in vitro and in vivo on many different synapses and using a variety of recording methods. Originally thought to be unique to the mammalian hippocampal formation, LTP is now known to occur in the mammalian peripheral nervous system, the arthropod neuromuscular junction and other invertebrate synapses, neocortical regions of mammals, and subcortical mammalian nuclei, such as the amygdala.

Brain Slices Are the Preferred Substrate for in Vivo Studies of Long-Term Potentiation

During the 1980s, a general appreciation of the numerous experimental advantages of brain slices for studies of synaptic physiology developed. Partly for this reason, most of the research that has been done on LTP thus far has used acute brain slices that remain viable for several hours. The hippocampal brain slice proved to be particularly useful in this respect because much of the intrinsic circuitry, along with the major cell classes, remains intact in a transverse slice. A schematic illustration of a rat hippocampal slice preparation is shown in Fig. 55.19. It is convenient to use as many as two or three stimulating electrodes plus one or two recording electrodes.

Within the hippocampus proper, by far the most commonly studied synapse is the Schaffer collateral/commissural (b/c/comm) input to pyramidal neurons of the CA1 region (Fig. 55.19). In fact, this is probably the most commonly studied synapse in the mammalian brain. Part of this interest is because the circuitry is relatively simple and its tansmitter organization makes it possible to extract useful data from extracellular recordings, which are easier to perform than intracellular recordings and preferable for some purposes. A much smaller number of laboratories have also been interested in the mossy-fiber input from the granule cells of the dentate gyrus to the pyramidal neurons of the CA3 region (Fig. 55.19).

The first study of LTP done under voltage-clamp conditions used microelectrodes in concert with a "switch clamp" to study the mossy-fiber synaptic input to CA3 in transverse rat hippocampal slices. More recent work on LTP in these synapses used "whole-cell recordings," with noise levels sufficiently low that unitary synaptic currents can be analyzed.

Examples of unitary mossy-fiber excitatory postsynaptic currents (EPSCs) recorded in rat brain slices before and after LTP induction are illustrated in Fig. 55.20. The upper waveforms are superimposed EPSCs before (Fig. 55.20A) and after (Fig. 55.20B) LTP induction, and the corresponding lower waveforms are their averages. LTP is indicated by the fact that the average responses to both the first and the second pair of stimulations were greater after LTP induction (Fig. 55.20B, bottom traces) than before (Fig. 55.20A, bottom traces). LTP was induced by stimulating the mossy-fiber syn-
apses with a short train of stimuli (5–10) at 100 Hz. In rat brain slices, mossy-fiber LTP can last for an hour or more, showing little if any decrement. Because brain slices have a limited life span, to study the full time course of hippocampal LTP typically requires in vivo studies. Of course, one gives up some degree of experimental control in such studies, and the measurements are typically limited to extracellular field-potential recordings.

The “Classical Properties” of Long-Term Potentiation Include Cooperativity, Associativity, and Input Specificity

Certain synapses of the hippocampal formation can exhibit a form of LTP characterized by properties that have been variously termed “cooperativity,” “associativity,” “input specificity,” and “spatiotemporal specificity.” As we shall see, these “classical properties” are not orthogonal but rather are different manifestations of the same underlying mechanism that is responsible for one type of LTP. This form of LTP is most commonly studied in the Sch/com synaptic input to pyramidal neurons of hippocampal region CA1. Other types of LTP, which are less commonly studied, have different signatures.

Cooperativity refers to the fact that the probability of inducing LTP, or the magnitude of the resulting change, increases with the number of stimulated afferents. The latter can be varied by changing the intensity of extracellular stimulation. Smaller or briefer currents (weak stimulation) activate fewer afferents than larger or longer currents (strong stimulation). The finding is that weak high-frequency stimulation often dist
not induce LTP, whereas strong stimulation at the same frequency and for the same duration produced LTP more reliably. This finding was termed cooperativity because it was thought that more axons were recruited with higher stimulation intensities, thus "cooperating" to trigger LTP.

Associativity was shown in preparations in which two distinct afferent inputs converged onto the same postsynaptic target. Levy and Steward explored this in vitro using the perforant-pathway inputs to the dentate gyrus, and Bartos and Brown explored it in vivo using Sch/Com inputs to hippocampal region CA1. The experiments were designed to examine interactions between weak (W, small number of stimulated afferents) and strong (S, large number of stimulated afferents) inputs (Figs. 55.19). Tetanic (high-frequency) stimulation of the W input by itself failed to produce LTP in that pathway unless this stimulation was paired with tetanic stimulation of the S input. Thus, LTP was induced in a W input only when its activity was associated with activity in the S input.

Input specificity means that LTP is restricted to only the inputs that received the tetanic stimulation. The spatiotemporal specificity of associative LTP induction was examined in detail in the CA1 region by using two sets of W inputs (W1 and W2) activated by two separate stimulating electrodes. Both W1 and W2 received the same tetanic stimulations, but only one of them was stimulated at the same time in the S input. Tetanic stimulation of the other input did not overlap temporally with tetanic stimulation of the S input. The finding was that LTP was induced only in the W input that was stimulated at the same time as the S input. The high degree of spatial specificity was emphasized by the fact that these W inputs were both Sch/Com synapses that were anatomically intertwined on the apical dendrites of the CA1 pyramidal neurons. Temporal specificity was evident from the fact that LTP was not induced in the W input if the tetanic stimulation terminated a fraction of a second before the onset of the S stimulation.

A Hebbian Mechanism Explains the Classical Properties of Long-Term Potentiation in the Hippocampus

How can one explain these classical properties of LTP in the hippocampal formation? In the late 1940s, the Canadian psychologist Donald Hebb advanced an idea regarding the conditions that cause synapses to change. His thinking proved to be influential and informed later experiments that probed the mechanisms behind LTP. According to Hebb's now-famous postulate:

When an axon of cell A is near enough to excite cell B repetitively or consistently takes place in firing it, some growth process or metabolic change takes place in one or both cells such that A is more effective, as one of the cells firing B, is increased. (Hebb, p.62)
In short, coincident activity in two, synaptically coupled neurons would cause increases in the synaptic strength between them. Hebb’s postulate could be thought of as the synthesis of William James’s “law of neural habit” and the synaptic hypothesis for memory. There are numerous modern interpretations of Hebb’s postulate, but most of them are captured by the mnemonic: “Cells that fire together, wire together.”

Could the classical properties of LTP all be consequences of synapses that obey a Hebbian rule? This could be true if a critical amount of postsynaptic depolarization was a necessary condition for inducing LTP in active synapses. According to this possibility, cooperativity would result when enough input fibers were stimulated to produce the critical amount of postsynaptic depolarization; associativity would emerge from the fact that the strong input(s) caused sufficient depolarization of the postsynaptic membrane during the presynaptic activity in the weak input(s); and the spatiotemporal specificity would occur because LTP was induced only in those W inputs to a neuron that were active at the same time that the cell was sufficiently depolarized by the S input to that neuron. In other words, these classical phenomena could all be manifestations of a single underlying Hebbian mechanism. It was natural, therefore, to ask whether Sch/comp synapses were in fact Hebbian.

An explicit effort to examine this possibility yielded unequivocal results based on a simple rationale. If the synapses were Hebbian, then it should be possible to induce LTP under experimental conditions in which one substitutes for the usual S input direct depolarization of the postsynaptic neuron via the recording microelectrode (see Fig. 55.21). On the other hand, if the synapses were non-Hebbian, and the critical role of the S input were instead, for example, to release an “LTP factor,” then pairing presynaptic stimulation of a W input with direct depolarization of the postsynaptic cell should fail to induce LTP.

Results of the first of a series of current- and voltage-clamp experiments, done to distinguish between these hypotheses, are shown in Fig. 55.21, which plots the mean amplitude of the excitatory postsynaptic potentials evoked in CA1 pyramidal neurons as a function of time and the various experimental manipulations. The EPSPs were tested every 12 s by delivering a single extracellular stimulation to the Sch/comp afferent inputs (Fig. 55.19). After a stable EPSP baseline was established, the postsynaptic cell was depolarized alone (in the absence of presynaptic tetanic stimulation) to demonstrate that this manipulation by itself was without effect (Fig. 55.21, Depol. alone). Next, a tetanic stimulation (100 Hz) was given to the W input while the postsynaptic soma was maintained at —80 mV under voltage-clamp conditions (Fig. 55.21, 100 Hz × voltageclamp). As indicated, this was sometimes done twice, with the same results—no LTP induction. Then the same presynaptic tetanic stimulation was given while the postsynaptic cell was depolarized under current-clamp conditions (Fig. 55.21, 100 Hz × depol.). In agreement with the Hebbian hypothesis, this manipulation did in fact consistently result in LTP induction. From these studies it is clear that Hebb-type synapses do exist in the hippocampus, a noncontroversial result that has been replicated in many laboratories around the world. Hebbian synapses were later found to exist also in the neocortex, and even in Aplysia, suggesting that this mechanism may have developed early in evolution and been conserved across phyla. However, we hasten to point out that not all forms of LTP are Hebbian (see Fig. 55.22), which means that the classical properties are not universal.

Mechanisms of Long-Term Potentiation
Must Account for Induction, Expression, and Maintenance

Even though a tremendous amount of research has been devoted to LTP, it remains a challenge to explain how this process occurs physiologically. Partially for pedagogical reasons, it is convenient to divide the discussion into three parts: induction, expression, and maintenance of LTP. Induction refers to the initial events.
that trigger or initiate the modification process; expression concerns the proximal cause of the final synaptic enhancement, and maintenance addresses the manner in which the enhancement is made to endure over time.

LTP Induction

With regard to LTP induction, we shall see that there are probably multiple mechanisms or at least multiple pathways that can lead to persistent synaptic enhancement. Multiple pathways may also contribute to expression and maintenance.

Glutamate receptors

To understand LTP induction we need to consider the neurotransmitter L-glutamic acid (glutamate) and the receptors that it activates. Most of the systems in which LTP has been
studied use glutamate as the neurotransmitter, although there are notable exceptions. In very broad terms, glutamate receptors (Glur) can be subdivided into ligand-gated ion channels and metabotropic receptors (mGlur) that are linked via a G protein to phospholi- pase C (PLC) activation and adenylyl cyclase inhibition. The ionotropic receptors in turn can be divided further into two subpopulations: those that respond optimally to N-methyl-D-aspartate versus those that respond to kainic acid (KA) or α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA).

Here, we distinguish between the NMDAR receptor (NMDAR) and the AMPA receptor (AMPAR), and we use these terms to refer to both the ligand-binding sites and the ionconducting channel. In interpreting some of the studies of LTP induction, it is a good idea to bear in mind that within the broad classification of Glur, there are further subtypes, and their pharmacology is more complex and uncertain than can be discussed here. (See Chapter 10 for additional details.)

**Calcium ions and LTP.** There is general agreement that some aspect of LTP induction depends on the intracellular concentration of calcium ions ([Ca++] in some key compartment of the pre- and/or postsynaptic cells. The exact role of calcium in the induction process depends on the particular form of LTP and the synaptic system. In the CA1 region of the hippocampus, LTP induction in the Sch synaptic receptors that input seems to depend critically on the postsynaptic Ca2+.

The current view is that many different routes modulate or control Ca2+ in the critical subcellular compartment(s) (Fig. 55.23). Three routes that have been studied extensively may be implicated in some aspect of LTP induction: calcium influx through ionotropic Glur, especially the NMDAR; calcium influx through voltage-gated calcium channels (VCCs); and calcium release from intracellular stores. We will elaborate on these three routes and their possible actions.

**NMDAR-dependent LTP.** Record that the classical form of LTP has properties that can be explained in terms of a Hebbian mechanism. For this form of LTP, considerable evidence shows a role for the NMDAR. Numerous pharmacological studies have shown that competitive antagonists of the NMDA receptor site, such as kynurenic acid, d-APV, or MK-801, block NMDA-dependent LTP. Noncompetitive antagonists such as 1,1-dihydro-5H-benzene[de]cyclohepten-5,10-imine (MK-801) can prevent the induction of one type of LTP.

The NMDAR has two properties that immediately suggest the potential for its role in LTP induction at Hebbian synapses. First, NMDARs are permeable to Ca2+ (in addition to Na+ and K+). This is significant because postsynaptic Ca2+ plays a critical role in inducing this form of LTP. Second, the channel permeability is a function of both pre- and postsynaptic factors. Channel opening requires the neurotransmitter glutamate (or some related agonist) to bind to the NMDA site. This in turn requires presynaptic activity for glutamate release. At the usual resting membrane potential, the ionic channels are normally blocked by magnesium ions (Mg2+), but this channel block is relieved by sufficient depolarization of the postsynaptic membrane containing the NMDAR. Thus, the NMDAR-mediated conductance is voltage dependent, allowing Ca2+ entry only when presynaptic release is combined with postsynaptic depolarization.

These interesting gating properties of the NMDAR, combined with a number of other assumptions and facts, easily give rise to quantitative models that can account for much of what is known about this form of LTP. Before proceeding further, we should distinguish between the properties of the NMDAR and those of the AMPAR. The AMPAR does not exhibit voltage-dependent Mg2+ block and has a relatively lower Ca2+ permeability. The AMPAR-mediated conductance is essentially voltage independent. Released glutamate can potentially act on both the AMPARs and the NMDARs associated with the membrane on the dendritic spine (Fig. 55.23). With this knowledge, it is easy to envision a possible role for the NMDAR in a Hebbian modification. Nearly concurrent presynaptic activity (producing glutamate release and binding) and postsynaptic activity (relieving the Mg2+ block) allow Ca2+ influx into the dendritic spine of the postsynaptic neuron. The increased Ca2+ in some critical region of the dendritic spine, presumably very close to the NMDAR, is thought to activate Ca2+-dependent enzymes, such as calmodulin-dependent kinase II (CAM kinase II), that are thought to play a key role in LTP induction (Fig. 55.23).

In qualitative terms, we can easily see how these molecular events could help account for the properties of cooperativity, associativity, and spatiotemporal specificity. Active synapses release glutamate which can bind to the NMDAR, causing Ca2+ influx into dendritic spines on the postsynaptic cell. This Ca2+ influx acts locally, resulting in input-specific LTP. However, the Ca2+ influx occurs only when the synaptic input is strong enough to depolarize the postsynaptic membrane sufficiently to relieve the Mg2+ block, giving rise...
to cooperativity (see Fig. 55.22). The depolarization itself is mediated in large part by the (voltage-independent) AMPARs, which are also co-localized on the dendritic spine (Fig. 55.23). Note that activity in a W input by itself would not depolarize the postsynaptic cell sufficiently to relieve the Mg²⁺ block unless this activity were properly timed in relationship to activity in an S input to the same cell. The combined depolarization of the two inputs gives rise to cooperativity and its spatio-temporal specificity (Fig. 55.22).

Efforts to create formal models of this process quickly led to the realization that the properties of the NMDAR alone were not sufficient to account for the classical properties of LTP. Characteristics of the dendritic spine itself seem to play a key role. The spine is presumed to compartmentalize and amplify the second messenger signal (∆Ca²⁺); and to perform a number of other functions, including binding and pumping Ca²⁺, that enable this mechanism to work (Fig. 55.23). Amplification of ∆[Ca²⁺] results from the small volume of the spine head coupled with restricted diffusion along the spine neck. Similarly, the restricted diffusion, resulting from the spine geometry, combined with Ca²⁺ binding, help to compartmentalize the largest transient ∆[Ca²⁺] to the head of the dendritic spine, where the relevant Ca²⁺-dependent enzymes are presumed to be located (Fig. 55.23).

Computer simulations were used to explore the role
of "back-propagating" action potentials—spikes initiated in the soma that actively propagate antidromically into the dendrites. The principal finding was that "anti-
dromic" Nm-spikes had little effect unless they in turn activated VGCCs, in which case they had a pronounced effect. In certain cells, we now know that there are back-propagating spikes, that they do activate voltage-
gated calcium channels, and that they may indeed participate in LTP induction.17

NMDAR-independent LTP

Most of the preceding accounts of the classical (Hebbian, NMDAR-depen-
dent) form of LTP are widely accepted and are as-
sumed to apply quite generally. Nevertheless, this ex-
planation may be applicable only to certain synapses
under some conditions, and even then it may be only
one piece of the story.18-23 In many synapses, LTP
induction does not appear to require the NMDA recep-
tor.14,15 Even within the hippocampus, some synapses
exhibit NMDAR-independent forms of LTP. The usual
case is the mossy fiber synaptic input to CA3 pyr-
oidal cells.21

The Sch/rom inputs to CA1 pyramidal neurons,
which are known to exhibit the classical Hebbian form
of LTP that relies on the NMDAR, can be shown, under
the appropriate conditions, to exhibit an NMDAR-in-
dependent type of LTP. Grover and Tevier24 demon-
strated that LTP can in fact be induced in these syn-
apses in the presence of the competitive antagonist
APV. They were careful to evaluate the possibility
that high-frequency tetanic release of glutamate might com-
petitively unlock the APV by using a relatively high
concentration of m-APV (200 mM) to saturate the APV-
binding site.25 The onset of NMDAR-independent LTP
was relatively slow (20-30 min), it exhibited input
specificity, and it was prevented by nifedipine, an L-
type Ca2+ channel blocker. Thus, they distinguish be-
tween "NMDA LTP" and "VGCC LTP."24

Using a combination of electrophysiological and
(Ca2+) imaging methods, Magee and Johnston26 fur-
ther examined NMDAR-independent LTP in the Sch/rom
synaptic input to CA1. They found that back prop-
agating dendritic spikes paired with stimulation of a
weak Sch/rom synaptic input to CA1 neurons pro-
duced LTP in the presence of 100 mM m-APV. Not
surprisingly, stimulation of the weak synaptic input
by itself failed to induce LTP in the presence of APV.
The back propagating spikes produced a strong Ca2+
signal in the dendrites, and, interestingly, the LTP pro-
duced by pairing was blocked by the Ca2+ channel
blockers nifedipine and Ni2+. NMDAR-independent
LTP is not restricted to the CA1 region of the hippocam-
pus.22 Other work has suggested that VGCCs are likely
to be responsible for certain types of LTP in the CA3
region of the hippocampus and in the visual cortex.

A cautious perspective is to recognize the possibility
that both NMDAR-dependent and NMDAR-indepen-
dent forms of LTP may co-occur in the same brain
region, among different classes of synaptic inputs onto
the same postsynaptic neuron, and even among the
same class of synaptic inputs to the same postsynaptic
neuron (Fig. 35.23). Given the possibilities, it is easy
to see that application of NMDAR antagonists in be-
havioral studies should not be expected to block all
forms of LTP.

LTP switch and mGluR

Another of the more re-
cently investigated LTP induction mechanisms in-
volves the "metabotropic" glutamate receptor
(mGluR).27 Like the NMDAR, this complex is found
on the postsynaptic cell, but unlike the NMDAR, it is
also found presynaptically. Not surprisingly, there are
a variety of mGluR receptors. Class I mGluR subtypes
(mGluR1 and mGluR5) are coupled to phosphatidylinositol
(PI), which enzymatically breaks down membrane
phospholipids to form diacylglycerol and inositol
1,4,5-trisphosphate (IP3) (see Fig. 35.23). DAG modu-
lates channel activity through protein kinase C (PKC),
which enzymatically breaks down membrane
phospholipids to form diacylglycerol and inositol
1,4,5-trisphosphate (IP3) (see Fig. 35.23).

The role of the mGluR has been studied most exten-
sively in the Sch/rom input to hippocampal region
CA1. Collingridge and co-workers reported28 that ap-
plication of the mGluR antagonist (+)-4-carboxymethyl-
leucine (CMEP) blocked LTP induction in synapses that
had not previously received high-
frequency stimulation (HFS) but did not prevent the
induction of additional LTP at synapses that had prior
exposure to HFS. A creative interpretation of these
and other experiments is that the mGluR acts as a
"molecular switch" that must be activated as a prereq-
uisite to LTP induction.29 This interesting idea has gen-
erated further experiments in other laboratories, but
thus far a consistent pattern has not emerged.30

Clearly, we need to know more about the factors that
affect the spatial distribution and relative proportions
of the various mGluR subtypes and their ultimate roles
in neuronal function.

VII. BEHAVIOR AND LEARNING NEUROCHEMISTRY
Long-term depression The mechanisms responsible for inducing LTP also may play a role in triggering another synaptic phenomenon, called long-term depression. First seen in the hippocampus and subsequently in many other brain regions, LTD is thought by many to be the mechanism by which learning is encoded in the cerebral cortex. As well as a process whereby LTD could be reversed in the hippocampus and neocortex. In the latter brain regions, brief, high-frequency stimulation (e.g., a trains of 10 shocks at 100 Hz) can induce classical LTD, whereas low-frequency stimulation (LFS) over longer periods (1 Hz for 10 min) can induce LTD. Some forms of LTD appear to be mediated by the NMDAR, and these forms of LTD seem to result from dephosphorylation (removal of LTP). In addition, there appear to be NMDAR-independent forms of LTD. One example of the latter comes from studies of the cerebral cortex. Reports on the layer 1/2 input to layer V neurons suggest that LTD and LTD are separate and superimposed processes and that LTD induction is not mediated by activation of NMDA receptors. Another example of NMDAR-independent LTD is found in the parallel fiber input to Purkinje cells in the cerebellum. From our discussion so far, it should be clear that there may be many forms of LTD and LTD and many pathways by which they operate. Furthermore, these forms may vary in different brain regions and sometimes among different inputs to the same brain region. Even within the CA1 region, the Sch/cortex input may exhibit both NMDAR-dependent and NMDAR-independent forms of LTD and the latter could be mediated partly by Ca²⁺ entry through VGCCs.

Bidirectional control by calcium ions With regard to NMDAR-dependent LTD, one interesting point on which there has been general agreement in studies of the Sch/cortex input to CA1 is that both LTD and LTD in CA1 appear to be Ca²⁺–dependent processes that can be blocked by injecting Ca²⁺ chelators into the postsynaptic cell. If both LTD and LTD are triggered by Ca²⁺ entry, then how are their induction processes different? The presumption is that more Ca²⁺ influx occurs during an LTD-inducing HFS than during an LTD-inducing LFS. One formal molecular model incorporates this Ca²⁺–dependent, bidirectional control of synaptic strength. In this model, high [Ca²⁺] (> 5 mM) activates a protein kinase that phosphorylates a protein that leads to LTD induction, whereas low [Ca²⁺] (< 5 mM) activates a protein phosphatase that dephosphorylates this protein and causes LTD. The synaptic strength thus depends on which of these competing processes is most active, which in turn will be a function of the pattern of activity experienced by the cell (Fig. 55.23). These ideas have led to testable biochemical predictions.

LTP Expression Up to this point we have emphasized evidence related to events in the causal chain that triggers the modification process. Another question follows naturally: What physical changes incorporate this modification once it has been triggered? This question addresses the process of LTP expression. A variety of hypotheses have been proposed to account for the synaptic enhancement associated with LTP expression. Since many of these models have not been conclusively evaluated yet, controversy still surrounds the details of LTP expression. In what follows, we will overview some of the possible expression mechanisms to reveal the logic behind this subfield of LTP research.

Most of the ideas about enhanced synaptic transmission concern either increased postsynaptic transmitter release or increased receptor affinity to released transmitter. The former entails presynaptic changes, the latter, postsynaptic. Although some of the induction mechanisms discussed previously implicated a postsynaptic increase in [Ca²⁺], this does not necessarily imply that expression also must be postsynaptic. There is ample evidence for ongoing two-way communication across the synaptic cleft, so that a postsynaptic trigger could in principle give rise to a pre- and/or postsynaptic modification. There are a large number of seemingly conflicting accounts in the literature regarding the nature of the changes responsible for the observed increase in synaptic efficacy following LTD induction.

Experimentally, the increase in synaptic efficacy that is characteristic of LTD was shown to be the result of an increase in the measured synaptic conductance (ΔG), and not a decrease in inhibition, a lowering of the spike threshold, or an increase in input resistance (see Fig. 55.24). This enhanced synaptic conductance could reflect an increase in either of two quantal parameters—m and g (see Chapter 7). The first parameter m is the mean number of quantal packages of neurotransmitter released per presynaptic action potential. The second parameter g is the average postsynaptic response produced by the release of each quantal package of neurotransmitter. The mean response amplitude is just the product of m and g.

Classical methods of quantal analysis have been used in an attempt to determine whether LTP results from an increase in m or g or both. In certain simple systems, like the crayfish neuromuscular junction, this analysis can be applied with some confidence. In this system, LTP seems to be due to an increase in the quantal parameter m, and there is no change in g.
Application of the methods of quantal analysis to an understanding of LTP in mammalian central synapses has been less successful. Different groups have often obtained seemingly contradictory results. In the mossy-fiber-synaptic input to pyramidal neurons in hippocampal region CA3, the results suggest an increase in n, but other synapses of the hippocampus need to be considered separately.

In a simple binomial model of transmitter release, \( m \) is the product of two other parameters—\( n \) and \( p \) (Fig. 55.24). The parameter \( n \) can be conceptualized as the number of eligible release sites. Even in the simplest binomial model, an increase in \( n \) can reflect any number of causes, two of which are indicated in Fig. 55.24. The first assumes that there are preexisting but "silent" release sites. They could be silent for several reasons—a failure of the neurotransmitter-containing vesicles to dock appropriately at the release site, an absence of functioning postsynaptic receptors opposite the release site, or an absence of functioning VGCCs immediately adjacent to the release site—but the point is that there could be a widening of existing sites (Fig. 55.24, USES). Alternatively, LTP could result from the formation of new sites (Fig. 55.24, FONS), which could even entail sprouting totally new synaptic boutons.

The binomial parameter \( p \) is the mean probability for each of the \( n \) active release sites of discharging a quantum when a presynaptic nerve impulse occurs. An increase in \( p \) could reflect any of a large number of causes, two of which are indicated in Fig. 55.24. First, a number of potentially modifiable causes are involved in coupling the neurotransmitter secretion to the arrival of an action potential in the nerve terminal. Here, there could be altered coupling of excitation to secretion (Fig. 55.24, ACES). Alternatively, there might be no alteration in excitation—secretion coupling for a given action potential, but instead, as in APPh, the action potential in the nerve terminal itself could change. Thus, the obvious alternative possibility entails greater excitation of the nerve terminal (Fig. 55.24, GENT).

Similarly, in the case of the binomial parameter \( n \) (the postsynaptic response), we can devise a large number of possible mechanisms for increasing its value. These include a decrease in the axial resistance of the dendritic spines (\( R_s \)), an increase in the probability that a released molecule of neurotransmitter will bind to a postsynaptic receptor (\( P_b \)), an increase in the number of neurotransmitter molecules in each vesicular packet (true indicated), and several others (see Fig. 55.24). An increase in \( P_b \) could be caused, for example, by an increase in the number of AMPA receptors, a possibility suggested by research in several laboratories. Nonetheless, there is reason to discount changes in spine resistance, based on the relationship of the reciprocal of the spine axial resistance (\( G_s \)) to the peak synaptic conductance (\( ac_{max} \). Theoretical work shows that the role of the spines is negligible if \( G_s \ll ac_{max} \), which may generally be the case. Still, a variety of other hypothesized mechanisms for increasing \( n \) remain possible.

LTP Maintenance

Regardless of the ultimate nature and locus of the modification that gives rise to LTP expression, the more general problem remains of how a synaptic change can endure over long periods of time in the face of constant molecular turnover. This is the problem of LTP maintenance.

Gene expression and protein synthesis

One favorite answer to this problem of how to maintain plastic changes is to assume that it ultimately involves alterations in gene expression. Just as genetic machinery...
can be used to maintain macroscopic aspects of an organism, such as eye color and height, the genome also could be used to maintain microscopic features of an organism. There is a difference, however, between eye color and synaptic plasticity. The former trait is relatively unchanged throughout the lifetime of an organism and is controlled by inherited genetic material. The latter is a rapid change that is hypothesized to be induced by experience.

Interestingly, there is indeed evidence for changes in gene expression after neuronal stimulation. High-frequency electrical stimulation in the rat hippocampus can raise levels of mRNA, which in turn could be used to maintain persistent structural changes. Furthermore, a large body of literature links protein synthesis to learning and memory.19 For example, application of protein synthesis inhibitors interferes with the formation and retention of memories.

This naturally raises the question whether protein synthesis inhibitors interfere with the maintenance of LTP. Several studies have revealed that maintaining long-lasting synaptic plasticity involves many stages, and not all of these stages are dependent on protein synthesis. In an illustrative experiment using hippocampal slices,20 the protein synthesis inhibitor actinomycin D (ACT D) was applied for 2 h, starting just before high-frequency stimulation was delivered. While the synaptic response indicated an enhancement, this increase persisted for only 2 h before steadily declining back to baseline levels. Other slices that did not receive ACT D showed a persistent enhancement lasting over 4 h, and slices that received only ACT D without high-frequency stimulation were unchanged.

These results suggest that synaptic enhancements induced by high-frequency stimulation have at least two stages—an early stage that lasts for about 2 h and is not dependent on protein synthesis, followed by a later stage that is dependent on protein synthesis. Interestingly, slices that received ACT D just 2 h after high-frequency stimulation showed no decline in enhancement, indicating that there is a critical time window during which protein synthesis might be necessary to maintain long-term plasticity. Other work, done in vivo, has pointed to a still later stage of LTP that lasts weeks and that also depends on protein synthesis.21

Similar stages of long-term synaptic plasticity have been identified in Aplysia, Drosophila, and Hermelindia, so that the two-stage process may be a general one present in a range of species.

Immediate-early genes and CREB The learning-related events that turn on gene expression in neurons are uncertain, but a plausible scheme has been put forward by Kendel and colleagues.22 They envision the first step to be Ca2+ binding to calmodulin, which activates adenylate cyclase, thereby promoting synthesis of cAMP. Prolonged elevation of cAMP can in turn trigger the activation of PKA, which may ultimately translocate to the nucleus. Once in the nucleus, PKA is thought to activate CREB (cAMP-responsive element-binding protein), which initiate the transcription of immediate-early genes such as c-fos or c-jun. These immediate-early genes could in turn transcribe late-effector genes whose role it would be to encode proteins underlying, for example, structural changes at the synapse. Protein synthesis is, of course, required for each of the above steps that involve transcription.

Gene expression and synaptic specificity Although this scheme is consistent with the data, it immediately raises the problem of synaptic input specificity. If neural activity ultimately affects gene expression in the nucleus, then the proteins produced in the soma could in principle travel to any synapse within the cell. The problem is to modify only the appropriate synapses. While this problem has not been solved experimentally, conceptually there is no shortage of reasonable possibilities. One solution is for the synapse to produce a local “marker” that makes it especially susceptible to proteins sent from the nucleus.23 To support a more permanent modification might require a self-perpetuating marker and/or an enduring modification in local transport.

Researchers Are Using Genetically Engineered “Knockout” Mice to Investigate the Links between Long-Term Potentiation and Learning Long-term potentiation has the kinds of properties that have long been considered necessary for the encoding and retrieval of information. Hebbian forms of LTP exhibit associativity, which seems like a desirable property, and all forms of LTP appear to be well suited to aspects of rapid learning. One of the more important and difficult remaining challenges entails linking LTP to learning and memory.24 Given what we now know about the molecular mechanisms underlying synaptic transmission and its modification, one can understand why the matter would not be easy to settle simply through pharmacological studies aimed at interfering with some aspect of LTP.

Some of the more promising work in this area has come from studies of genetically engineered knockout mice. The first generation of knockouts prevented the expression of some factor that was thought to be necessary for LTP, such as CAM kinase II. Although these studies were intriguing, they suffered from the fact that the outcomes of a gene knockout could alter
brain development and could not be confined to a specific part of the brain under study. Some of these problems have been overcome in a second generation of knockouts that have temporal as well as spatial specificity. In one example of what will surely prove to be a widely used approach, one group has developed a mutant mouse that lacks one form of the NMDAR only in hippocampal region CA1. Furthermore, this mutation is only expressed after the third postnatal week, thereby reducing influences on early development.

Tests of these mice have revealed that they have LTP deficits in hippocampal region CA1, but not in the dentate gyrus, where the NMDAR was expressed. The behavioral effects of this manipulation also are interesting and include impaired performance on tests of spatial memory, as well as altered firing properties of CA1 pyramidal cells during navigational behaviors. The firing rate of a CA1 pyramidal cell commonly increases when the mouse visits certain spatial locations, which constitute the "place field" for that neuron. Cells coding for overlapping place fields also tend to show correlated firing. In knockout mice, however, the place fields were spatially more diffuse than those seen in the controls, and the firing patterns of neurons from overlapping place fields did not correlate with each other as highly. These results are consistent with the hypothesis that the NMDAR participates in some aspect of the formation of place fields through a Hebbian mechanism, and deficits in spatial memory could result, in part, from antuned and/or improperly associated place fields.

Although this knockout study does not directly connect LTP to learning and memory, it is impressive because it combines evidence and ideas from molecular, cellular, systems, and behavioral levels into a consistent picture. One looks forward to future variants of knockout mice that have inducible molecular lesions that can be triggered at the will of the investigator. This general approach holds tremendous promise for testing hypotheses about the functional role of various forms of LTP in specific brain regions.

Summary

The search for the biological basis of learning and memory has led many of the 20th century's leading neuroscientists to investigate the synapse. As a result of this focus, tremendous effort has been directed toward understanding the cellular and molecular mechanisms behind changes in synaptic efficacy. Our understanding of synaptic plasticity is rapidly evolving and constantly changing. Whereas the N-methyl-d-aspartate receptor was once the pivotal focus of long-term potentiation (LTP) research, it is now clear that voltage-gated calcium channels and metabotropic glutamate receptors as well as other mechanisms should be considered. It also has become evident that high-frequency stimulation is but one end of a spectrum of stimulations that can induce synaptic changes. Lower stimulation frequencies can induce long-term depression (LTD), which may share some common molecular mechanisms with LTP. Finally, several stages in the maintenance of LTP have been identified, and probably more will be discovered. The most daunting remaining challenge is to clarify the varieties of LTD and LTP mechanisms and to demonstrate their functional significance by establishing convincing links to the encoding and retrieval of information and to the development and maturation of the nervous system.

References

General


Cited


VI. Behavioral and Cognitive Neuroscience
generator potentials in identified B photoreceptors in
vironmental light but not short-term in Hermissenda is dependent upon
blocks long-term enhancement of generator potentials pro-
duced by one or twice in continuous conditioning in Hermissenda. Proc.
57. Crow, T., Forester, J., Williams, M. S., Kirshman, M. N., and Neary,
induced enhancement in Hermissenda B photoreceptors. Neuro-
kinase C blocks the intrinsic short- and long-term enhancement produced by one or two trials-conditioning of
dependent increase in protein phosphorylation following one or two-trial enhancement in Hermissenda. J. Neurosci. 16:
1764-1774.
60. Crow, T., Xu-Bian, J. I., Seligson, V., Kang, Y., and Neary, J. T.
(1998). Phosphorylation of reno-onactivated protein kinase by
one-trial and multi-trial classic conditioning. J. Neurosci. 18:
4260-4267.
theory: Relationships between Pavlovian conditioning and trans-
Twenty years of clinical conditioning research with the rabbit.
In Prog. in Psychol. Psychol. (J. M. Sprague and A. N. Epstein,
Physiological Activity of the Central Cortex (G. V. Anrep, trans.).
London.
64. Thompson, R. F., and Kim, J. J. (1966). Memory-systems in the
1336-1344.
wink: Electrical stimulation of cerebellar precursor cortex of the cat.
66. Woody, C. D., Yacovone, P., Owens, J., Black-Cleaves, W., and
37: 954-964.
not affect learning or performance of the eyelid response in
isms of Startle Behavior (R. E. Estes, ed.), pp. 287-313. Plenum,
New York.
substrate of oculobulbar conditioning in the hippocampus. Science.
192: 405-485.
70. Berger, T. W., and Thompson, R. F. (1976). Identification of pyramidal cells as the critical elements in hippocampal neu-
1572-1576.
71. Berger, T. W., Berry, S. D., and Thompson, R. F. (1986). Role of
the hippocampus in classical conditioning of aversive and
appetitive behaviors. In The Hippocampus (R. J. Leibson and
74. Thompson, R. F. (1986). Neural mechanisms of classical condi-
Classical conditioning with temporal life-limited cues in man: im-
campic formations disrupt trace eyeblink conditioning in rabbits.
Behav. Neural. 13: 243-252.
78. Solomon, P. R., Yonel, Shavit, E. R., Thompson, R. F., and
Wisco, D. L. (1986). Hippocampus and trace conditioning of the
rabbit's classically conditioned nictitating membrane response.
Behav. Neural. 110: 729-744.
pus contribution to memory of recently and not remotely
acquired trace eyeblink conditioned responses. Behav. Neural.
119: 195-203.
campus: formation and function in the time-limited trace memory
activity of dentate granule cells during nictitating membrane
83. Maren, S., Tocco, G., Stadig, S., Baudry, M., and Thompson,
R. F. (1993). Paminocarya factors in the expression of long-
term potentiation (E-LP) increased glutamate receptor binding,
2665-2668.
84. Tocco, G., Maren, S., Shaw, T., Baudry, M., and Thompson,
R. F. (1992). Long-term potentiation is associated with increased
in-vivo synaptic binding in the hippocampus. Brain Res. 572: 224-234.
campus-dependent learning facilitated by a monoclonal anti-
30: 25-61.
88. Thompson, R. F. (1986). The neurobiology of learning and
89. Thompson, R. F., and Kocak, D. (1964). Organization of mem-
alian brain substrates of aversive classical conditioning. Annu.
Rev. Psychol. 44: 317-342.
Essential involvement in the classically conditioned eyelid re-
human eyelid conditioning combined with regional cere-
bral glucose metabolism and positron-emission tomography.
94. Steinmetz, J. E., Lavond, D. G., Iverson, D., Logan, G. C., and
Thompson, R. F. (1992). Disruption of classically eyelid condition-
ing after cerebellar lesions: damage to a memory trace system or a simple performance deficit? J. Neurosci.
12: 4403-4420.


