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36

Cellular Mechanisms of Learning and Memory

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Neuronal Changes Associated with Learning Provide Insights into Psychiatric Disorders

Throughout this book we have emphasized that all behavior is a function of the brain and that malfunctions of the brain are expressed in characteristic disturbances of behavior. All functions of the brain, in turn, are the product of interactions between genetic and developmental processes on the one hand, and learning, on the other. In this final chapter we focus on the mechanisms by which learning alters the structure and function of nerve cells and their connections.

Many aspects of behavior result from the ability to learn from experience. Indeed, we are who we are largely because of what we learn and remember. As we have seen in Chapter 33, we are able to communicate experiences and thereby create cultures that are maintained over generations because we learn language. We also learn dysfunctional behaviors and these can, in the extreme, constitute psychological disorders. Fortunately, what is learned can sometimes be unlearned. Thus, insofar as psychotherapy is successful in treating behavioral disorders, it presumably does so because treatment teaches the patient to acquire new patterns of behavior.

In the last chapter we saw that learning is not a single process but has at least two major forms. Implicit forms of learning are covert and often reflexive and do not require conscious attention, while explicit forms do require conscious awareness. In this chapter we first examine the cellular and molecular mechanisms that underlie simple implicit and explicit forms of learning in both invertebrate and vertebrate experimental animals. We then consider how these mechanisms may contribute to individuality through differences in life experience.

Simple Forms of Implicit Learning Lead to Changes in the Effectiveness of Synaptic Transmission

Most of the progress in the cellular study of implicit forms of learning and memory has come from examining elementary modifications of behavior: habituation, sensitization, and classical conditioning. These elementary modifications have been analyzed in the nervous system of invertebrates and in simple vertebrate behavioral systems, such as the eye-blink response (see Chapter 35). Most of these modifications involve changes in the effectiveness of specific synaptic connections.

Habituation Involves Depression of Synaptic Transmission

As we saw in Chapter 35, habituation is the simplest form of implicit learning. It is a nonassociative form in which an animal learns about the properties of a novel stimulus that is harmless, as that stimulus is repeated. An animal first responds to a new stimulus with a series of orienting reflexes. If the stimulus is neither rewarding nor harmful, the animal learns to suppress its response to the stimulus through repeated encounters with it. This learned suppression of response is *habituation*.

Habituation was first investigated in animals by Ivan Pavlov and Charles Sherrington. While studying posture and locomotion, Sherrington observed that certain reflex forms of behavior, such as the withdrawal of the limb in response to a tactile stimulus (a flexion reflex), habituated with repeated stimulation and only recurred after many seconds of rest. He suggested that the habituation was due to a functional decrease in the synaptic effectiveness of the pathways to the motor neurons that had been repeatedly activated.

This problem was later investigated at the cellular level by Alden Spencer and Richard Thompson. They first carried out a series of behavioral experiments and found close parallels between habituation of the spinal flexion reflex in the cat and habituation of more complex behavioral responses in humans. They thus felt confident that habituation of spinal reflexes is a good model for studying habituation. Next, by recording intracellularly from motor neurons in the spinal cord of cats, they found that habituation does not affect the initial synapse in the spinal cord between the sensory neurons innervating the skin and their central target skills. Rather, habituation leads to a decrease in the strength of the synaptic connection at the next synaptic relay in the chain, that between the interneurons and the motor neurons.

Since the organization of the interneurons in the spinal cord of vertebrates is quite complex, it proved difficult to analyze further the cellular mechanisms of habituation in the flexion reflex. As a result, investigation of habituation required still simpler systems in which the behavioral response could be examined in a series of monosynaptic connections.

This sort of analysis has been carried out in the marine snail *Aplysia californica*, which has a simple nervous system containing only about 20,000 central nerve cells. *Aplysia* has a repertory of defensive reflexes for withdrawing its tail, gill, and siphon, a small fleshy spout above the gill used to expel seawater and waste (Figure 36–1). These reflexes are similar to the leg-flexion reflex studied by Spencer and Thompson. For example, a mild tactile stimulus delivered to the siphon elicits withdrawal of both the siphon and gill; a tactile stimulus to the tail elicits tail withdrawal. With repeated stimulation these reflex withdrawals habituate. As we shall see later, these responses can also be sensitized and classically conditioned.

Gill withdrawal has been studied in detail. In response to a newly encountered stimulus to the siphon, sensory neurons innervating the siphon generate excitatory synaptic potentials in the interneurons and motor cells (Figure 36–1). These synaptic potentials summate both temporally and spatially and cause the motor cells to discharge strongly, leading to a strong reflex withdrawal of the gill. If the stimulus is repeatedly presented, the synaptic potentials produced by the sensory neurons in the interneurons and motor cells become progressively smaller. The synaptic potentials produced by some of the excitatory interneurons in the motor neurons also become weaker, with the net result that the strength of the reflex response is reduced.

The decrease in synaptic transmission in the sensory neurons results from a decrease in the amount of the chemical transmitter (glutamate) released

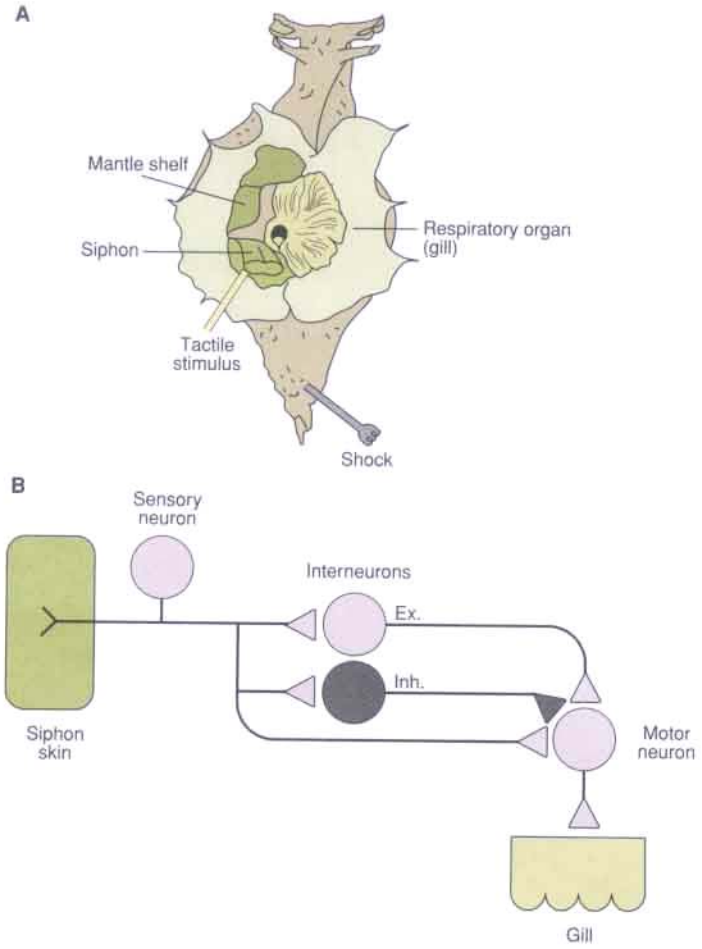


Figure 36–1 The marine snail *Aplysia* has a simple nervous system that makes it an ideal animal model for studying the neural mediation of reflexes at the cellular level. The cellular mechanisms of habituation have been investigated in the animal's gill-withdrawal reflex.

A. A dorsal view of the animal illustrates the respiratory organ, the gill, and the mantle shelf, which ends in a fleshy spout called the siphon.

B. This simplified circuit shows key elements involved in the gill-withdrawal reflex as well as sites involved in habituation. In this circuit about 24 sensory neurons (mechanoreceptors) in the abdominal ganglion innervate the siphon skin. These sensory cells use glutamate as their transmitter and terminate on a cluster of six motor neurons that innervate the gill and on several groups of excitatory (Ex) and inhibitory (Inh) interneurons that synapse on the motor neurons. (For simplicity, only one of each type of neuron is illustrated here.) Repeated stimulation of the siphon leads to a depression of synaptic transmission between the sensory and motor neurons as well as between certain interneurons and the motor cells.

from the presynaptic terminal. How this occurs is not understood. Part of the decrease is thought to be due to a decrease in the ability of transmitter vesicles to be mobilized to the active zone so as to be available for release (see Chapter 15).

This reduction in the effectiveness of the synaptic connections between the sensory neurons and their target cells, the interneurons and motor neu-

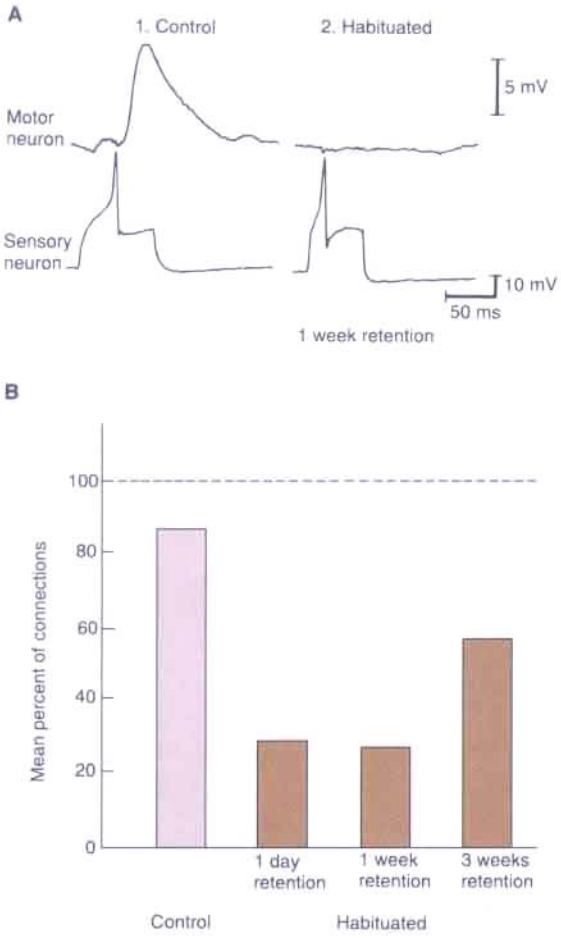


Figure 36–2 Long-term habituation of the gill-withdrawal reflex in *Aplysia* is represented on the cellular level by a dramatic depression of synaptic effectiveness between the sensory and motor neurons. (Adapted from Castellucci, Carew, and Kandel, 1978.)

A. Comparison of the synaptic potentials in a sensory neuron and a motor neuron in a control (untrained) animal and in an animal that has been subjected to long-term habituation. In the habituated animal the synaptic potential in the motor neuron is still undetectable one week after training.

B. The mean percentage of physiologically detectable connections in habituated animals at several points in time after long-term habituation training.

rons, can last many minutes. Similarly, enduring changes occur in the synaptic connections between several interneurons and motor neurons in this circuit. Thus, the storage of even a simple reflexive memory is not restricted to one site but is distributed to several sites within the neural circuit. These distributed *plastic* changes in the functional strength of the synaptic connections represent the short-term memory process for habituation.

Synaptic depression of the connections made by sensory neurons or interneurons, or both, seems to be a fairly common mechanism of habituation. Similarly distributed plastic changes account for habituation of escape responses in crayfish and cockroaches and in the startle reflexes in vertebrates. In each of the instances cellular analyses have shown that memory storage for implicit forms of learning does not depend on specialized "memory neurons" whose only function is to store information. Rather, memory storage results from changes in neurons that are functional components of the normal reflex pathway. It is therefore likely that in the human brain, too, memory can be stored in nerve cells that have a function other than storing information.

What are the limits of this plasticity in neuronal function? How much can the effectiveness of a synapse change and how long can the change last? Can changes in synaptic effectiveness give rise to long-term memory for habituation lasting days, weeks, or years? In *Aplysia* a single training session of 10 stimuli leads to a short-term habituation lasting minutes; four such training sessions spaced over time lead to a long-term memory lasting up to three weeks (Figure 36–2). Whereas 90% of the sensory neurons in control animals make physiologically detectable connections onto an identified motor neuron, in long-term habituated animals the incidence of detectable connections between sensory neurons and motor cells is reduced to 30%. This low incidence persists for one week and does not completely recover until three weeks after habituation training. As we shall see later, this long-term inactivation of synaptic transmission is accompanied by structural changes in the sensory cells.

Not all synapses in *Aplysia* are plastic and adaptable—some synaptic connections in the nervous system do not change their strength, even with repeated activation. However, at synapses involved in learning, such as the connections between the sensory neurons and the motor neurons as well as

at some of the interneuronal connections in the withdrawal reflex, a relatively small amount of training can produce large and enduring changes in synaptic strength.

Sensitization Involves Enhancement of Synaptic Transmission

After an animal encounters a harmful stimulus, it typically learns to respond more vigorously to a variety of *other* stimuli, even harmless ones. In particular, its defensive reflexes become sharpened in preparation for withdrawal and escape. This change, called *sensitization*, is a more complex form of nonassociative learning than habituation. Like habituation, sensitization has both a short-term form lasting minutes and a long-term form lasting days and weeks.

Short-term sensitization has been examined at the cellular level in *Aplysia*. After a single noxious stimulus to the head or tail, a number of different synaptic connections in the neural circuit of the gill-withdrawal reflex become modified, including those of the sensory neurons on the motor neurons and interneurons. Thus, a single set of synaptic connections can participate in at least two different forms of learning: they can be depressed by habituation or enhanced by sensitization.

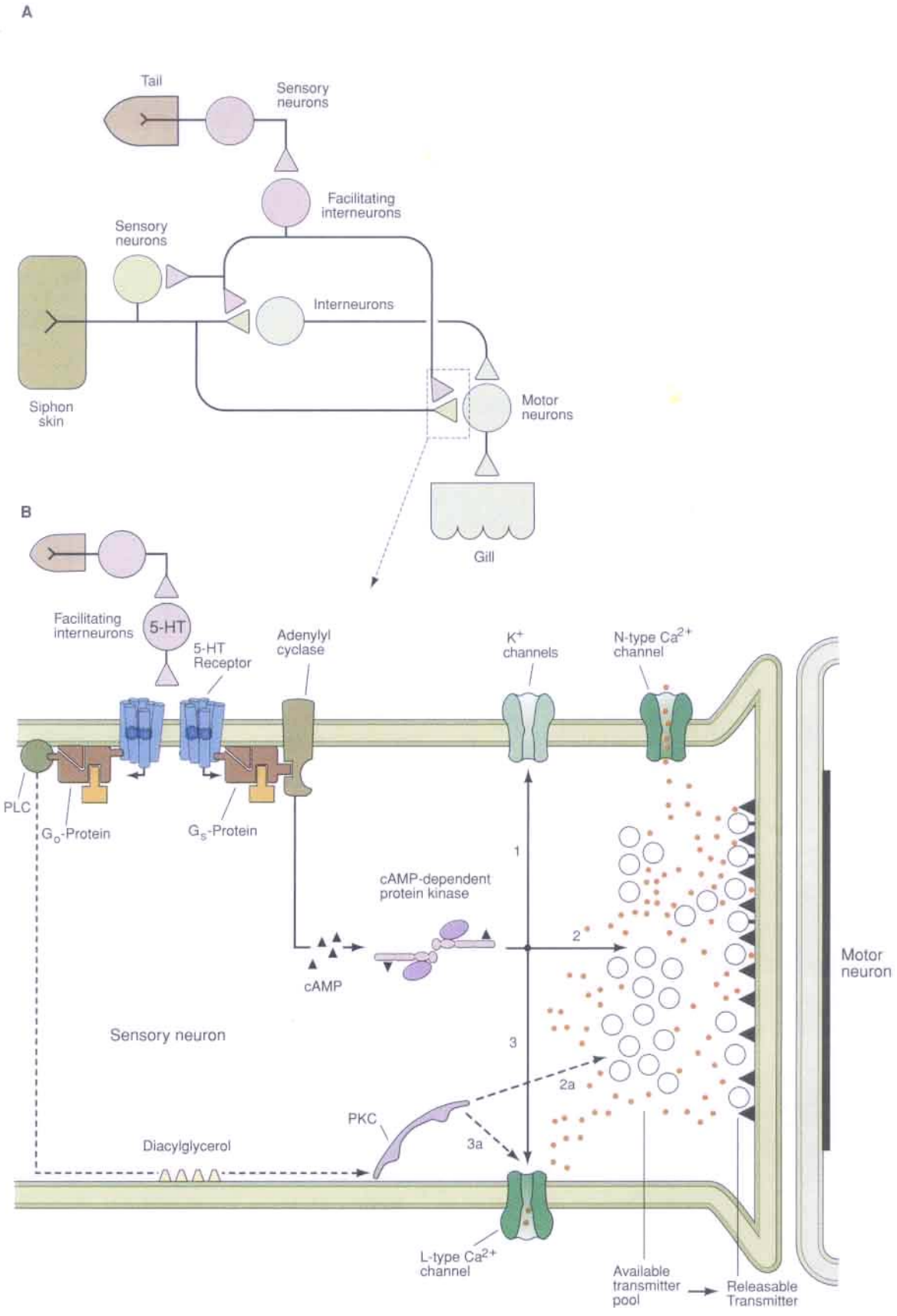
Habituation leads to a *homosynaptic depression*, a decrease in synaptic strength resulting from sustained activity in the stimulated pathway. In contrast, sensitization involves *heterosynaptic facilitation*: the sensitizing stimulus activates a group of interneurons that form synapses on the sensory neurons, including axo-axonal synapses of the sort described in Chapter 15 (Figure 36–3A). These facilitating neurons, some of which are serotonergic, enhance transmitter release from the sensory neurons by increasing the amount of the second messenger cAMP in the sensory neurons.

The sequence of biochemical steps in sensitization of this monosynaptic pathway has been pieced together on the basis of pharmacological and biochemical studies (Figure 36–3B). Serotonin (and the other neurotransmitters released by the facilitating neurons) activate receptors that engage a GTP-binding protein (G_S), which activates the enzyme adenylyl cyclase and increases the concentration of cAMP in the sensory neurons. Cyclic AMP activates the cAMP-dependent protein kinase, which phosphorylates a number of substrate proteins. As discussed in Chapter 14, phosphorylation can lead to an increase or decrease in the activity of a protein by changing its conformation. In sensitization, activation of the cAMP-dependent protein kinase has at least three short-term consequences.

First, the kinase phosphorylates and closes two classes of K^+ channels, thereby reducing two components of the K^+ current that normally repolarizes the action potential. Reduction of these K^+ currents prolongs the action potential and allows the N-type Ca^{2+} channels to be activated for longer periods. More Ca^{2+} is able to enter the terminals, thereby further enhancing transmitter release (Figure 36–3B). Second, the kinase acts to enhance the mobilization of transmitter vesicles and the efficiency of the transmitter release apparatus through a calcium-independent mechanism. Third, the kinase alters an L-type Ca^{2+} channel, whose influx does not directly affect release but increases the mobilization of transmitter vesicles through a calcium-dependent mechanism. In its effects on mobilization and release efficiency, the cAMP-dependent protein kinase works in parallel with protein kinase C, which is also activated by serotonin, presumably through a different receptor (Figure 36–3B).

Long-Term Memory Requires the Synthesis of New Proteins and the Growth of New Synaptic Connections

As with habituation and most other forms of learning, practice makes perfect and prolongs the memory for sensitization. In *Aplysia* a single training trial (or a single application of serotonin) gives rise to short-term sensitization lasting only minutes, whereas four training trials produce long-term sensitization lasting one day, and further repetition produces sensitization that persists for a week or longer. These behavioral studies in *Aplysia* and similar ones in vertebrates suggest that short-term and long-term memory are two points of a graded process. Several findings point to this interpretation. First, both short and long-term sensitization are associated with changes in synaptic strength at the *same* locus: the connections between the sensory and motor neurons (Figure 36–4). Second, in both the long-term and short-term processes the increase in synaptic strength is due to the enhanced release of transmitter. Third, serotonin, a modulatory transmitter that produces the short-term facilitation following a single exposure, pro-


A
Tail
Sensory neurons
Facilitating interneurons
Sensory neurons
Interneurons
Siphon skin
Motor neurons
Gill
B
Facilitating interneurons
5-HT
5-HT Receptor
Adenylyl cyclase
K+ channels
N-type Ca2+ channel
PLC
Go-Protein
Gs-Protein
1
cAMP-dependent protein kinase
2
cAMP
Motor neuron
Sensory neuron
3
2a
PKC
3a
Diacylglycerol
L-type Ca2+ channel
Available transmitter pool
Releasable Transmitter

← *Figure 36–3* Short-term sensitization of the gill-withdrawal reflex in *Aplysia* involves presynaptic facilitation.

A. Sensitization of the gill is produced by applying a noxious stimulus to another part of the body, such as the tail. Stimuli to the tail activate sensory neurons that excite facilitating interneurons. The facilitating cells, some of which use serotonin as their transmitter form synapses on the terminals of the sensory neurons innervating the siphon skin. There they enhance transmitter release from the sensory neurons by means of presynaptic facilitation.

B. Presynaptic facilitation in the sensory neuron is thought to occur by means of the following biochemical steps. The action of serotonin (5-hydroxytryptamine, 5-HT) and other facilitating transmitters leads to enhanced transmitter release by directly modulating the release process, as well as causing the closure of K^+ channels, which results in broadening of the action potential and a consequent increase in Ca^{2+} influx through a Ca^{2+} channel. Serotonin produces these actions by binding to a receptor that engages a G-protein, which increases the activity of adenylyl cyclase. The adenylyl cyclase converts ATP to cyclic AMP, thereby increasing the level of cyclic AMP in the terminal of the sensory neuron. The cAMP activates the cAMP-dependent protein kinase by attaching to its regulatory subunit, which releases its active catalytic subunit. The catalytic subunit then phosphorylates K^+ channels, thereby changing the conformation of the channel and decreasing the K^+ current (**pathway 1**). This prolongs the action potential, increases the influx of Ca^{2+}, and thus augments transmitter release.

Serotonin also leads to an increase in the availability of transmitter by mobilizing vesicles from a transmitter pool to the releasable pool at the active zone and also directly enhances the efficiency of the machinery of the exocytotic release of transmitter (**pathway 2**). Serotonin also leads to the opening of L-type Ca^{2+} channels (**pathway 3**). The second and third pathways reflect joint action of the cAMP-dependent protein kinase and protein kinase C, a second kinase activated by 5-HT (**pathways 2a and 3a**). Protein kinase C (PKC) is activated by 5-HT through another receptor, which engages another G-protein that activates a phospholipase that in turn stimulates diacylglycerol in the membrane. Diacylglycerol activates protein kinase C.

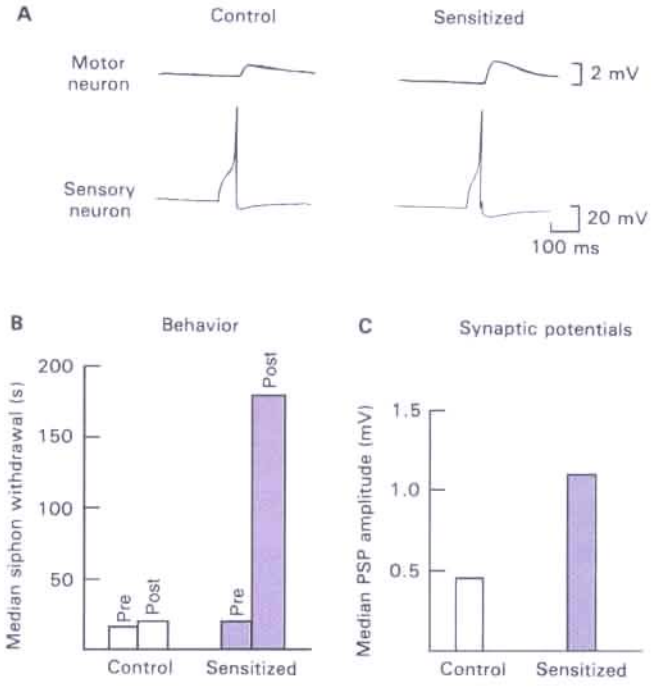


Figure 36–4 Long-term sensitization involves facilitation of the connections between sensory and motor neurons. (Adapted from Frost et al., 1985.)

A. Representative synaptic potentials in a siphon sensory neuron and a gill motor neuron in a control animal and an animal that has received long-term sensitization training by stimulating its tail. The record was obtained one day after the end of training.

B. The median duration of withdrawal of the siphon is used as a measure of the strength of the reflex. A comparison of control and sensitized groups illustrates the effect of sensitization. (Pre = score before training; post = score after training.) The experimental group was tested one day after the end of training.

C. Median values of the amplitudes of postsynaptic potentials (PSP) in an identified gill motor neuron for the control group and for sensitized animals one day after the end of training.

duces long-term facilitation after four or five repeated exposures. Finally, cAMP, an intracellular second messenger involved in the short-term process, also turns on the long-term change.

However, certain clinical conditions such as seizure and head trauma can selectively affect short- or long-term memory in humans. An even clearer behavioral separation between memory processes can be obtained in experimental animals using inhibitors of protein or mRNA synthesis, which selectively block long-term memory without affecting short-term memory. This dependency on macromolecular synthesis, which is also evident in the faciliation at the synapse between the sensory and motor neurons, suggests that genes and proteins not directly involved in short-term facilitation are required for long-term facilitation.

What is the function of these genes and proteins? Molecular studies indicate that with repeated training (or repeated application of serotonin) the cAMP-dependent protein kinase translocates to the nucleus of the sensory neurons to phosphorylate one or more cAMP-dependent transcriptional regulatory proteins such as CREB, the cAMP response element binding protein (Chapter 14). These transcriptional regulators activate genes whose protein products have two long-term consequences.

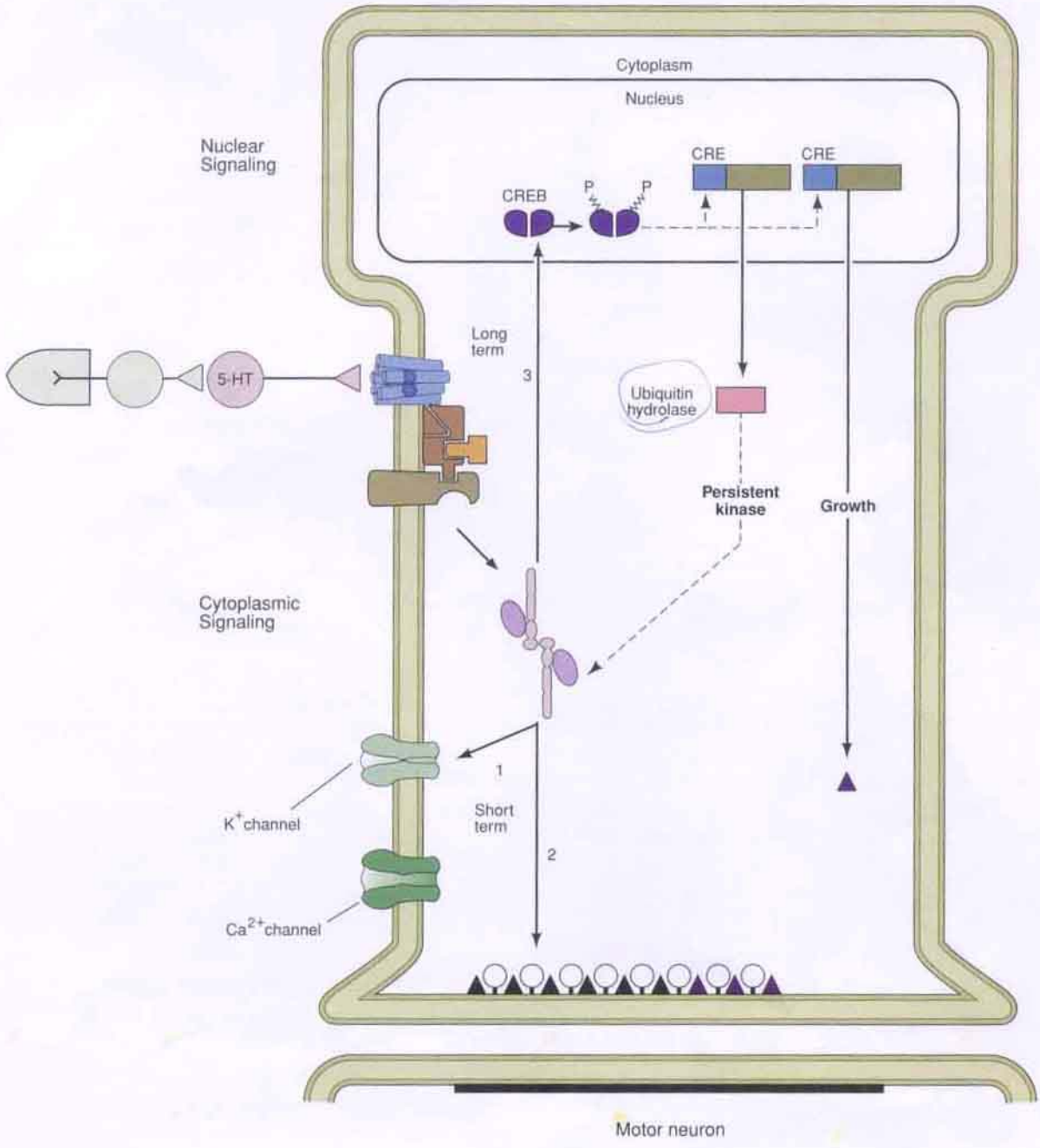
One consequence is a persistent activation of the cAMP-dependent protein kinase, a heterodimer consisting of two regulatory subunits that inhibit the two catalytic subunits (Chapter 14). With long-term training, the amount of the regulatory subunit in the sensory cells decreases relative to that of the catalytic subunit. This decrease in the regulatory subunit does not occur at the level of transcription but at the level of protein turnover. Indeed, one of the genes induced in long-term memory encodes a protein that is an enzyme in the ubiquitin proteolytic pathway that degrades the regulatory subunits (Figure 36–5). In turn, this decrease in the regulatory subunits causes the kinase to be persistently active, even though the level of cAMP has returned to its basal level. As a result, the short-term phosphorylation of the substrate proteins (Figure 36–3B) can be maintained in the long term. This persistent phosphorylation may explain why long-term facilitation appears to be a graded extension of the short-term process.

A second and more enduring consequence of gene activation is the growth of synaptic connec-

Figure 36–5 Schematic outline of the two major sets of changes in the sensory neurons of the gill-withdrawal reflex that accompany long-term memory for sensitization in *Aplysia*: (1) persistent activity of protein kinase A and (2) structural changes. Serotonin (5-HT), a transmitter released by facilitatory neurons, acts on a sensory neuron to initiate both the short-term facilitation and the long-term facilitation that contribute to the memory processes.

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

Long-term facilitation (lasting one or more days) involves the synthesis of new proteins. The switch for this inductive mechanism is initiated by the protein kinase A, which translocates to the nucleus where it is thought to phosphorylate one or more transcriptional activators that bind to *c*yclic AMP *r*egulatory *e*lements (CRE) located in the upstream region of cAMP-inducible genes. The transcriptional activators, thought to belong to the protein family of *c*yclic AMP *r*esponse *e*lement-*b*inding (CREB) proteins, activate two classes of effector genes that encode two classes of proteins. Inhibiting protein synthesis during learning blocks the expression of these two classes of proteins. These two sets of proteins have distinct functions. One protein (■), a ubiquitin hydrolase, is a component of a specific protease that leads to downregulation of the regulatory subunit. This results in persistent activity of kinase A, leading to persistent phosphorylation of the substrate proteins of **pathways 1 and 2**. The second set of proteins (▲) is important for the growth of active zones and the development of new synaptic connections.


Cytoplasm
Nucleus
Nuclear Signaling
CRE
CRE
CREB
P
P
Long term
3
5-HT
Ubiquitin hydrolase
Persistent kinase
Growth
Cytoplasmic Signaling
1
Short term
2
K+ channel
Ca2+ channel
Motor neuron

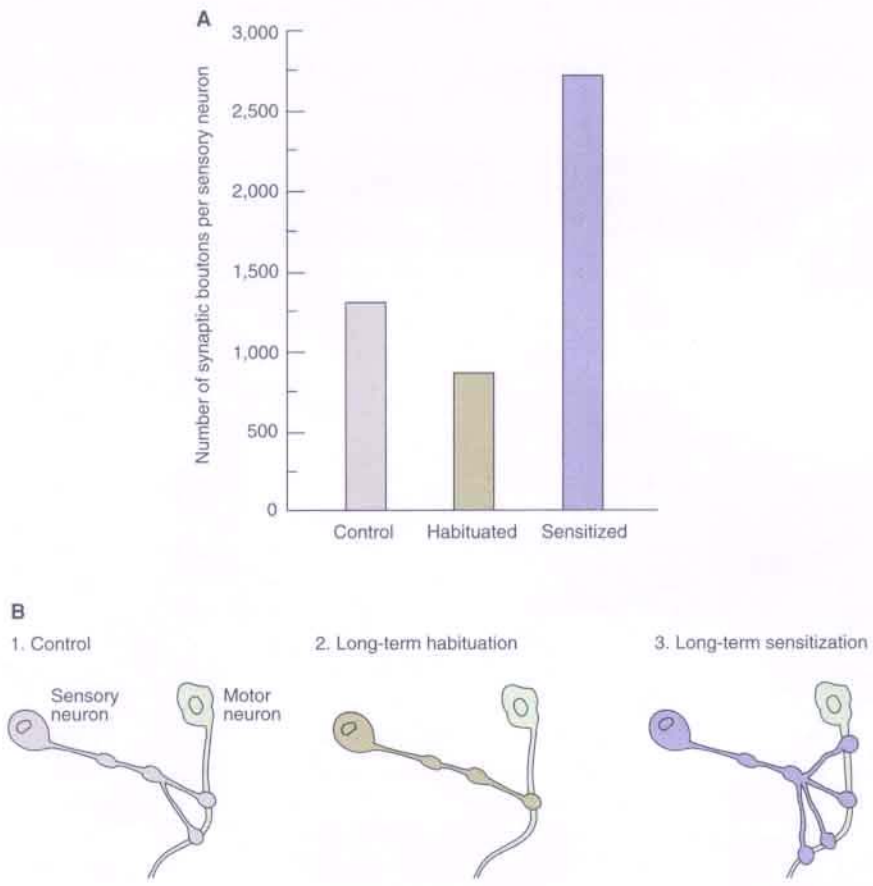


Figure 36–6 Long-term habituation and sensitization involve structural changes in the presynaptic terminals of sensory neurons. (Adapted from Bailey and Chen, 1983.)

A. This histogram compares the number of presynaptic terminals in control animals with those in long-term habituated and sensitized animals. The number is highest in the sensitized animals.

B. Long-term habituation leads to a loss of synapses, and long-term sensitization to an increase.

tions. This change was delineated in the sensory and motor cells involved in the gill-withdrawal reflex by examining the synaptic terminals with the electron microscope. The sensory neurons in sensitized animals had twice as many presynaptic terminals as those in untrained animals (Figure 36–6). Moreover, long-term sensitization increased the number of active zones from 40% of the synaptic terminals in untrained animals to 65% in trained animals. Finally, in the sensitized animals the dendrites of the motor neurons grew to accommodate the additional synaptic input. Such morphological changes seem to be characteristic only of long-term sensitization; they do not occur with short-term sensitization (see Figure 36–5).

In contrast to long-term sensitization, long-term habituation leads to *pruning* of synaptic connections: the inactivation of the functional connections between sensory and motor neurons reduces the number of terminals per neuron by one-third (Figure 36–6) and the proportion of terminals with active zones is reduced from 40% to 10%.

Classical Conditioning Involves an Associative Enhancement of Presynaptic Facilitation That Is Dependent on Activity

Classical conditioning is a more complex form of learning than sensitization. Rather than learning about the properties of one stimulus, the subject learns to associate one type of stimulus with another (see Chapter 35). In classical conditioning an initially weak or ineffective stimulus becomes highly effective in producing a response after it has been paired or associated with a strong unconditioned stimulus. For reflexes that can be modified by both sensitization and classical conditioning, classical conditioning is more effective in enhancing the responsiveness of the reflex, and it lasts longer than sensitization. As we shall see, the cellular mechanism of certain types of classical conditioning is an elaboration of the mechanism for sensitization.

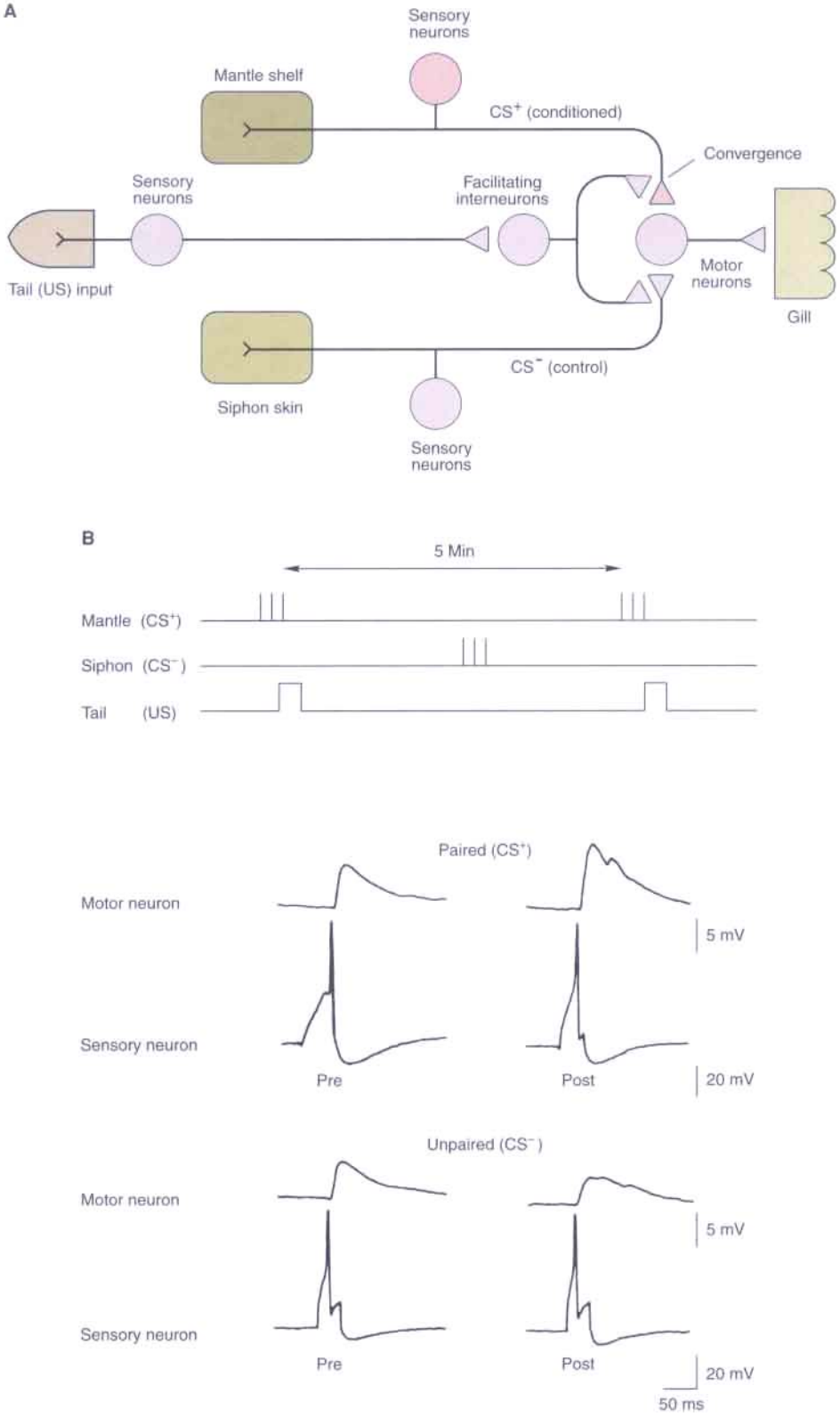
The siphon- and gill-withdrawal reflexes of *Aplysia* can be enhanced by classical conditioning as well as sensitization. These reflexes can be elicited by stimulating, respectively, the siphon and a nearby structure called the mantle shelf. Each of these areas is separately innervated by distinct populations of sensory neurons. Thus, each neural pathway can be conditioned independently by pairing a stimulus to the appropriate area of the body (either the siphon or the mantle shelf) with an unconditioned stimulus (a strong shock to the tail). After such training, the response of the conditioned pathway to stimulation is significantly stronger than that of the unconditioned pathway (Figure 36–7).

Unlike nonassociative learning, timing is critical to associative learning. For classical conditioning to work, the conditioned stimulus must *precede* the unconditioned stimulus, and often it must do so within a critical interval of about 0.5 second. What cellular mechanisms are responsible for this requirement for the temporal pairing of stimuli? In classical conditioning of the gill-withdrawal reflex of *Aplysia*, one important mechanism for the temporal specificity is the convergence of the conditioned and unconditioned stimuli on individual sensory neurons. As we have seen, the unconditioned stimulus to the tail activates facilitating interneurons that have axo-axonic connections with the sensory neurons of the conditioned stimulus. This gives rise to presynaptic facilitation and behavioral sensitization. However, if the two stimuli are timed so that the interneurons are activated by the unconditioned stimulus immediately after the sensory neurons begin to fire in response to the conditioned stimulus, an even greater presynaptic facilitation is produced (Figure 36–7). In contrast, no enhancement of facilitation occurs if the interneurons are activated before the sensory neurons begin firing, that is, if activity in the sensory neurons *follows* the unconditioned stimulus.

This novel property of presynaptic facilitation, whereby the facilitation is amplified if the conditioned stimulus produces action potentials in the sensory neurons just before the unconditioned stimulus arrives, is called *activity dependence*. Thus, one component of the cellular mechanism of classical conditioning in the monosynaptic component of the withdrawal reflex in *Aplysia* is an elaboration of presynaptic facilitation, the mechanism of sensitization in this component of the reflex. A similar enhancement of sensory neurons occurs in the tail of *Aplysia*.

How is activity-dependent presynaptic facilitation achieved? The Ca^{2+} that flows into the cell following an action potential is thought to act through calmodulin to amplify the activation of the adenylyl cyclase by serotonin and other modulatory transmitters (Figure 36–8). Much of the cyclase in the brain is sensitive to Ca^{2+}/calmodulin and generates more cAMP when it is bound to Ca^{2+}/calmodulin than when it is not.

Genetic analyses of learning have also implicated the cAMP system. The fruit fly *Drosophila* can be classically conditioned, and single-gene mutants deficient in learning have been isolated. Three of these mutants, called *dunce*, *rutabaga*, and *amnesiac*, have been studied in detail and show two interesting features. First, all of the mutants that fail to show classical conditioning also fail to show sensitization. Second, all three mutants have a defect in the cAMP cascade. The *dunce* mutant lacks a phosphodiesterase, an enzyme that degrades cAMP. As a result, this fly has abnormally high levels of cAMP that are thought to be out of the range of normal modulation. The *rutabaga* mutant has a defect in the Ca^{2+}/calmodulin-dependent adenylyl cyclase and a low basal level of cAMP. The *amnesiac* mutation lacks a peptide that regulates the activity of the adenylyl cyclase. Finally, blocking the action of the CREB transcription


A
Sensory neurons
Mantle shelf
CS+ (conditioned)
Convergence
Sensory neurons
Facilitating interneurons
Motor neurons
Tail (US) input
Gill
CS− (control)
Siphon skin
Sensory neurons
B
5 Min
Mantle (CS+)
Siphon (CS−)
Tail (US)
Paired (CS+)
Motor neuron
5 mV
Sensory neuron
Pre
Post
20 mV
Unpaired (CS−)
Motor neuron
5 mV
Sensory neuron
Pre
Post
20 mV
50 ms

← *Figure 36–7* Classical conditioning of the gill-withdrawal reflex in *Aplysia*. A conditioned stimulus (CS) applied to the mantle is paired with an unconditioned stimulus (US) to the tail; as a control, a CS applied to the siphon is not paired with the US. (Adapted from Hawkins et. al., 1983).

A. This simplified diagram shows the neural pathways involved. A shock to the tail (US) excites facilitatory interneurons that synapse on the presynaptic terminals of sensory neurons innervating the mantle shelf and siphon. This is a mechanism of sensitization. However, when the mantle pathway is activated by a CS just prior to the US, the activity primes the mantle sensory neurons so that they are more responsive to subsequent stimulation from the facilitatory interneurons in the US pathway. This is a mechanism of classical conditioning; it both amplifies the response of the CS pathway and restricts the amplification to that pathway.

B. The activity of individual cells is modified by classical conditioning. Two sensory neurons are each stimulated independently. Stimulation of the mantle sensory neuron (CS^+) is paired with the US (tail shock), while, stimulation of the siphon sensory neuron (CS^-) is not paired with the US. Recordings of the excitatory postsynaptic potentials produced in an identified motor neuron by the two sensory neurons were made before training (Pre) and one hour after training (Post). After training the excitatory postsynaptic potential due to the paired sensory neuron is considerably greater than that due to the unpaired neuron.

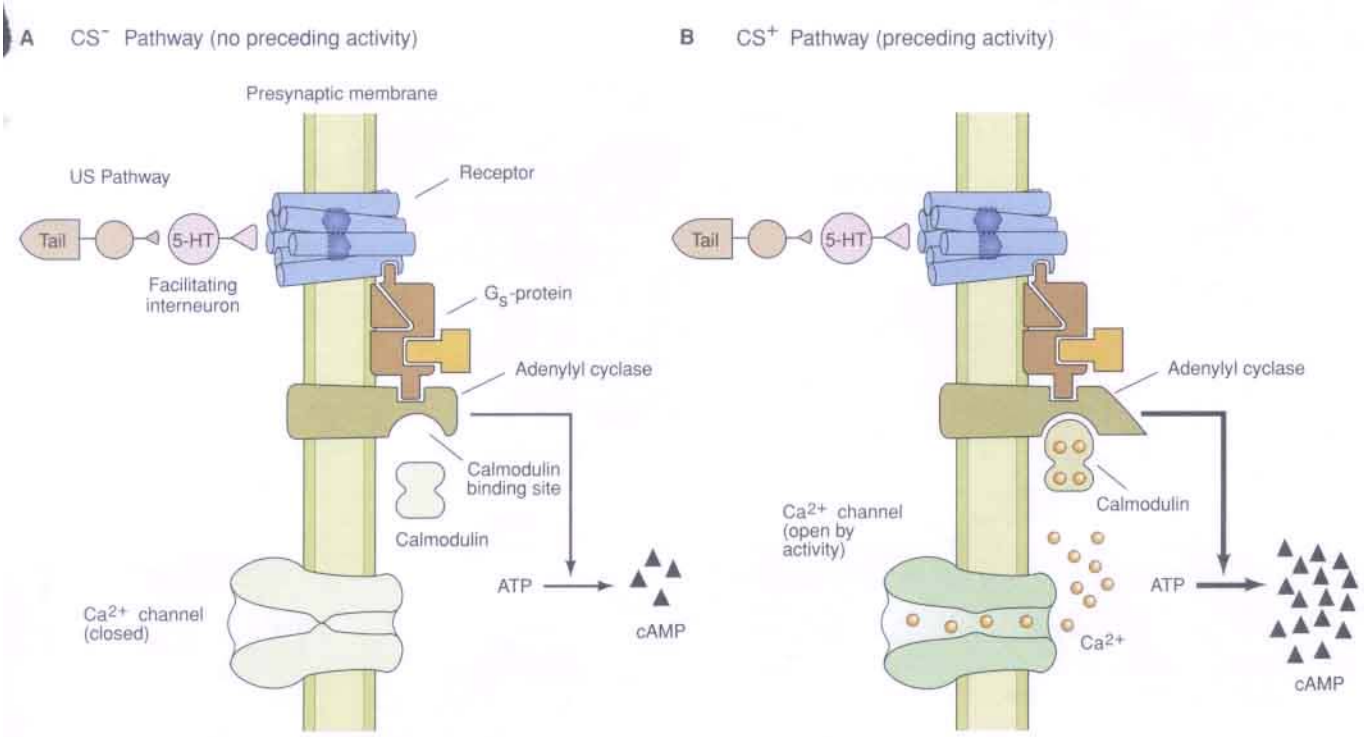


Figure 36–8 A molecular model of the synaptic action underlying classical conditioning. The model is based on the hypothesis that activity in the sensory neurons mediating the conditioned stimulus prior to the presentation of the unconditional stimulus permits an influx of Ca^{2+} that enhances the activity of calcium-dependent adenylyl cyclase.

A. In the unpaired pathway (CS^-) the sensory neuron is not active prior to presentation of the CS, so its Ca^{2+} channels are closed when the unconditioned stimulus (US) is presented. (5-HT, serotonin.)

B. In the paired pathway (CS^+) the sensory neuron is active prior to the CS and thus its Ca^{2+} channels are open when the US is presented. The intracellular Ca^{2+} binds to calmodulin and in turn interacts with adenylyl cyclase. As a result, the adenylyl cyclase undergoes a conformational change that enhances its ability to synthesize cAMP in response to serotonin released in the US pathway. The greater amount of cAMP activates more cAMP-dependent protein kinase and leads to a substantially greater amount of transmitter release than would occur without paired activity.

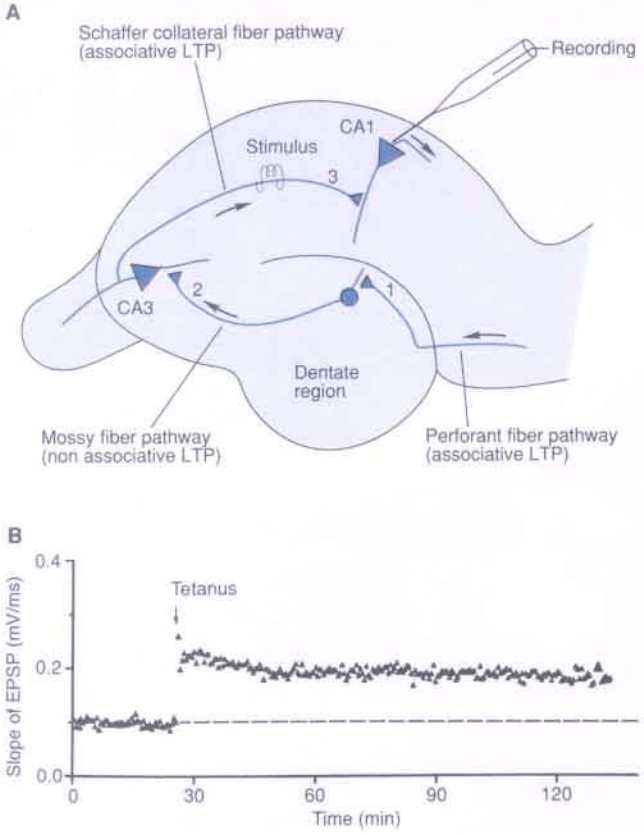


Figure 36–9 Long-term potentiation (LTP) in the hippocampus.

A. There are three major afferent pathways in the hippocampus. (**Arrows** denote the direction of impulse flow.) The *perforant pathway* (**1**) from the entorhinal cortex forms excitatory connections with the granule cells of the dentate gyrus. The granule cells give rise to axons that form the *mossy fiber pathway* (**2**). This pathway connects with the pyramidal cells in area CA3 of the hippocampus. The CA3 cells project to the pyramidal cells in CA1 by means of the *Schaffer collaterals* (**3**).

B. The effect of long-term potentiation in a cell in the CA1 region of the hippocampus is shown in this plot of the slope (rate of rise) of the excitatory postsynaptic potentials in the cell. The slope is a measure of synaptic efficacy. Excitatory postsynaptic potentials (EPSPs) were recorded from outside the cell. A test stimulus was given every 10 seconds. To elicit long-term potentiation two trains of stimuli for 1 second each at 100 Hz tetani and separated by 20 seconds were delivered to the Schaffer collaterals. The resulting LTP lasts several hours. (Adapted from Nicoll et al., 1988.)

factor in *Drosophila* (thereby preventing the expression of cAMP-dependent genes) selectively blocks the protein synthesis-dependent long-term memory in the fly without interfering with learning or short-term memory.

Thus, both cellular studies of *Aplysia* and genetic studies of *Drosophila* indicate that the cAMP cascade is important for certain elementary forms of learning and memory storage. However, the cAMP cascade is not the only second-messenger system important for synaptic plasticity related to implicit forms of learning. In other instances of learning other second messenger cascades are recruited.

That the cellular mechanisms of classical conditioning in *Aplysia* may be an elaboration of those involved in sensitization suggests that, at least in certain instances, more complex forms of learning can be built up from the molecular components of simpler forms. By this means a variety of distinct forms of behavioral modifications could be achieved by a small set of molecular mechanisms.

Storage of Explicit Memory in Mammals Involves Long-Term Potentiation in the Hippocampus

What about explicit forms of learning? Can specific cellular mechanisms for these more complex forms of learning be identified? As we saw in Chapter 35, the hippocampus is important for storage of explicit memory, and there is evidence that neurons in the hippocampus have the plasticity of the sort that would be required for explicit memory.

The hippocampus has three major afferent pathways running from the entorhinal cortex to the CA1 region (Figure 36–9A). The *perforant pathway* runs from the entorhinal cortex to the granule cells in the hilus of the dentate gyrus. The axons of the granule cells form a bundle, the *mossy fiber pathway*, that runs to the pyramidal cells lying in the CA3 region of the hippocampus. Finally, the pyramidal cells in the CA3 region send excitatory collaterals, the *Schaffer collaterals*, to the pyramidal cells in CA1. A brief high-frequency train of stimuli to any one of these pathways increases the excitatory postsynaptic potentials in the hippocampal neurons, an increase that can last for hours and, in the intact animal, for days and even weeks. This facilitation is called *long-term potentiation* (Figure 36–9B). Long-term potentiation is not produced in the same way in all three of these pathways, however, and we discuss the differences next.

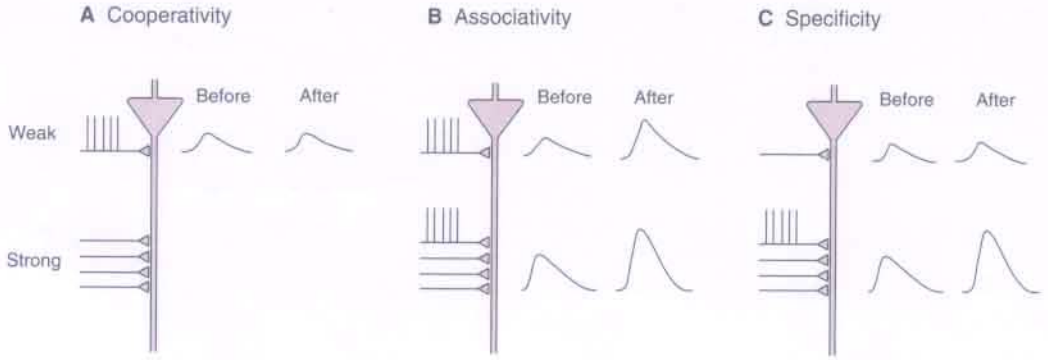


Figure 36–10 Long-term potentiation in area CA1 of the hippocampus shows cooperativity, associativity, and specificity. In the figure a single pyramidal cell receives weak and strong synaptic inputs from two different fascicles of the Schaffer collateral pathway. (Adapted from Nicoll et al., 1988.)

A. Tetanic stimulation of the weak input alone does not cause long-term potentiation in the pathway (compare the potential before and after tetanus).

B. Tetanic stimulation of the strong and weak pathways together causes long-term potentiation in both pathways.

C. Tetanic stimulation of the strong input alone causes long-term potentiation in the strong pathway but not in the weak.

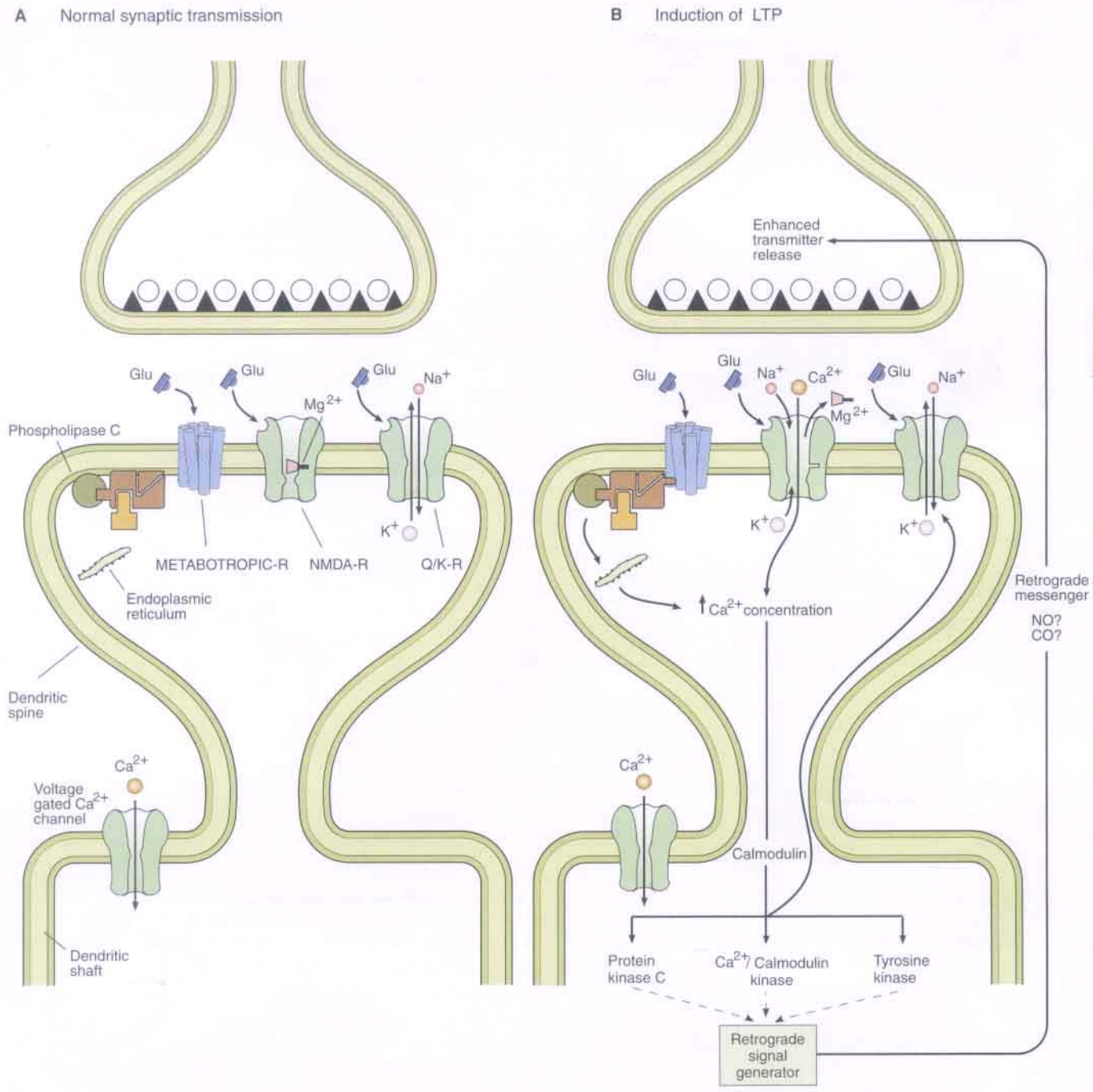
Long-Term Potentiation in the CA1 Region Is Associative

Long-term potentiation (LTP) can be produced in the Schaffer axon collateral pathway, which connects the pyramidal cells of the CA3 region of the hippocampus with those of the CA1 region (Figure 36–9A). To produce LTP it is necessary to use a strong stimulus that activates several afferent fibers together. This *cooperative* activity has associative features similar to those encountered in classical conditioning. When separate weak and strong excitatory inputs arrive at the same region of the dendrites of a pyramidal cell, the weak input will become potentiated only if it is activated in association with the strong one. Finally, LTP is specific to those synapses that are activated by the stimulus. For example, LTP produced by an input to the apical dendrites does not affect an independent input onto the basilar dendrites. These features of LTP in the CA1 region are illustrated in Figure 36–10.

What accounts for these three features? What appears critical for the induction of LTP in the CA1 region of the hippocampus is that the postsynaptic cell be adequately depolarized. LTP can be induced when a weak stimulus train, or even a single test stimulus, not sufficient in itself to produce LTP, is paired repeatedly with a depolarizing current pulse injected in a single postsynaptic cell. Conversely, hyperpolarizing the postsynaptic cell during the tetanus can prevent LTP.

Thus, LTP requires simultaneous firing in both the postsynaptic and presynaptic neurons. This finding provides the first direct evidence for *Hebb's rule*, proposed in 1949 by the psychologist Donald Hebb: "When an axon of cell A . . . excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased." As we have seen in Chapter 25, a similar principle seems to be involved in the fine tuning of synaptic connections during the late stages of development.

Why is the simultaneous firing of the pre- and postsynaptic cells important for LTP? The Schaffer collateral axons from the CA3 region of the hippocampus that terminate on the pyramidal cells of the CA1 region use glutamate as their transmitter. Glutamate acts on its target cells in the CA1 region by binding to both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors (Chapter 13). In normal synaptic transmission the non-NMDA receptors dominate as the NMDA receptor–channels are


A Normal synaptic transmission
B Induction of LTP
Glu
Glu
Glu
Na+
Mg2+
Phospholipase C
K+
METABOTROPIC-R
NMDA-R
Q/K-R
Endoplasmic reticulum
Dendritic spine
Ca2+
Voltage gated Ca2+ channel
Dendritic shaft
Enhanced transmitter release
Glu
Glu
Na+
Ca2+
Glu
Na+
Mg2+
K+
K+
↑Ca2+ concentration
Retrograde messenger
NO?
CO?
Ca2+
Calmodulin
Protein kinase C
Ca2+/ Calmodulin kinase
Tyrosine kinase
Retrograde signal generator

blocked by Mg^{2+}. They become unblocked, and thus activated, only when the postsynaptic cell is adequately depolarized by strong (cooperative) inputs from many presynaptic neurons. This depolarization causes the positively charged Mg^{2+} ion to pop out of the channel mouth, allowing Na^+ and particularly Ca^{2+} to flow through the channel into the cell. The influx of Ca^{2+} is the signal for the induction of LTP.

Thus, the NMDA receptor–channel is unusual in being a *doubly gated channel*. The channel becomes functional only when glutamate binds to the receptor *and* the membrane is depolarized. This critical depolarization is normally achieved through the activation of many non-NMDA receptors by the firing of many presynaptic neurons (Figure 36–11). Artificially it can be obtained by simply depolarizing the postsynaptic cell.

← *Figure 36–11* A model for the induction of long-term potentiation. According to this model *N*-methyl-D-aspartate (NMDA) and non-NMDA (quisqualate/kainate) receptor–channels are located near each other in dendritic spines. (Adapted from Gustafsson and Wigström, 1988.)

A. During normal low-frequency synaptic transmission glutamate is released from the presynaptic terminal and acts on both the NMDA and non-NMDA (Q/K) receptors. Sodium and K^+ flow through the non-NMDA receptor–channels but not through the NMDA receptor–channels, owing to Mg^{2+} blockade of these channels at the resting level of membrane potential.

B. When the postsynaptic membrane is depolarized by the actions of the non-NMDA receptor channels, as occurs during a high-frequency tetanus that induces long-term potentiation (LTP), the depolarization relieves the Mg^{2+} blockade of the NMDA channel. This allows Na^+, K^+, and Ca^{2+} to flow through the NMDA channel. The resulting rise in Ca^{2+} in the dendritic spine triggers calcium-dependent kinases (calcium/calmodulin kinase and kinase C) that induce LTP. Once LTP is induced, the postsynaptic cell releases (in ways that are still not understood) a retrograde messenger that is thought to act on kinases in the presynaptic terminal to produce the sustained enhancement of transmitter release that underlies the persistence of LTP.

Calcium influx through the unblocked NMDA receptor–channel is critical for LTP. Blocking Ca^{2+} influx prevents induction of LTP; conversely, injecting Ca^{2+} into the postsynaptic cell initiates the early phase of LTP. In principle, Ca^{2+} could pass through either a voltage-gated Ca^{2+} channel or the NMDA-gated channel. However, the Ca^{2+} influx critical for LTP normally enters through the NMDA receptor–channel, not through the voltage-gated Ca^{2+} channels. Blocking the NMDA receptor–channel blocks LTP.

Receptors for NMDA seem to be clustered on the heads of the spines of dendrites, not on the shafts of the dendrites. (Spines, as we have seen in Chapter 13, are specialized lateral protrusions on the shafts of dendrites that receive excitatory synaptic input.) The activation of non-NMDA receptor–channels depolarizes the spines sufficiently to remove the Mg^{2+} blockade of the NMDA receptor–channels, allowing Ca^{2+} to enter the spines. The spine acts as a functional compartment that restrains the diffusion of Ca^{2+}, so that the synaptic action is restricted to the synapses that are active.

Calcium initiates the persistent enhancement of synaptic transmission by activating two calcium-dependent serine–threonine protein kinases—the Ca^{2+}/calmodulin kinase and protein kinase C—and a tyrosine protein kinase. One of these kinases, an isoform of protein kinase C, then becomes persistently active.

Thus, the induction of LTP in the CA1 region depends on postsynaptic depolarization, Ca^{2+} influx, and Ca^{2+} activation of a second-messenger system. Maintenance of LTP in the CA1 region, however, involves in addition an increase in *presynaptic* transmitter release (Figure 36–12). This finding is based on three lines of evidence. First, LTP is accompanied by an enhancement of glutamate release. Second, LTP involves an increased probability of transmitter release as well as a change in the sensitivity of the non-NMDA glutamate receptors in the postsynaptic cell. Third, the induction of LTP by depolarization of a single postsynaptic cell produces LTP in a small population of surrounding neurons. If the LTP mechanism were strictly postsynaptic, the LTP would be restricted to the cell that was depolarized.

Since the induction of LTP requires a postsynaptic event (activation of NMDA receptors and Ca^{2+} influx) and maintenance of LTP involves a presynaptic event (increase in transmitter release), a mes-

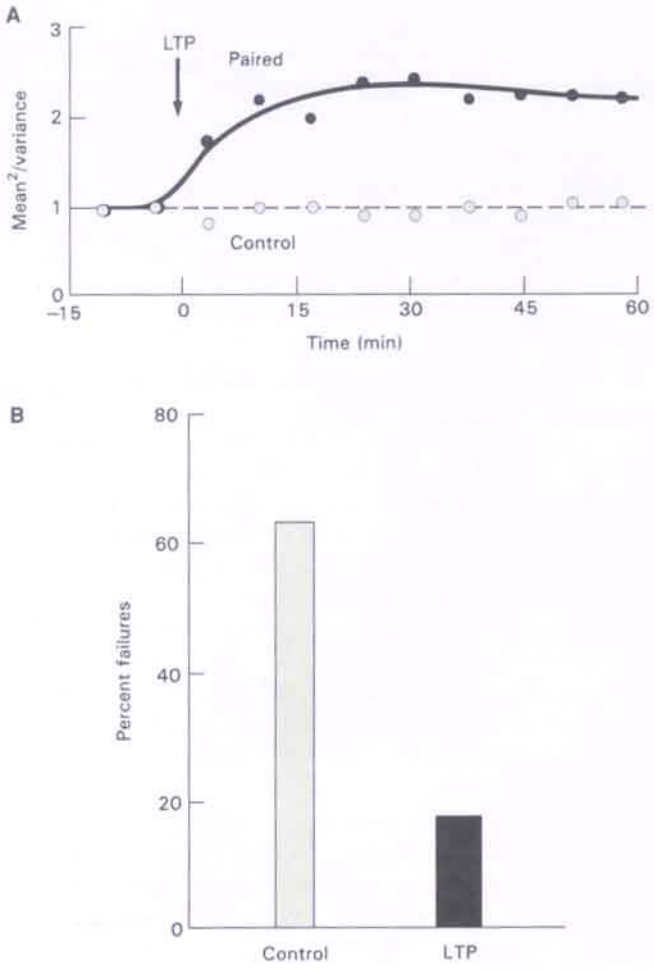


Figure 36–12 Maintenance of long-term potentiation (LTP) in the CA1 region of the hippocampus depends on an increase in presynaptic transmitter release. Quantal analysis of long-term potentiation in area CA1 is based on a coefficient of variation of evoked responses. This analysis assumes that the number of quanta of transmitter released follows a binomial distribution, where the coefficient of variation (mean²/variance) provides an index of transmitter release from the presynaptic terminal that is independent of quantal size. (From Malinow and Tsien, 1990.)

A. With long-term potentiation there is an increase in the mean²/variance, indicating an increase in transmitter release. This increase occurs only in the pathway that is paired with depolarization of the postsynaptic cell. It does not occur in a control pathway that is not paired.

B. At normal rates of stimulation the number of failures in transmission is significant. In 60% of cases stimulation of the presynaptic axons leads to no release. Following LTP the percentage of failures decreases to 20%, another indication that LTP is presynaptic.

sage must be sent from the postsynaptic to the presynaptic neurons. There is now evidence that the Ca^{2+}-activated second messenger, or perhaps Ca^{2+} acting directly, causes the release of one or more *retrograde messengers* from the dendritic spines of the active postsynaptic cell. This retrograde factor diffuses to the presynaptic terminals to activate one or more second messengers that act to enhance transmitter release and thereby maintain LTP. The actions of a membrane-permeable retrograde messenger seems to be restricted to recently active presynaptic cells. Indeed, to account for the pathway specificity of LTP, the action of the retrograde messenger must be restricted. Two gases that diffuse readily from cell to cell, nitric oxide and carbon monoxide, have properties that have made them interesting candidates, acting either alone or jointly with other molecules, for the retrograde messenger of LTP.

According to this view, LTP in the CA1 region of the hippocampus uses two associative mechanisms in series: a Hebbian mechanism and activity-dependent presynaptic facilitation. However, LTP differs from the activity-dependent presynaptic facilitation found in *Aplysia* in that the facilitatory substance is released from the postsynaptic target cell by activation of NMDA receptors, rather than from a facilitatory interneuron with diffuse projections, as in *Aplysia*.

What might be the advantage of combining in the hippocampus two associative cellular mechanisms in series (the postsynaptic NMDA receptor and activity-dependent presynaptic facilitation)? One possible advantage is spatial amplification of the signal. The retrograde factor can recruit other nearby presynaptic fibers in addition to those that synapse directly on the active postsynaptic cell.

Associative Long-Term Potentiation May Be Important for Spatial Memory

The finding that LTP occurs in many areas of the brain, including the cerebral cortex and hippocampus, a region known to be important for memory storage, raises the question: Is LTP involved in memory storage? Evidence for this has come from the analysis of a spatial memory task in which a rat has to make its way through a maze in a pool filled with a whitish opaque fluid to find a platform hidden under the fluid. The animal is released at random locations around the pool and must use spa-

tial cues—markings on the walls of the room in which the pool is located—to find the platform. In a simple visual (nonspatial) version of this task, the platform is raised above the water surface or marked with a flag so that it is visible; thus, the rat can navigate to the platform by means of direct sight rather than spatial cues.

When NMDA receptors in the hippocampus are blocked by the injection of an antagonist into the ventricle, the animal can successfully navigate the maze in the simple visual version of the task but cannot find his way to the platform in the spatial version of the task. These experiments suggest that an NMDA receptor mechanism in the hippocampus, perhaps LTP, is involved in spatial learning. Further evidence for this correlation comes from mice with altered genes. Ablation of the gene coding for the calcium/calmodulin-dependent protein kinase or the gene coding for the nonreceptor tyrosine kinase (*fyn*) reduces LTP and also blocks spatial learning in the water maze (Box 36–1 and Figure 36–13).

Long-Term Potentiation in the CA3 Region Is Nonassociative

Although LTP occurs at several synapses in the hippocampus and in many regions of the cerebral cortex, the mechanisms for the induction of LTP are not the same everywhere. Some do not work through the NMDA receptor and do not depend on either Ca^{2+} influx or the activation of calcium/calmodulin-dependent kinases in the postsynaptic cell.

Neurons in the CA3 region of the hippocampus release glutamate as their transmitter, but the synapses they form utilize NMDA receptors only in a minor way. In fact, LTP at these synapses is not blocked by the standard NMDA receptor antagonist. Moreover, this potentiation is not associative—the input need not be paired with another input or with depolarization of the postsynaptic cells.

Blocking Ca^{2+} influx into the postsynaptic cells in the CA3 region does not affect LTP in these cells. Indeed, LTP can be obtained after washing the postsynaptic cell with fluoride, which disrupts various intracellular second-messenger pathways in the postsynaptic cell. Rather, this form of LTP seems to depend on presynaptic Ca^{2+} influx as a result of the tetanus. The Ca^{2+} influx in turn activates a Ca^{2+}/calmodulin dependent (Type I) adenyl cyclase, which increases the level of cAMP and activates the cAMP-dependent protein kinase, much as occurs in the *Aplysia* sensory neurons (Figure 36–14).

Is There a Molecular Alphabet for Learning?

The changes in synaptic efficacy that we have encountered in studies of both implicit and explicit forms of learning raise these surprising reductionist possibilities in a neurobiological approach to learning.

First, synaptic changes can be associative without depending on complex features of the neural *network*. This fact means that the associative activity that contributes to implicit and explicit learning represents a *basic cellular process*. In the two instances we have considered here—activity-dependent enhancement of presynaptic facilitation and associative LTP—the plastic properties of cells seem to derive from the properties of specific proteins, such as the adenylyl cyclase and the NMDA receptor, which are capable of responding to two independent signals.

Second, the finding that the associative forms of synaptic plasticity in *Aplysia* and in the hippocampus are related in certain instances to nonassociative forms suggests that there may be a molecular alphabet for synaptic plasticity—simpler forms of plasticity might represent elements of more complex forms. Of course, these elementary cellular mechanisms are embedded in neural circuitry with considerable additional computational power, which can add substantial complexity to these elementary mechanisms. Finally, there is evidence for a late phase of LTP that parallels long-term memory in requiring gene expression and new protein synthesis. In both the CA1 and the CA3 region this late phase requires cAMP-inducible genes, much as does the long-term memory-related plasticity for implicit learning in *Aplysia* and *Drosophilia*. Thus, even though implicit and explicit forms of learning use different mechanisms for short-term memory storage, both forms of learning seem to share a restricted number of mechanisms for long-term memory storage.