66

Cellular Mechanisms of Implicit Memory Storage and the Biological Basis of Individuality

Storage of Implicit Memory Involves Changes in the Effectiveness of Synaptic Transmission

- Habituation Results from an Activity-Dependent Presynaptic Depression of Synaptic Transmission
- Sensitization Involves Presynaptic Facilitation of Synaptic Transmission
- Classical Conditioning of Fear Involves Coordinated Pre- and Postsynaptic Facilitation of Synaptic Transmission

Long-Term Storage of Implicit Memory Involves Changes in Chromatin Structure and Gene Expression Mediated by the cAMP-PKA-CREB Pathway

- Cyclic AMP Signaling Has a Role in Long-Term Sensitization
- Long-Term Synaptic Facilitation Is Synapse Specific
- Long-Term Facilitation Requires a Prion-Like Protein Regulator of Local Protein Synthesis for Maintenance

Classical Fear Conditioning in Flies Uses the cAMP-PKA-CREB Pathway

Memory for Learned Fear in Mammals Involves the Amygdala

Habit Learning and Memory Require the Striatum

Learning-Induced Changes in the Structure of the Brain Contribute to the Biological Basis of Individuality

An Overall View

Throughout this book we have emphasized that all behavior is a function of the brain and that malfunctions of the brain produce characteristic disturbances of behavior. Behavior is also shaped by experience. How does experience act on the neural circuits of the brain to change behavior? How is new information acquired by the brain and, once acquired, how is it remembered?

In the previous chapter we saw that memory is not a single process but has at least two major forms. Implicit memory operates unconsciously and automatically, as in the memory for habits and perceptual and motor skills, whereas explicit memory operates consciously, as in the memory for people, places, and objects. Long-term storage of explicit memory begins in the hippocampus and the medial temporal lobe of the neocortex, whereas long-term storage of implicit memory requires a family of structures: the neocortex for priming, the striatum for skills and habits, the amygdala for learned fear, the cerebellum for learned motor skills, and certain reflex pathways for nonassociative learning such as habituation and sensitization (Figure 66–1).

Over time, explicit memories are transferred to different regions of the neocortex. In addition, many cognitive, motor, and perceptual skills that we initially store in explicit memory ultimately become so ingrained with practice that they become stored as implicit memory.

The transference from explicit to implicit memory and the difference between them is dramatically demonstrated in the case of the English musician and conductor Clive Waring, who in 1985 sustained a viral infection of his brain (herpes encephalitis) that affected the hippocampus and temporal cortex. Waring was left with a devastating loss of memory for events or people he had encountered even a minute or two earlier.

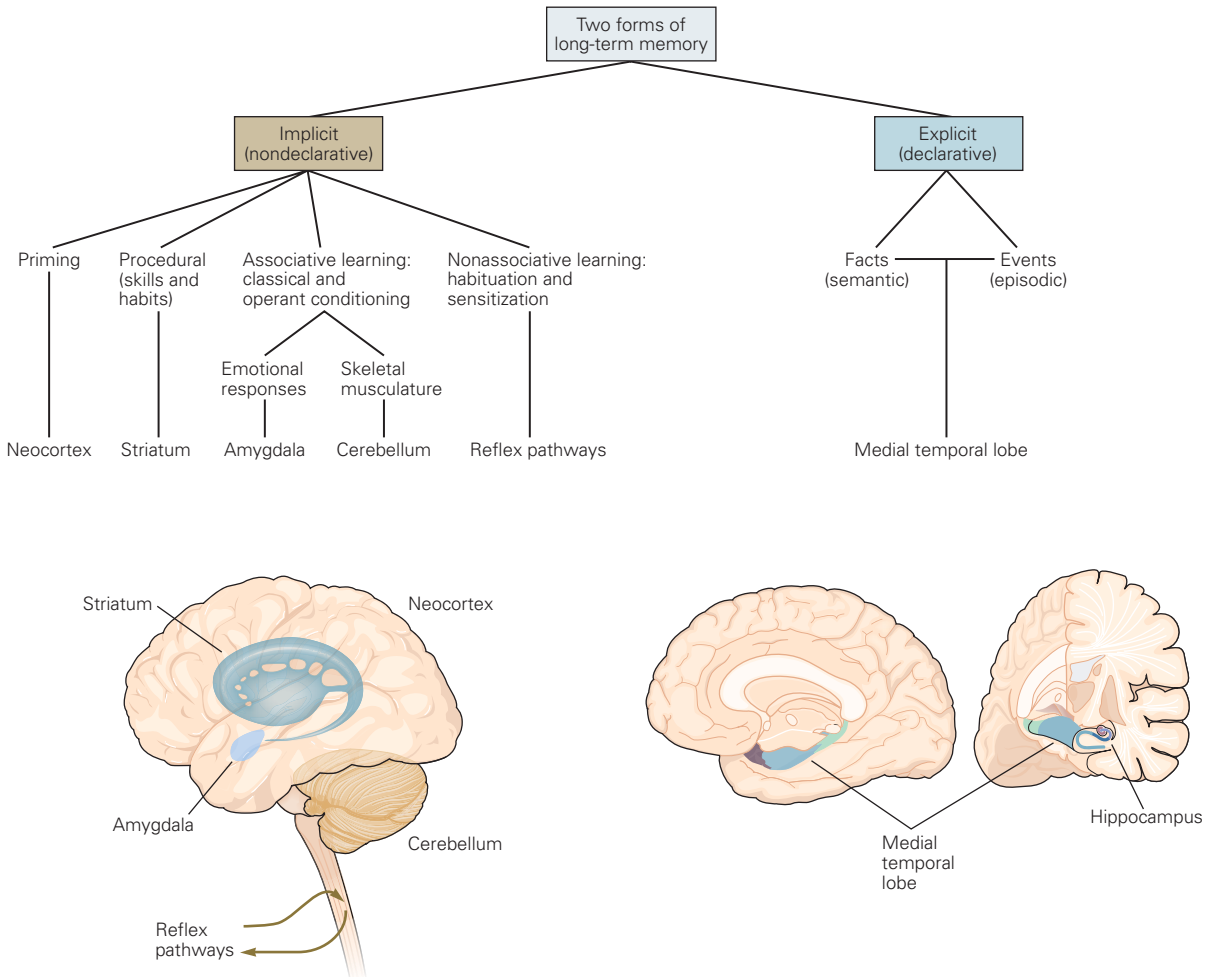


Figure 66–1 Two forms of long-term memory involve different brain systems. Implicit memory involves the neocortex, striatum, amygdala, cerebellum, and in the simplest cases the reflex pathways themselves. Explicit memory requires the medial temporal lobe and the hippocampus, as well as certain areas of neocortex (not shown).

Yet he could still read music, perform on the piano, and conduct a chorale. Under these circumstances it was clear that many aspects of his basic personality—the biological basis of his individuality—were still intact. Once a performance was completed, however, he could not remember a thing about it.

Similarly, William deKooning, the abstract expressionist painter, developed Alzheimer disease and severe disturbances of explicit memory. As the disease progressed and his memory for people, places, and objects deteriorated, he nevertheless continued to produce important and interesting paintings. This aspect of his creative personality was relatively untouched.

In this chapter we examine the cellular and molecular mechanisms that underlie implicit memory in invertebrate and vertebrate animals. In the next chapter we examine the biology of explicit memory storage in mammals.

Storage of Implicit Memory Involves Changes in the Effectiveness of Synaptic Transmission

Studies of elementary forms of implicit learning—habituation, sensitization, and classical conditioning—provided the groundwork for understanding the neural mechanisms of memory storage. Such learning has been analyzed in simple invertebrates and in a variety of vertebrate reflexes, such as the flexion reflexes, fear

responses, and the eye blink. These simple forms of implicit learning involve changes in the effectiveness of the synaptic pathways that mediate the behavior.

Habituation Results from an Activity-Dependent Presynaptic Depression of Synaptic Transmission

Habituation is the simplest form of implicit learning. It occurs, for example, when an animal learns to ignore a novel stimulus. An animal reacts to a new stimulus with a series of orienting responses. If the stimulus is neither beneficial nor harmful, the animal learns to ignore it after repeated exposure.

The physiological basis of habituation was first investigated by Charles Sherrington while studying posture and locomotion in the cat. Sherrington observed a decrease in the intensity of certain reflexes in response to repeated electrical stimulation of the motor pathways. He suggested that this decrease, which he called *habituation*, is caused by diminished synaptic effectiveness in the stimulated pathways. Habituation was later investigated at the cellular level by Alden Spencer and Richard Thompson. They found close cellular and behavioral parallels between habituation of a spinal flexion reflex in cats (the withdrawal of a limb from a noxious stimulus) and habituation of more complex human behaviors. They showed that during habituation the strength of the input from local excitatory interneurons onto motor neurons in the spinal cord decreased. Connections to interneurons from sensory neurons innervating the skin were unaffected.

Because the organization of interneurons in the vertebrate spinal cord is quite complex, it was difficult to analyze further the cellular mechanisms of habituation in the flexion reflex. Progress required a simpler system. The marine mollusk *Aplysia californica*, which has a simple nervous system of about 20,000 central neurons, proved to be an excellent system for studying implicit forms of memory.

Aplysia has a repertory of defensive reflexes for withdrawing its respiratory gill and siphon, a small fleshy spout above the gill used to expel seawater and waste (Figure 66–2A). These reflexes are similar to the withdrawal reflex of the leg studied by Spencer and Thompson. Mild touching of the siphon elicits reflex withdrawal of both the siphon and gill. With repeated stimulation these reflexes habituate. As we shall see, these responses can also be dishabituated, sensitized, and classically conditioned.

The neural circuit mediating the gill-withdrawal reflex in *Aplysia* has been studied in detail. Touching the siphon excites a population of mechanoreceptor sensory neurons that innervate the siphon. The release of glutamate from sensory neuron terminals generates fast excitatory postsynaptic potentials (EPSPs) in interneurons and motor cells. The EPSPs from the sensory cells and interneurons summate on motor cells both temporally and spatially, causing them to discharge strongly, thereby producing vigorous withdrawal of the gill. If the stimulus is repeated, the monosynaptic EPSPs produced by sensory neurons in both interneurons and motor cells progressively decrease, paralleling the habituation of gill withdrawal. In addition, repeated stimulation also leads to a decrease in the strength of synaptic transmission from the excitatory interneurons to the motor neurons; the net result is that the reflex response diminishes (Figure 66–2B,C).

What reduces the effectiveness of synaptic transmission between the sensory neurons and their postsynaptic cells during repeated stimulation? Quantal analysis revealed that the amount of glutamate released from presynaptic terminals of sensory neurons decreases. That is, fewer synaptic vesicles are released with each action potential in the sensory neuron; the sensitivity of the postsynaptic glutamate receptors does not change. Because the reduction in transmission occurs in the activated pathway itself and does not require another modulatory cell, the reduction is referred to as *homosynaptic depression*. This depression lasts many minutes.

An enduring change in the functional strength of synaptic connections thus constitutes the cellular mechanism mediating short-term habituation. As change of this type occurs at several sites in the gill-withdrawal reflex circuit, memory is distributed and stored throughout the circuit. Depression of synaptic transmission by sensory neurons, interneurons, or both is a common mechanism underlying habituation of escape responses of crayfish and cockroaches as well as startle reflexes in vertebrates.

How much can the effectiveness of a synapse change and how long can the change last? In *Aplysia* a single session of 10 stimuli leads to short-term habituation of the withdrawal reflex lasting minutes. Four sessions separated by periods ranging from several hours to 1 day produce long-term habituation, lasting as long as 3 weeks (Figure 66–3).

Anatomical studies indicate that long-term habituation is caused by a decrease in the number of synaptic contacts between sensory and motor neurons. In naïve animals 90% of the sensory neurons make physiologically detectable connections with identified motor neurons. In contrast, in animals trained for long-term habituation the incidence of connections is reduced to 30%; the reduction in number of synapses persists for a week and does not fully recover even 3 weeks later.

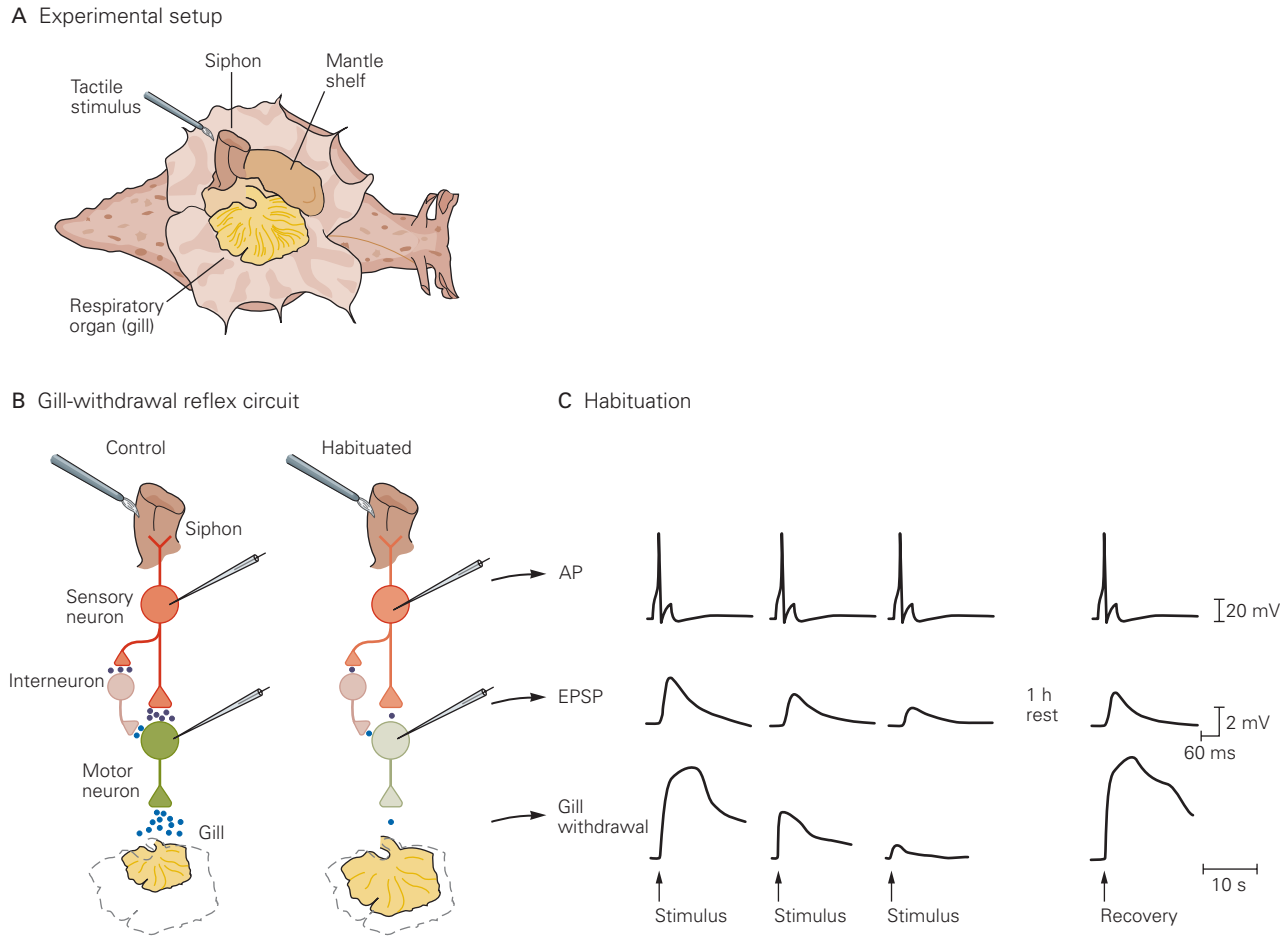


Figure 66–2 Short-term habituation of the gill-withdrawal reflex of the marine snail *Aplysia*.

A. A dorsal view of *Aplysia* illustrates the respiratory organ (gill) and the mantle shelf, which ends in the siphon, a fleshy spout used to expel seawater and waste. Touching the siphon elicits the gill-withdrawal reflex. Repeated stimulation leads to habituation.

B. This simplified circuit shows key elements of the gill-withdrawal reflex as well as sites involved in habituation. Approximately 24 mechanoreceptor neurons in the abdominal ganglion innervate the siphon skin. These sensory cells make excitatory synapses onto a cluster of six motor neurons that innervate the gill as well as on interneurons that modulate the firing of the motor neurons. (For simplicity only one of each type of neuron is illustrated here.) Touching the siphon leads to withdrawal of the gill (**dashed outline** shows original gill size; **solid outline** shows maximal withdrawal).

C. Repeated stimulation of the siphon sensory neuron **(top traces)** leads to a progressive depression of synaptic transmission between the sensory and motor neurons, seen as a decrease in size of the motor neuron EPSP with no change in the action potential (**AP**) in the presynaptic sensory neuron. In a separate experiment repeated stimulation of the siphon results in a decrease in gill withdrawal (habituation). One hour after repetitive stimulation both the EPSP and gill withdrawal have recovered. Habituation is now known to involve a decrease in transmitter release at many synaptic sites throughout the reflex circuit (part B). (Adapted, with permission, from Pinsker et al. 1970; Castellucci and Kandel 1974.)

As we shall see later, long-term sensitization of synaptic transmission is associated with an increase in the number of synapses between sensory and motor neurons.

Not all synapses are equally modifiable. In *Aplysia* the strength of some synapses rarely changes, even with repeated activation. In synapses specifically involved in learning (such as the connections between sensory and motor neurons in the withdrawal reflex circuit) a relatively small amount of training can produce large and enduring changes in synaptic strength.

Sensitization Involves Presynaptic Facilitation of Synaptic Transmission

When an animal repeatedly encounters a harmless stimulus, its responsiveness to the stimulus habituates.

In contrast, with a *harmful* stimulus the animal typically learns fear; it responds vigorously not only to the harmful stimulus but also to other concurrent stimuli, even harmless ones. As a result, defensive reflexes for withdrawal and escape become heightened. This enhancement of reflex responses is called *sensitization.*

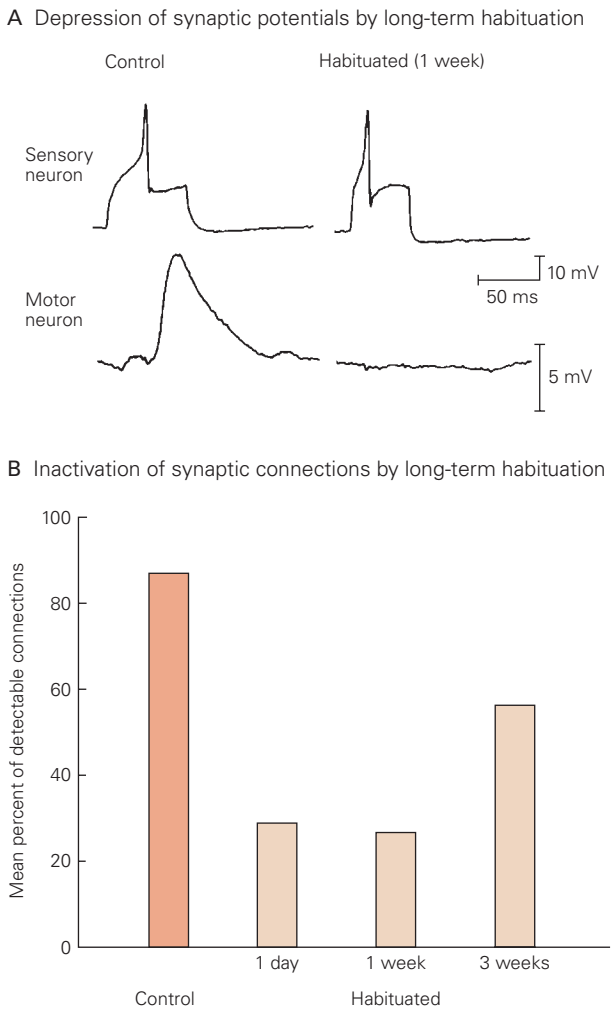


Figure 66–3 Long-term habituation of the gill-withdrawal reflex in *Aplysia.* (Adapted, with permission, from Castellucci, Carew, and Kandel 1978.)

A. Comparison of the action potentials and synaptic potentials in sensory and motor neurons, respectively, in an untrained animal (control) and one that has been subjected to long-term habituation. In the habituated animal 1 week after training no synaptic potential occurs in the motor neuron in response to the sensory neuron action potential.

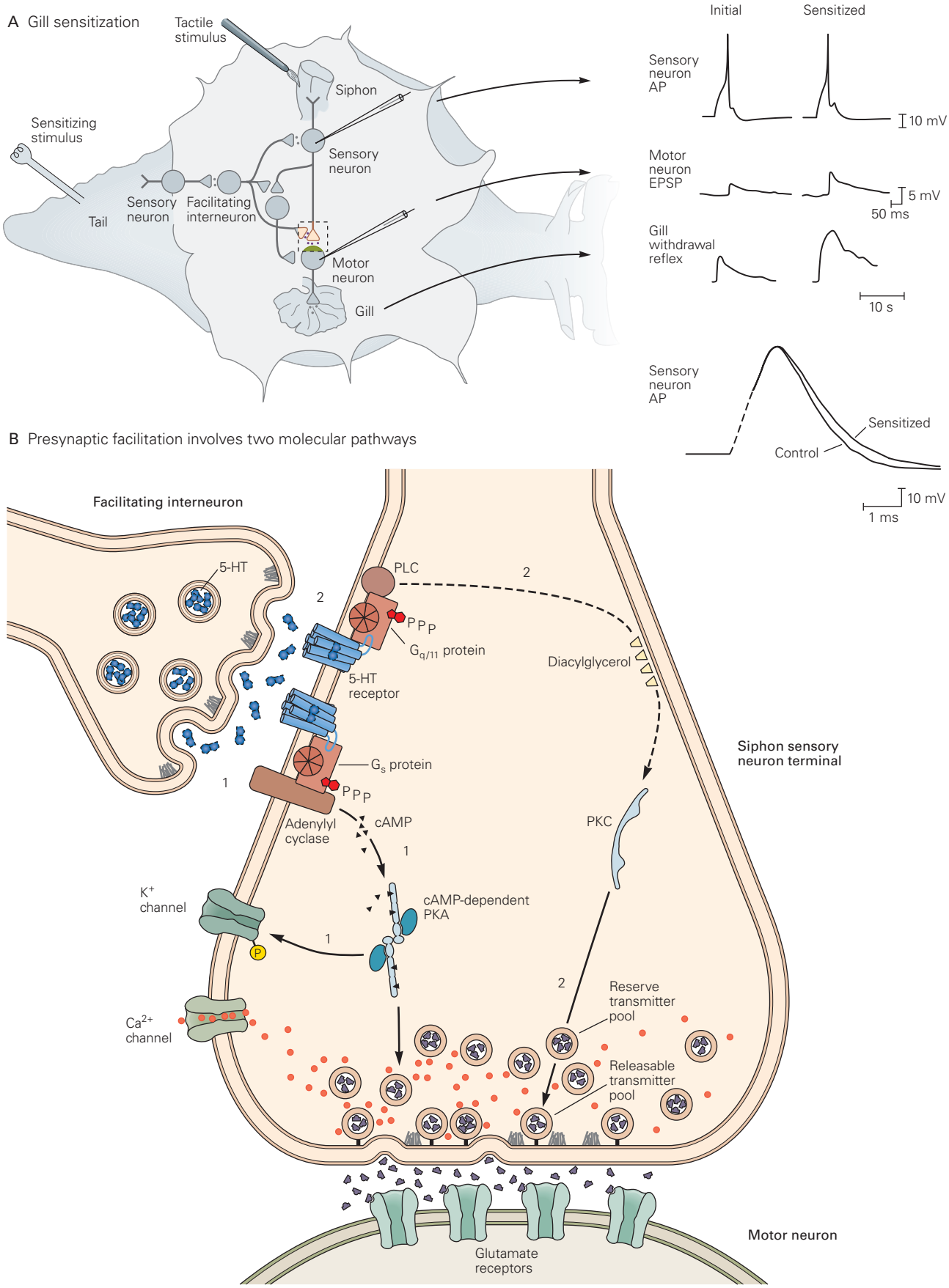
B. The mean percentage of sensory neurons making physiologically detectable connections with motor neurons in habituated animals is decreased at three points in time after long-term habituation training.

Like habituation, sensitization can be transient or long lasting. A single shock to the tail of an *Aplysia* produces short-term sensitization of the gill-withdrawal reflex that lasts minutes; five or more shocks to the tail produce sensitization lasting days to weeks. Tail shock is also sufficient to overcome the effects of habituation and enhance a habituated gill-withdrawal reflex, a process termed *dishabituation*.

Sensitization and dishabituation result from an enhancement in synaptic transmission at several connections in the neural circuit of the gill-withdrawal reflex, including the connections made by sensory neurons with motor neurons and interneurons—the same synapses depressed by habituation (Figure 66–4A). Typically, modifiable synapses can be regulated bidirectionally, participate in more than one type of learning, and store more than one type of memory. The bidirectional synaptic changes that underlie habituation and sensitization are the result of different cellular mechanisms. In *Aplysia* the same synapses that are weakened by habituation through a homosynaptic process can be strengthened by sensitization through a *heterosynaptic* process that depends on modulatory interneurons activated by the harmful stimulus to the tail.

At least three groups of modulatory interneurons are involved in sensitization. The best studied use serotonin as a transmitter. The serotonergic interneurons form synapses on many regions of the sensory neurons, including axo-axonic synapses on the presynaptic terminals of the sensory cells. The serotonin released from the interneurons after a single tail shock binds to a type of receptor in the sensory neurons that is coupled to a stimulatory G protein that increases the activity of adenylyl cyclase. This action produces the second messenger cyclic adenosine monophosphate (cAMP), which in turn activates the cAMP-dependent protein kinase (PKA) (see Chapter 11). Serotonin also activates a second type of G protein-coupled receptor that leads to the hydrolysis of phospholipids and the activation of protein kinase C (PKC).

The protein phosphorylation mediated by PKA and PKC enhances the release of transmitter from sensory neurons through at least two mechanisms (Figure 66–4B). In one action PKA phosphorylates a K^+ channel, causing it to close. This broadens the action potential and thus enhances Ca^{2+} influx through voltage-gated Ca^{2+} channels, which in turn enhances transmitter release. In a second action protein phosphorylation through PKC enhances the functioning of the release machinery directly. Presynaptic facilitation in response to release of serotonin by a tail shock lasts for a period of many minutes. Repeated noxious stimuli can strengthen synaptic activity for days.


A Gill sensitization
Tactile stimulus
Siphon
Sensitizing stimulus
Sensory neuron
Facilitating interneuron
Tail
Sensory neuron
Motor neuron
Gill
Initial
Sensitized
Sensory neuron AP
10 mV
Motor neuron EPSP
5 mV
50 ms
Gill withdrawal reflex
10 s
Sensory neuron AP
Sensitized
Control
10 mV
1 ms
B Presynaptic facilitation involves two molecular pathways
Facilitating interneuron
5-HT
PLC
2
P P P
$G_{q/11}$ protein
5-HT receptor
Diacylglycerol
G_s protein
P P P
Adenylyl cyclase
cAMP
PKC
1
K^+ channel
cAMP-dependent PKA
P
Siphon sensory neuron terminal
Ca^{2+} channel
Reserve transmitter pool
Releasable transmitter pool
Glutamate receptors
Motor neuron

Classical Conditioning of Fear Involves Coordinated Pre- and Postsynaptic Facilitation of Synaptic Transmission

Classical conditioning is a more complex form of learning. Rather than learning about the properties of one stimulus, as in habituation and sensitization, the animal learns to associate one type of stimulus with another. As described in Chapter 65, an initial weak conditioned stimulus (such as the ringing of a bell) becomes highly effective in producing a response when paired with a strong unconditioned stimulus (such as presentation of food). In reflexes that can be enhanced by both classical conditioning and sensitization, like the defensive withdrawal reflexes of *Aplysia,* classical conditioning results in greater and longer-lasting enhancement.

For classical conditioning of the *Aplysia* gill-withdrawal reflex, a weak touch to the siphon serves as the conditioned stimulus while a strong shock to the tail serves as the unconditioned stimulus. When the gill withdrawal reflex is classically conditioned, gill withdrawal in response to siphon stimulation alone is greatly enhanced. This enhancement is even more dramatic than the enhancement produced in an unpaired pathway by tail shock alone (sensitization). In classical conditioning the timing of the conditioned and unconditioned stimuli is critical. To be effective, the conditioned stimulus (siphon touch) must *precede* (and predict) the unconditioned stimulus (tail shock), often within an interval of about 0.5 seconds (Figure 66–5).

The convergence in individual sensory neurons of the signals initiated by the conditioned and unconditioned stimuli is critical. A strong shock to the tail excites serotonergic interneurons that form synapses on presynaptic terminals of the siphon sensory neurons, resulting in presynaptic facilitation associated with sensitization (Figure 66–5A). However, when the tail shock (unconditioned stimulus) immediately follows a slight tap on the siphon (conditioned stimulus), the serotonin from the interneurons produces even greater presynaptic facilitation, a process termed *activity-dependent facilitation* (Figure 66–5B).

How does this work? During conditioning the modulatory interneurons activated by tail shock release serotonin shortly *after* siphon touch has triggered an action potential in the sensory neurons. The action potential triggers an influx of Ca^{2+} into the sensory neuron's presynaptic terminals, the Ca^{2+} binds to calmodulin, and the complex in turn binds to adenylyl cyclase. This primes the adenylyl cyclase so that it responds more vigorously to the serotonin released following the unconditioned stimulus at the tail. As a result, the production of cAMP is enhanced, which increases the amount of presynaptic facilitation. If the order of stimuli is reversed so that serotonin release precedes Ca^{2+} influx in the presynaptic terminals, there is no potentiation and no classical conditioning.

Thus the cellular mechanism of classical conditioning in the monosynaptic pathway of the withdrawal reflex is largely an elaboration of the mechanism of sensitization, with the added feature that the adenylyl cyclase serves as a *coincidence detector* in the presynaptic sensory neuron, recognizing the temporal order of

Figure 66–4 (Opposite) Short-term sensitization of the gill-withdrawal reflex in *Aplysia*.

A. Sensitization of the gill-withdrawal reflex is produced by applying a noxious stimulus to another part of the body, such as the tail. A shock to the tail activates tail sensory neurons that excite facilitating (modulatory) interneurons, which form synapses on the cell body and terminals of the mechanoreceptor sensory neurons that innervate the siphon. Through these axo-axonic synapses the modulatory interneurons enhance transmitter release from the siphon sensory neurons onto their postsynaptic gill motor neurons (presynaptic facilitation), thus enhancing gill withdrawal. Presynaptic facilitation results, in part, from a prolongation of the sensory neuron action potential (**bottom traces**). (Adapted, with permission, from Pinsker et al. 1970; Klein and Kandel 1980.)

B. Presynaptic facilitation in the sensory neuron is thought to occur by means of two biochemical pathways. The diagram shows details of the synaptic complex in the **dashed box** in part A.

Pathway 1: A facilitating interneuron releases serotonin (**5-HT**), which binds to metabotropic receptors in the sensory neuron terminal. This action engages a G protein (G_s), which in turn increases the activity of adenylyl cyclase. The adenylyl cyclase converts ATP to cAMP, which binds to the regulatory subunit of PKA, thus activating its catalytic subunit. The catalytic subunit phosphorylates certain K^+ channels, thereby closing the channels and decreasing the outward K^+ current. This prolongs the action potential, thus increasing the influx of Ca^{2+} through voltage-gated Ca^{2+} channels and thereby augmenting transmitter release.

Pathway 2: Serotonin binds to a second class of metabotropic receptor that activates the $G_{q/11}$ class of G protein that enhances the activity of phospholipase C (**PLC**). The PLC activity leads to production of diacylglycerol, which activates protein kinase C (**PKC**). Phosphorylation of presynaptic proteins by PKC results in the mobilization of vesicles containing glutamate from a reserve pool to a releasable pool at the active zone, increasing the efficiency of transmitter release.

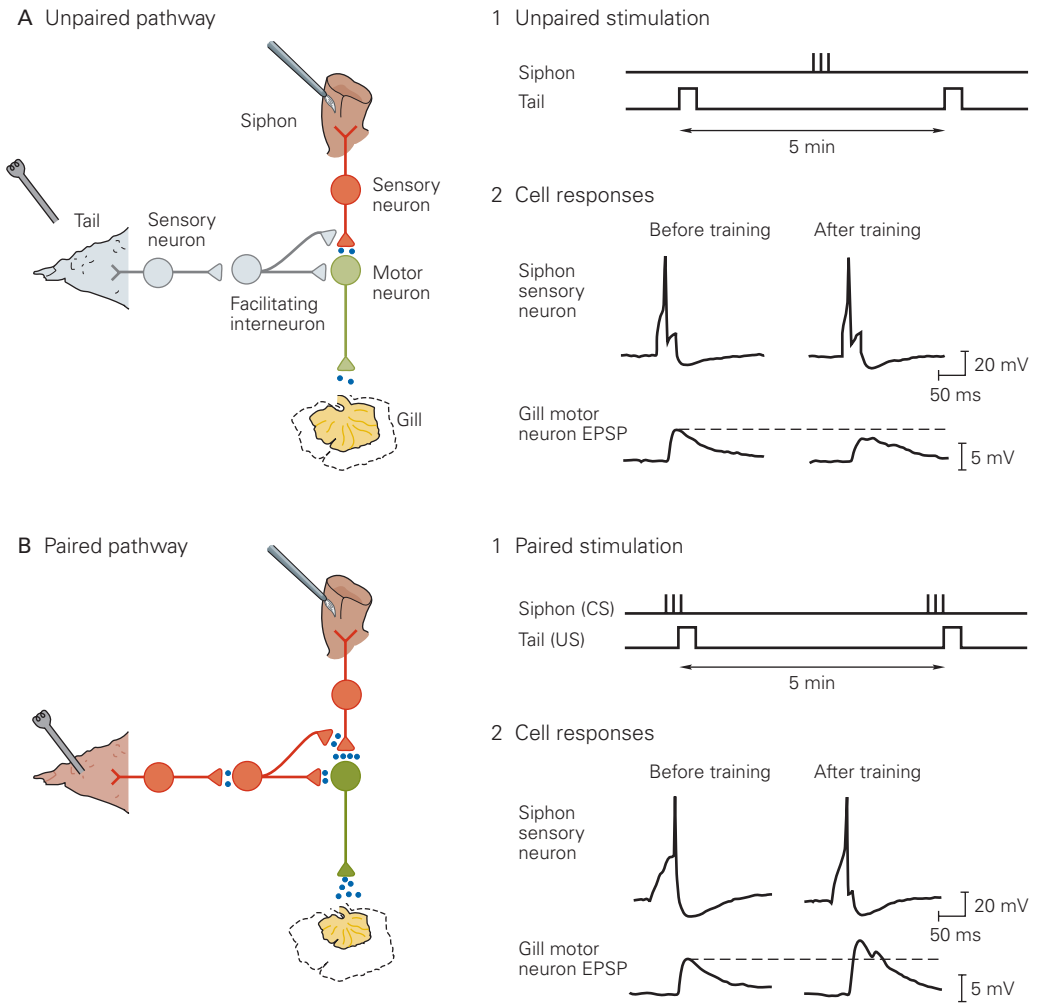


Figure 66–5 Classical conditioning of the gill-withdrawal reflex in *Aplysia*. (Adapted, with permission, from Hawkins et al. 1983.)

A. The siphon is stimulated by a light touch and the tail is shocked, but the two stimuli are not paired in time. The tail shock excites facilitatory interneurons that form synapses on the presynaptic terminals of sensory neurons innervating the mantle shelf and siphon. This is the mechanism of sensitization. 1. The pattern of unpaired stimulation during training. 2. Under these conditions the size of the motor neuron EPSP is only weakly facilitated by the tail shock. In this example the EPSP actually decreases slightly despite the tail shock because repeated unpaired stimulation of the siphon leads to synaptic depression.

B. The tail shock is paired with stimulation of the siphon. 1. The siphon is touched (conditioned stimulus or CS) immediately prior to shocking the tail (unconditioned stimulus or US). As a result, the siphon sensory neurons are primed to be more responsive to input from the facilitatory interneurons in the unconditioned pathway. This is the mechanism of classical conditioning; it both amplifies the response of the conditioned pathway and restricts the amplification to that pathway. 2. Recordings of EPSPs in an identified motor neuron produced by the siphon sensory neurons before training and one hour after training. After training the EPSP in the motor neuron produced by paired sensory input is considerably greater than either the EPSP before training or the EPSP following unpaired tail shock (shown in part A2). This produces a more vigorous gill withdrawal.

the physiological representations of both the unconditioned stimulus (tail shock) and conditioned stimulus (siphon touch).

In addition to the presynaptic component of activity-dependent facilitation, a postsynaptic component is triggered by Ca^{+} influx into the motor neuron when it is highly excited by the siphon sensory neurons. The properties of this postsynaptic mechanism are similar to those of long-term potentiation of synaptic transmission in the mammalian brain (discussed later in this chapter and in Chapter 67).

Long-Term Storage of Implicit Memory Involves Changes in Chromatin Structure and Gene Expression Mediated by the cAMP-PKA-CREB Pathway

Cyclic AMP Signaling Has a Role in Long-Term Sensitization

In all forms of learning practice makes perfect. Repeated experience converts short-term memory into a long-term form. In *Aplysia* the form of long-term memory that has been most intensively studied is long-term sensitization. Like the short-term form, long-term sensitization of the gill-withdrawal reflex involves changes in the strength of connections at several synapses, including those between sensory and motor neurons. However, it also involves the growth of new synaptic connections.

Five spaced training sessions (or repeated applications of serotonin) over approximately 1 hour produce long-term sensitization and long-term synaptic facilitation lasting 1 or more days; continued spaced training over several days produces sensitization that persists for 1 or more weeks. Long-term sensitization, like the short-term form, requires protein phosphorylation that is dependent on increased levels of cAMP (Figure 66–6).

The conversion of short-term memory into long-term memory, called *consolidation,* requires synthesis of messenger RNAs and proteins in the neurons in the circuit. Thus specific gene expression is required for long-term memory. The transition from short-term to long-term memory depends on the prolonged rise in cAMP that follows repeated applications of serotonin. This leads to prolonged activation of PKA, allowing the catalytic subunit of the kinase to translocate into the nucleus of the sensory neurons. It also leads indirectly to activation of a second protein kinase, the mitogen-activated protein kinase (MAPK), a kinase commonly associated with cellular growth (see Chapter 11). Within the nucleus the catalytic subunit of PKA phosphorylates and thereby activates the transcription factor CREB-1 (*c*AMP *r*esponse *e*lement *b*inding protein 1), which binds a promoter element called CRE (*c*AMP *r*ecognition *e*lement) (Figures 66–6 and 66–7).

To turn on gene transcription, phosphorylated CREB-1 recruits a transcriptional coactivator, CREB-binding protein (CBP), to the promoter region. CBP has two important properties that facilitate transcriptional activation: it recruits RNA polymerase II to the promoter, and it functions as an acetyltransferase, adding acetyl groups to certain lysine residues on its substrate proteins. One of the most important substrates of CBP are the histone proteins, which are components of nucleosomes, the fundamental building blocks of chromatin. The histones contain a series of positively charged basic residues that strongly interact with the negatively charged phosphates of DNA. This interaction causes DNA to become tightly wrapped around the nucleosomes, much like string is wrapped around a spool, thereby preventing necessary transcription factors from accessing their gene targets.

The binding of CBP to CREB-1 leads to histone acetylation, which causes a number of important structural and functional changes at the nucleosome level. For example, acetylation neutralizes the positive charge of lysine residues in the histone tail domains, decreasing the affinity of histones for DNA. Also, specific classes of transcriptional activators can bind to acetylated histones and facilitate the repositioning of nucleosomes at the promoter region. Together these and other types of chromatin modifications serve to regulate the accessibility of chromatin to the transcriptional machinery, and thus enhance the ability of a gene to be transcribed. As we will see in Chapter 67, a mutation in the gene encoding CBP underlies Rubinstein-Taybi syndrome, a disorder associated with mental retardation.

The turning on of transcription by PKA also depends on its ability to indirectly activate the MAPK pathway (see Chapter 11). MAPK phosphorylation of the transcription factor CREB-2 relieves an inhibitory action of CREB-2 on transcription (Figure 66–6B). The combined effects of CREB-1 activation and relief of CREB-2 repression induces a cascade of new gene expression important for learning and memory (Figure 66–7).

The presence of both a repressor (CREB-2) and an activator (CREB-1) of transcription at the first step in long-term facilitation suggests that the threshold for long-term memory storage can be regulated. Indeed, we see in everyday life that the ease with which short-term memory is transferred into long-term memory varies greatly with attention, mood, and social context.

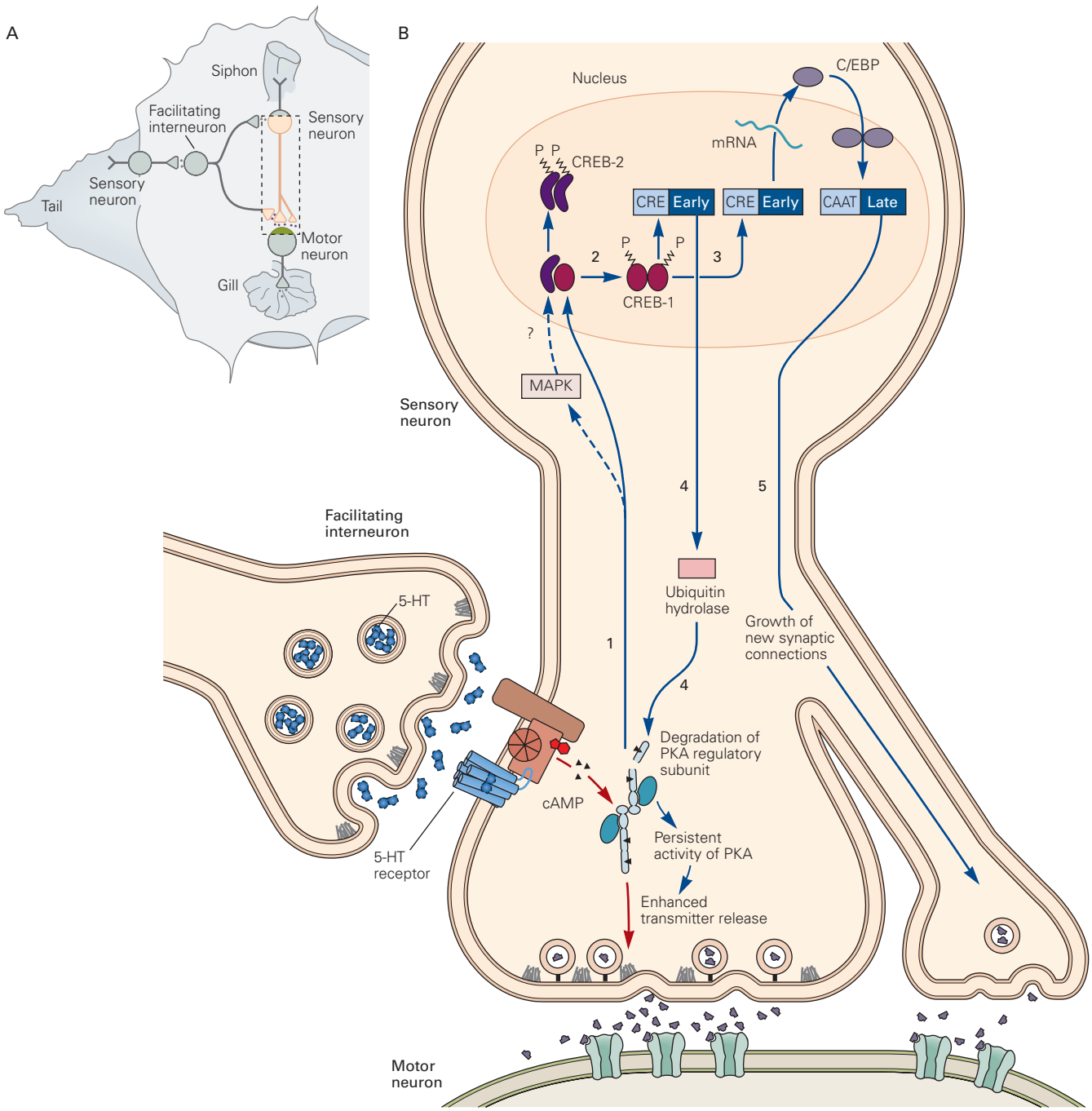


Figure 66–6 Long-term sensitization involves synaptic facilitation and the growth of new synaptic connections.

A. Long-term sensitization of the gill-withdrawal reflex of *Aplysia* following repeated tail shocks involves long-lasting facilitation of transmitter release at the synapses between sensory and motor neurons.

B. Long-term sensitization of the gill-withdrawal reflex leads to persistent activity of PKA, resulting in the growth of new synaptic connections. Repeated tail shock leads to more pronounced elevation of cAMP, producing long-term facilitation (lasting 1 or more days) that outlasts the increase in cAMP and recruits the synthesis of new proteins. This inductive mechanism is initiated by translocation of PKA to the nucleus (**pathway 1**), where PKA phosphorylates the transcriptional activator CREB-1 (cAMP response element binding protein 1) (**pathway 2**). CREB-1 binds cAMP regulatory elements (CRE) located in the upstream region of several cAMP-inducible genes, activating gene transcription (**pathway 3**). PKA also activates the mitogen-activated protein kinase **(MAPK)**, which phosphorylates the transcriptional repressor CREB-2 (cAMP response element binding protein 2), thus removing its repressive action. One gene activated by CREB-1 encodes a ubiquitin hydrolase, a component of a specific ubiquitin proteasome that leads to the proteolytic cleavage of the regulatory subunit of PKA, resulting in persistent activity of PKA, even after cAMP has returned to its resting level (**pathway 4**). CREB-1 also activates the expression of the transcription factor C/EBP, which leads to expression of a set of unidentified proteins important for the growth of new synaptic connections (**pathway 5**).

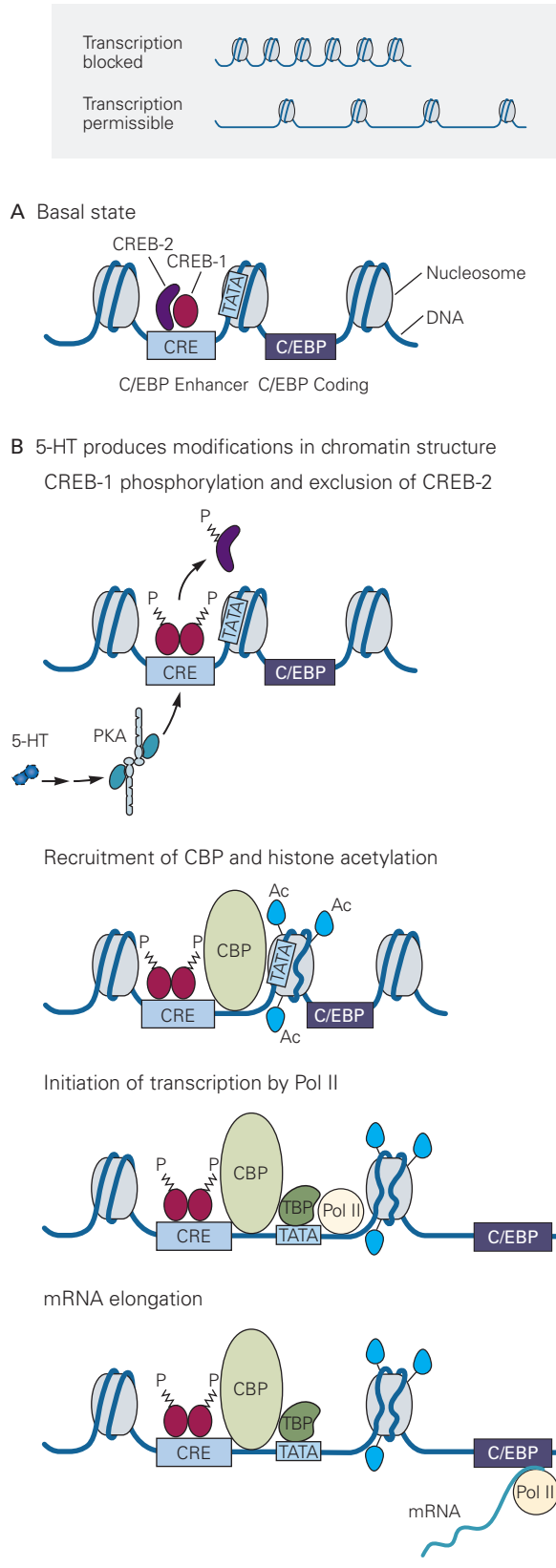


Figure 66–7 Regulation of histone acetylation by serotonin, CREB-1, and CBP.

A. Under basal conditions the activator CREB-1 (here in complex with CREB-2) occupies the binding site for CRE (cAMP recognition element) within the promoter region of its target genes. In the example shown here, CREB-1 binds to the CRE within the C/EBP promoter. In the basal state CREB-1 binding is not able to activate transcription because the TATA box, the core promoter region responsible for recruiting RNA polymerase II (**Pol II**) during transcription initiation, is inaccessible because the DNA is tightly bound to histone proteins in the nucleosome.

B. Serotonin (**5-HT**) activates PKA, which phosphorylates CREB-1 and indirectly enhances CREB-2 phosphorylation by MAPK, causing the repressor to dissociate from the promoter. This allows CREB-1 to form a complex at the promoter with CREB binding protein (**CBP**). Activated CBP acetylates specific lysine residues of the histones, causing them to bind less tightly to DNA. Along with other changes in chromatin structure, acetylation facilitates the repositioning of the nucleosome that previously blocked access of the Pol II complex to the TATA box. This repositioning allows Pol II to be recruited to initiate transcription of the C/EBP gene. (**TBP**, TATA binding protein).

Two of the genes expressed in the wake of CREB-1 activation and the consequential alteration in chromatin structure are important in the early development of long-term facilitation. One is a gene for ubiquitin carboxyterminal hydrolase. The other is a gene for a transcription factor, CAAT box enhancer binding protein (C/EBP), a component of a gene cascade necessary for synthesizing proteins needed for the growth of new synaptic connections (Figure 66–6, 66–7).

The hydrolase, which facilitates ubiquitin-mediated protein degradation (see Chapter 3), helps enhance activation of PKA. Protein kinase A is made up of four subunits; two regulatory subunits inhibit two catalytic subunits (see Chapter 11). With long-term training and the induction of the hydrolase, approximately 25% of the regulatory subunits are degraded in the sensory neurons. As a result, free catalytic subunits can continue to phosphorylate proteins important for the enhancement of transmitter release and the strengthening of synaptic connections, including CREB-1, long after cAMP has returned to its basal level (Figure 66–6B). Formation of a constitutively active enzyme is therefore the simplest molecular mechanism for long-term memory. With repeated training a second-messenger kinase critical for short-term facilitation can remain persistently active for up to 24 hours without requiring a continuous activating signal.

The second and more enduring consequence of CREB-1 activation is the activation of the transcription factor C/EBP. This transcription factor forms both a homodimer with itself and a heterodimer with another transcription factor called *activating factor*. Together these factors act on downstream genes that trigger the growth of new synaptic connections that support long-term memory.

With long-term sensitization the number of presynaptic terminals in the sensory neurons in the gill-withdrawal circuit doubles (Figure 66–8). The dendrites of the motor neurons also grow to accommodate the additional synaptic input. Thus long-term structural changes in both post- and presynaptic cells increase the number of synapses.

Long-term habituation, in contrast, leads to *pruning* of synaptic connections. The long-term inactivation of the functional connections between sensory and motor neurons reduces the number of terminals of each sensory neuron by one-third (Figure 66–8A).

Long-Term Synaptic Facilitation Is Synapse Specific

A typical neuron in the mammalian brain makes 10,000 synapses with a wide range of target cells. It is therefore generally thought that long-term memory storage should be synapse specific—that is, only those synapses that actively participate in learning should be enhanced. However, the finding that long-term facilitation involves gene expression—which occurs in the nucleus, an organelle that is far removed from a neuron's synapses—raises some fundamental questions regarding information storage.

Is long-term memory storage indeed synapse specific, or do the gene products recruited during long-term memory storage alter the strength of every presynaptic terminal in a neuron? And if long-term memory is synapse specific, what are the cellular mechanisms that enable the products of gene transcription to selectively strengthen just some synapses and not others?

Kelsey Martin and her colleagues addressed these questions regarding long-term facilitation by using a cell culture system consisting of an isolated *Aplysia* sensory neuron with a bifurcated axon that makes separate synaptic contacts with two motor neurons. The sensory neuron terminals on one of the two motor neurons were activated by focal pulses of serotonin, thus mimicking the neural effects of a shock to the tail. When only one pulse of serotonin was applied, those synapses showed short-term facilitation. The synapses on the second motor neuron, which did not receive serotonin, showed no change in synaptic transmission (Figure 66–9).

When five pulses of serotonin were applied to the same synapses, those synapses displayed both short-term and long-term facilitation, and new synaptic connections were formed with the motor neuron. Again the synapses that did not receive serotonin showed no enhancement of synaptic transmission (Figure 66–9B). Thus both short-term and long-term synaptic facilitation are synapse specific and manifested only by those synapses that receive the modulatory serotonin signal.

But how are the nuclear products able to enhance transmission at certain synapses only? Are the newly synthesized proteins somehow targeted to only those synapses that receive serotonin? Or are they shipped out to all synapses but used productively for the growth of new synaptic connections only at those synapses that have been activated—or marked—perhaps by only a single pulse of serotonin?

To test this question Martin and her colleagues again selectively applied five pulses of serotonin to the synapses made by the sensory neuron onto one of the motor neurons. This time, however, the synapses with the second motor neuron were simultaneously activated by a single pulse of serotonin (which by itself produces only short-term synaptic facilitation lasting

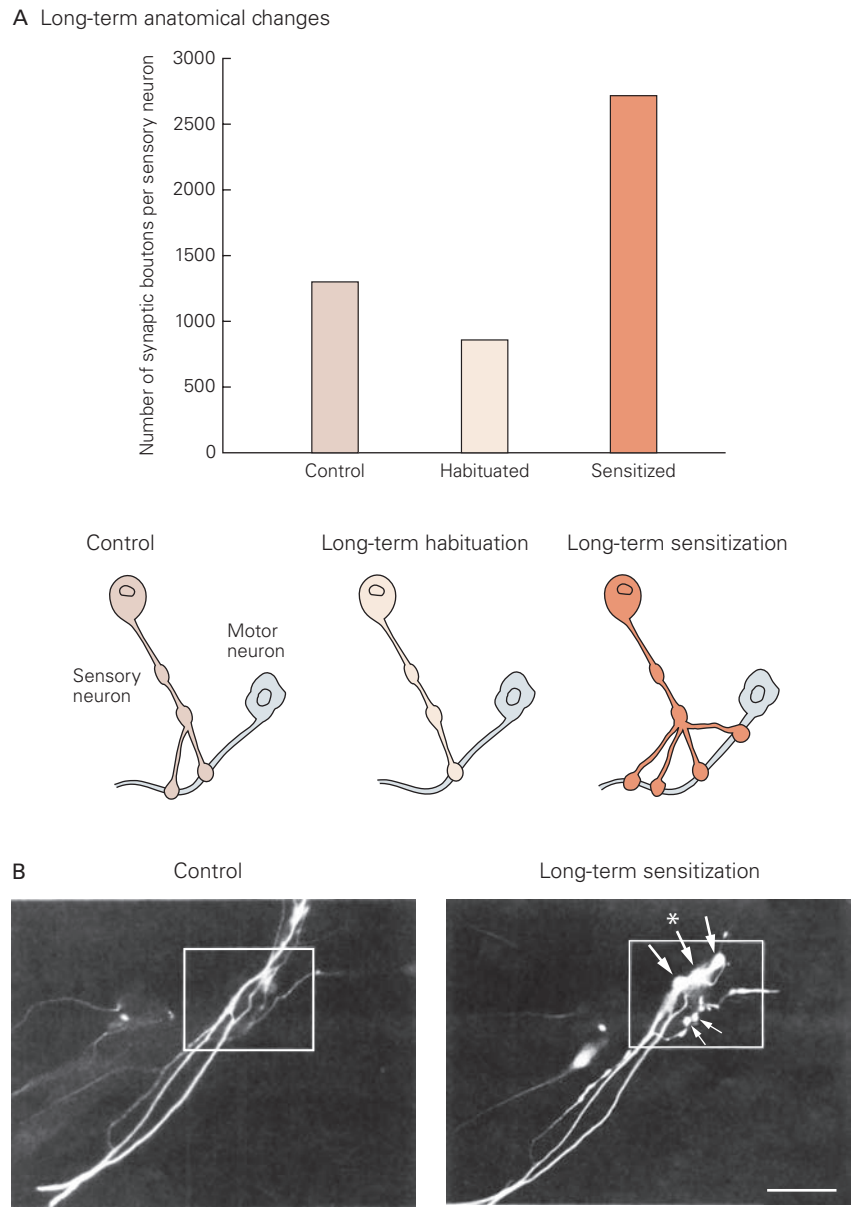


Figure 66–8 Long-term habituation and sensitization involve structural changes in the presynaptic terminals of sensory neurons.

A. Long-term habituation leads to a loss of synapses, and long-term sensitization leads to an increase in number of synapses. When measured either 1 day (shown here) or 1 week after training, the number of presynaptic terminals (or boutons) relative to control levels is increased in sensitized animals and reduced in habituated animals. The drawings below the graph illustrate changes in the number of synaptic contacts. Swellings or varicosities on sensory neuron processes are presynaptic terminals. (Adapted, with permission, from Bailey and Chen 1983.)

B. Fluorescence images of the axon of a sensory neuron contacting a motor neuron in culture before (**left**) and 1 day after (**right**) five brief exposures to serotonin. The resulting increase in varicosities simulates the synaptic changes associated with long-term sensitization. Prior to serotonin application no presynaptic varicosities are visible in the outlined area (control). After serotonin the growth of several new varicosities is apparent (**arrows**), indicative of formation of new synapses. Boutons can be seen at the arrows in the right, some of which contain a fully developed zone, identified by the asterisk, or have small, immature active zones. Scale bar = 50 μm. (Reproduced, with permission, from Glanzman, Kandel, and Schacher 1990.)

minutes). Under these conditions the single pulse of serotonin was sufficient to induce long-term facilitation and growth of new synaptic connections at the contacts between the sensory neuron and the second motor neuron. Thus application of the single pulse of serotonin onto the synapses at the second branch enabled those synapses to use the nuclear products produced in response to the five pulses of serotonin onto the synapses of the first branch, a process called *capture*.

These results suggest that newly synthesized gene products, both mRNAs and proteins, are delivered by a fast axonal transport mechanism to all the synapses of a neuron but are functionally incorporated only at synapses that have been tagged or marked by previous synaptic activity.

For a synapse to use the new proteins and mRNAs for long-term facilitation, it must first be marked by serotonin. Although one pulse of serotonin at a synapse is insufficient to turn on new gene expression in the cell body, it is sufficient to allow that synapse to make productive use of new proteins generated in the soma in response to the five pulses of serotonin at another synapse. This idea, developed by Martin and

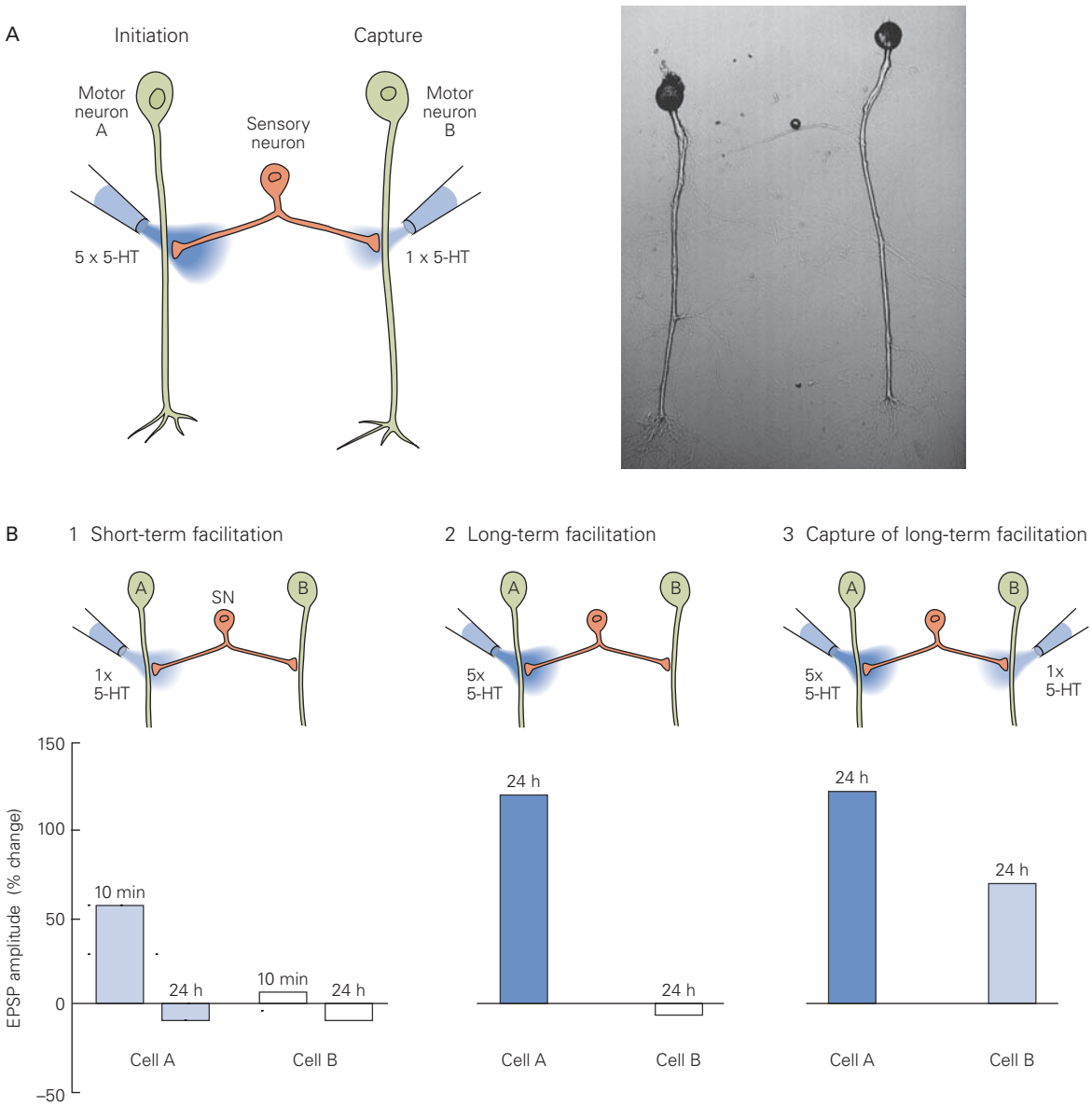


Figure 66–9 **Long-term facilitation of synaptic transmission is synapse-specific.** (Adapted, with permission, from Martin et al. 1997.)

A. The experiment uses a single presynaptic sensory neuron that contacts two postsynaptic motor neurons (**A** and **B**). The pipette on the left is used to apply five pulses of serotonin (**5-HT**) to a sensory neuron synapse with motor neuron A, initiating long-term facilitation at these synapses. The pipette on the right is used to apply one pulse of 5-HT to a sensory neuron synapse with motor neuron B, allowing this synapse to make use of (capture) new proteins produced in the cell body in response to the five pulses of 5-HT at the synapses with motor neuron A. The image at the right shows the actual appearance of the cells in culture.

B. 1. One pulse of 5-HT applied to the synapses with motor neuron A produces only short-term (10 min) facilitation of the excitatory postsynaptic potential (EPSP) in the neuron. By 24 hours the EPSP has returned to its normal size. There is no significant change in EPSP size in cell B. **2**. Application of five pulses of 5-HT to the synapses with cell A produces long-term (24 hour) facilitation of the EPSP in that cell but no change in the size of the EPSP in cell B. **3**. However, when five pulses of 5-HT onto the synapses with cell A are paired with a single pulse of 5-HT onto the synapses with cell B, cell B displays long-term facilitation and an increase in EPSP size after 24 hours.

her colleagues for *Aplysia* and independently by Frey and Morris for the hippocampus in rodents, is called *synaptic capture* or *synaptic tagging*.

These findings raise the question, what is the nature of the synaptic mark that allows the capture of the gene products for long-term facilitation? When an inhibitor of PKA was applied locally to the synapses receiving the single pulse of serotonin, those synapses could no longer capture the gene products produced in response to the five pulses of serotonin (Figure 66–10). This indicates that phosphorylation mediated by PKA is required for capturing the long-term process.

In the early 1980s Oswald Steward discovered that ribosomes, the machinery for protein synthesis, are situated locally at the synapse in addition to being present in the cell body. Martin examined the importance of local protein synthesis in long-term synaptic facilitation by applying a single pulse of serotonin together with an inhibitor of local protein synthesis onto one set of synapses while simultaneously applying five pulses of serotonin to the other set of synapses. Normally long-term facilitation and synaptic growth would persist for up to 72 hours in response to synaptic capture. In the presence of the protein synthesis inhibitor, synaptic capture could still generate long-term synaptic facilitation at the synapses exposed to only one pulse of serotonin for at least 24 hours (Figure 66–10B), but synaptic growth and facilitation at these synapses collapsed after 24 hours, indicating that the maintenance of learning-induced

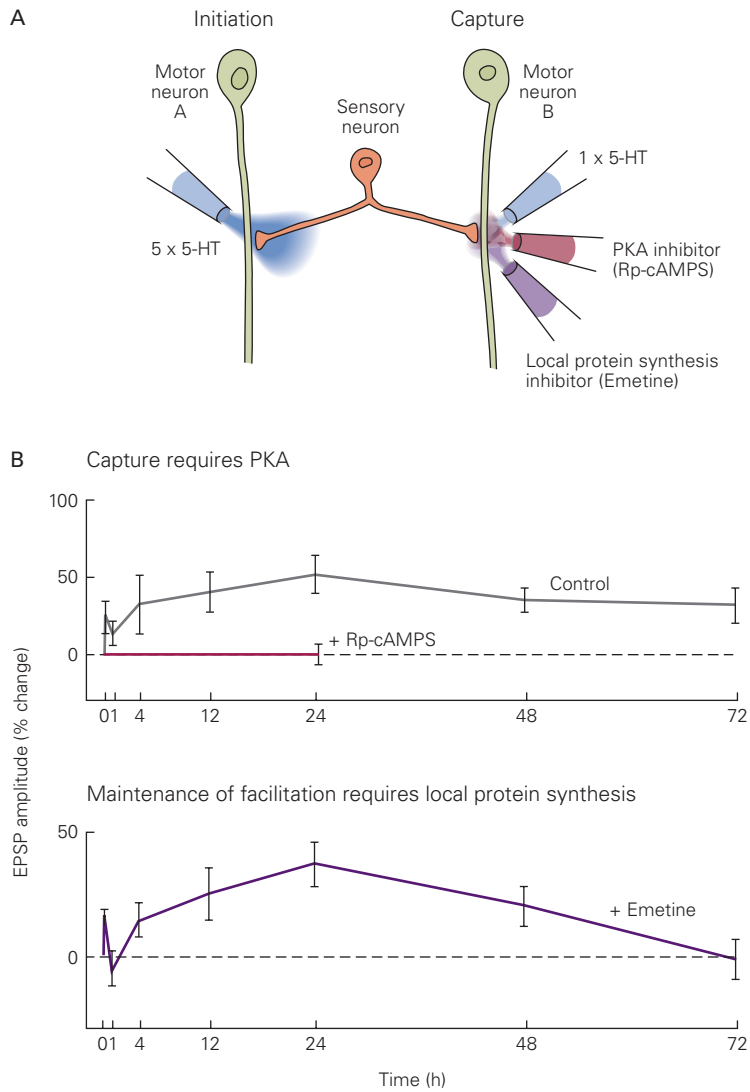


Figure 66–10 Long-term facilitation requires both cAMP-dependent phosphorylation and local protein synthesis. (Adapted, with permission, from Casadio et al., 1999.)

A. Five pulses of serotonin (**5-HT**) are applied to the synapses on motor neuron A and a single pulse is applied to those of cell B. Inhibitors of protein kinase A (**Rp-cAMPS**) or local protein synthesis (**emetine**) are applied to synapses on cell B.

B. Rp-cAMPS blocks the capture of long-term facilitation completely at the synapses on neuron B. Emetine has no effect on the capture of facilitation or the growth of new synaptic connections measured 24 hours after 5-HT application, but by 72 hours it fully blocks synaptic enhancement. The outgrowth of new synaptic connections is retracted and long-term facilitation decays after 1 day if capture is not maintained by local protein synthesis. (**Rp-cAMPS**, Rp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate.)

synaptic growth requires new local protein synthesis at the synapse.

Martin and her colleagues thus found that regulation of protein synthesis at the synapse plays a major role in controlling synaptic strength at the sensory-to-motor neuron connection in *Aplysia.* As we shall see in Chapter 67, local protein synthesis is also important for the later phases of long-term potentiation of synaptic strength in the hippocampus.

These findings indicate there are two distinct components of synaptic marking in *Aplysia.* The first component, lasting about 24 hours, initiates long-term synaptic plasticity and synaptic growth, requires transcription and translation in the nucleus, and recruits local PKA activity, but does not require local protein synthesis. The second component, which stabilizes the long-term synaptic change after 72 hours, requires local protein synthesis at the synapse. How might this local protein synthesis be regulated?

Long-Term Facilitation Requires a Prion-Like Protein Regulator of Local Protein Synthesis for Maintenance

The fact that mRNAs are translated at the synapse in response to marking of that synapse by one pulse of serotonin suggests that these mRNAs may initially be dormant and under the control of a regulator of translation recruited by serotonin. Joel Richter found that in *Xenopus* (frog) oocytes the maternal mRNAs have a short tail of adenine nucleotides, poly(A), at their 3′ end and are silent until activated by the cytoplasmic polyadenylation element binding protein (CPEB), which binds to a site on mRNAs and recruits poly(A) polymerase, leading to the elongation of the poly(A) tail. Kausik Si and his colleagues found that serotonin increases the local synthesis of a novel, neuron-specific isoform of CPEB in *Aplysia* sensory neuron processes (Figure 66–11). The induction of CPEB is independent of transcription but requires new protein synthesis. Blocking CPEB locally at an activated synapse blocks the long-term maintenance of synaptic facilitation at the synapse but not its initiation and maintenance for 24 hours.

How might CPEB stabilize the late phase of long-term facilitation? Most biological molecules have a relatively short half-life (hours to days) compared to the duration of memory (days, weeks, even years). How then can the learning-induced alterations in the molecular composition of a synapse be maintained for such a long time? Most hypotheses rely on some type of self-sustained mechanism that can somehow modulate synaptic strength and synaptic structure.

Si and his colleagues found that the neuronal isoform of *Aplysia* CPEB indeed appears to have self-sustaining properties that resemble those of prion proteins. Prions were discovered by Stanley Prusiner, who demonstrated that these proteins were the causative agents of Jacob-Creutzfeldt disease, a terrible neurodegenerative human disease, and of mad cow disease. Prion proteins can exist in a soluble form and an aggregated form that is capable of self-perpetuation. *Aplysia* CPEB also has two conformational states, a soluble form that is inactive and an aggregated form that is active. This switch involves an N-terminal domain of CPEB that is rich in glutamine, similar to prion domains in other proteins.

In a naïve synapse CPEB exists in the soluble, inactive state, and its basal level of expression is low. However, in response to serotonin the local synthesis of CPEB increases until a threshold concentration is reached that switches CPEB to the aggregated, active state, which is then capable of activating the translation of dormant mRNAs. Once the active state is established, it becomes self-perpetuating by recruiting soluble CPEB to aggregates. Dormant mRNAs, made in the cell body and distributed cell-wide, are translated only at synapses with active CPEB.

Because the activated CPEB is self-perpetuating, it could promote a self-sustaining, synapse-specific long-term molecular change and provide a mechanism for the stabilization of learning-related synaptic growth and the persistence of memory storage (Figure 66–11). This proposed mechanism, albeit self-perpetuating, is different from conventional prion mechanisms, which are pathogenic (the aggregated state of most prion proteins causes cell death). By contrast, CPEB is a new form of a prion-like protein. It is a functional prion; the active self-perpetuating form of the protein does not kill cells but rather has an important physiological function.

Classical Fear Conditioning in Flies Uses the cAMP-PKA-CREB Pathway

Do the mechanisms for implicit memory found in *Aplysia* have parallels in other animals? Studies on fear learning in both the fruit fly *Drosophila* and mouse indicate that the molecular mechanisms of implicit memory are conserved throughout evolution.

The fruit fly is particularly convenient for the study of implicit memory storage because its genome is easily manipulated and, as first demonstrated by Seymour Benzer and his colleagues, the fly can be classically conditioned.

In a typical classical conditioning paradigm an odor is paired with repeated electrical foot shocks. The extent of learning is then examined by allowing

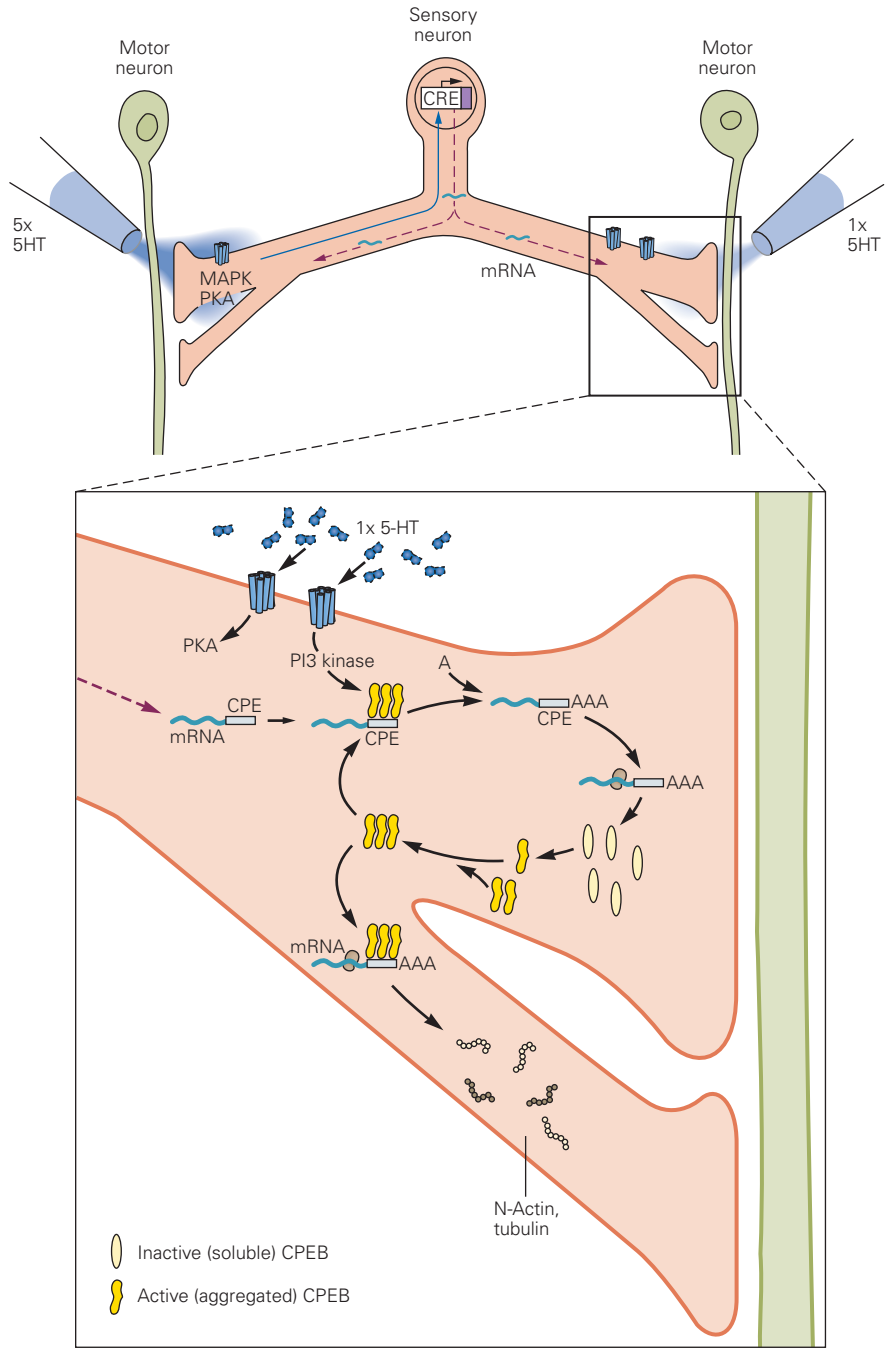


Figure 66–11 CPEB may be a self-perpetuating switch of protein synthesis at axon terminals and synapse-specific growth. According to this model (based on Bailey, Kandel, and Si, 2004) five pulses of serotonin (**5-HT**) set up a signal that goes back to the nucleus to activate synthesis of mRNA. Newly transcribed mRNAs and newly synthesized proteins made in the cell body are then sent to all terminals by fast axonal transport. However, only those terminals that have been marked by exposure to at least one pulse of serotonin can use the proteins productively to grow new synapses and produce long-term facilitation. The marking of a terminal involves two components: (**1**) protein kinase A (**PKA**), which is necessary for the immediate synaptic growth initiated by the proteins transported to the terminals, and (**2**) phosphoinositide 3 kinase (**PI3 kinase**), which initiates the local translation of mRNAs required to maintain synaptic growth and long-term facilitation past 24 hours. Some of the mRNAs at the terminals encode CPEB, a regulator of local protein synthesis. In the basal state CPEB is thought to exist in a largely inactive conformation as a soluble monomer that cannot bind to mRNAs. Through some as yet unspecified mechanism activated by serotonin and PI3 kinase, some copies of CPEB convert to an active conformation that forms aggregates. The aggregates function like prions in that they are able to recruit monomers to join the aggregate, thereby activating the monomers. The CPEB aggregates bind the cytoplasmic polyadenylation element (**CPE**) site of mRNAs. This binding recruits the poly(A) polymerase machinery and allows poly(A) tails of adenine nucleotides (A) to be added to dormant mRNAs. The polyadenylated mRNAs can now be recognized by ribosomes, allowing the translation of these mRNAs to several proteins. For example, in addition to CPEB, this leads to the local synthesis of N-actin and tubulin, which stabilize newly grown synaptic structures.

the flies to choose between two arms of a maze, where one arm contains the conditioned odor and the other arm contains an unpaired odor. Following training, a large fraction of wild-type flies avoids the arm with the conditioned odor. Several fly mutants have been identified that do not learn to avoid the conditioned odor. These learning-defective mutants have been given imaginatively descriptive names such as *dumb*, *dunce*, *rutabaga*, *amnesiac*, and *PKA-R1*. Of great interest, all of these mutants have defects in the cAMP cascade.

Olfactory fear conditioning depends on a region of the fly brain called the mushroom bodies. Neurons of the mushroom bodies, called Kenyon cells,

receive olfactory input from the antennal lobes, structures similar to the olfactory lobes of the mammalian brain. The Kenyon cells also receive input from neurons that release dopamine in response to aversive stimuli, such as a foot shock. The dopamine binds to a metabotropic receptor (encoded by the *dumb* gene) that activates a stimulatory G protein and a specific type of Ca^{2+}/calmodulin-dependent adenylyl cyclase (encoded by the *rutabaga* gene), similar to the cyclase involved in classical conditioning in *Aplysia*. The convergent action of dopamine released by the unconditioned stimulus (foot shock) and a rise in intracellular Ca^{2+} triggered by olfactory input leads to the synergistic activation of adenylyl cyclase, producing a large increase in cAMP.

Recent experiments have demonstrated that flies can be classically conditioned when an odorant is paired with direct stimulation of the dopaminergic neurons, bypassing the foot shock. In these experiments the mammalian P2X receptor (an ATP-gated cation channel), is expressed as a transgene (see Box 3–3) in the dopaminergic neurons. The flies are then injected with a caged derivative of ATP. As a result the dopamine neurons can be excited to fire action potentials by shining light on the flies to release ATP from its cage and activate the P2X receptors. When the dopamine neurons are activated in the presence of an odor, the flies undergo fear conditioning; they learn to avoid the odor. Thus the unconditioned stimulus activates a modulatory signal mediated by dopamine that conveys aversive reinforcement, much as serotonin acts as an aversive reinforcement signal for learned fear in *Aplysia.*

A reverse genetic approach has also been used to explore memory formation in *Drosophila.* In these experiments various transgenes are placed under the control of a promoter that is heat-sensitive. The heat sensitivity permits the gene to be turned on at will. This was done in mature animals to minimize any potential effect on the development of the brain. When the catalytic subunit of PKA was blocked by transient expression of an inhibitory transgene, flies were unable to form short-term memory, indicating the importance of the cAMP signal transduction pathway for associative learning and short-term memory in *Drosophila.*

Long-term memory in *Drosophila* requires new protein synthesis just as in *Aplysia* and other animals. Like *Aplysia, Drosophila* expresses a CREB activator gene. Knockout of this gene selectively blocks long-term memory without interfering with short-term memory. Conversely, when the gene is overexpressed a training procedure that ordinarily produces only short-term memory produces long-term memory.

As in *Aplysia,* certain forms of long-term memory in *Drosophila* also involve CPEB and may depend on prion-like activity in this protein. Male flies learn to suppress their courtship behavior as a result of exposure to unreceptive females. When the N-terminal domain of CPEB is deleted genetically, there is a loss of long-term courtship memory. This N-terminal domain is rich in glutamine residues and corresponds to the glutamine rich prion-like domain of CPEB in *Aplysia.* Thus several molecular mechanisms involved in implicit memory are conserved from *Aplysia* to flies, and, as we will see next, this conservation extends to mammals.

Memory for Learned Fear in Mammals Involves the Amygdala

Innate fear, the ability to recognize and respond to danger, is necessary for survival. Not only snails and flies but all animals as well as humans need to distinguish predators from prey and hostile environments from safe ones. Because innate fear has been conserved throughout the evolution of species, one can readily discern and study fear in a variety of experimental animals.

At the beginning of the 20th century both Pavlov and Freud independently discovered that fear can also be learned. A previously neutral stimulus, such as a tone, can become associated with a fearful stimulus, such as a painful shock, so that the tone leads to conditioned fear, a form of what Freud called "signal anxiety." Both Freud and Pavlov also appreciated that learned fear—anticipatory defensive responses to danger signals—is biologically adaptive and therefore also conserved in evolution. Learned fear prepares the individual for fight or flight if there is even the suggestion of external danger.

From the work of Joseph LeDoux, Michael Davis, and Michael Fanselow we now have a good understanding of the neural circuits for both instinctive and learned fear in mammals. In particular, we know that both are centered on the amygdala, which participates in the detection and evaluation of a broad range of significant and potentially dangerous environmental stimuli (see Chapter 48). The amygdala receives information about unconscious fear responses (emotional state) directly and information about the cognitive processing of fear (feelings) indirectly by means of connections from the cingulate cortex.

In addition to its innate ability to respond to routine, natural threats, the amygdala-based defense system is also able to learn quickly about new dangers. It can associate a new neutral (conditioned) stimulus with a known threatening (unconditioned) stimulus on a single paired exposure and this learned fear can be remembered throughout life. The input nucleus of the amygdala, the lateral nucleus, is the site of convergence for the signals

from the unconditioned stimulus (such as a shock) and the conditioned stimulus (such as a tone). Both signals are carried by a rapid direct pathway that goes directly from the thalamus to the amygdala and a slower indirect pathway that goes from the thalamus to the cortex and from there to the amygdala. These parallel pathways are important for conditioning of fear (Figure 66–12).

Long-term memory for learned fear in mammals requires CREB, as it does in *Aplysia* and *Drosophila*. In fact, in studies of the amygdala, Alcino Silva and his colleagues have found that neurons in the amygdala are recruited for long-term memory based on their basal levels of CREB expression. Neurons with large amounts of the CREB switch, required for long-term memory, are selectively recruited in fear learning. Indeed, the relative activity of CREB at the time of learning determines whether a neuron is recruited. Conversely, if those neurons with a large amount of CREB are selectively ablated after learning, the memory of fear is blocked.

Pavlovian classical conditioning modifies the strength of synaptic transmission in the amygdala. In response to a tone, an extracellular electrophysiological signal proportional to the excitatory synaptic response is recorded in the lateral nucleus. Following pairing of the tone with a shock, the electrophysiological response is enhanced because of an increase in synaptic transmission (Figure 66–13).

What causes the enhanced synaptic response of learned fear? This question has been addressed by examining synaptic transmission in isolated brain slices containing the input pathways and nuclei of the amygdala. High-frequency tetanic stimulation of either the direct or indirect pathways induces a long-lasting increase in the synaptic response to these inputs (Figure 66–14). This change is a form of homosynaptic plasticity called *long-term potentiation* (LTP), which we examine in detail in Chapter 67 in connection with explicit memory and the hippocampus, where this mechanism was first identified.

Long-term potentiation in the lateral nucleus of the amygdala is triggered by Ca^{2+} influx into the postsynaptic neurons in response to strong synaptic activity. The Ca^{2+} entry is mediated by the opening of both NMDA-type glutamate receptors and L-type voltage-gated Ca^{2+} channels in the postsynaptic cell. Calcium influx triggers a biochemical cascade that enhances synaptic transmission through both the insertion of additional AMPA-type glutamate receptors in the postsynaptic membrane and an increase in transmitter release from the presynaptic terminals. The persistence of the memory for learned fear and the synaptic changes also require both cAMP-dependent protein kinase and MAPK, which activate the transcription factor CREB to initiate gene expression, much like learned fear in *Aplysia* and *Drosophila.*

Are these experimentally induced activity-dependent synaptic changes important for the induction of learned fear or are they only corollary or parallel phenomena? Two types of genetic experiments support the idea that LTP provides a cellular mechanism for memory storage of learned fear. In one experiment genetic disruption of the GluN2B (NR2B) subunit of the NMDA receptor was found to interfere both with fear conditioning and

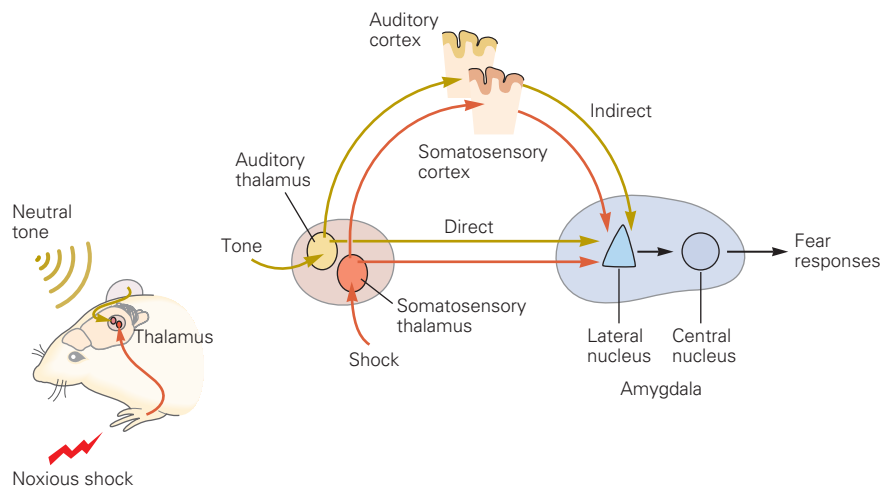


Figure 66–12 The neural pathways recruited during learned fear. The signal for the conditioned stimulus, here a neutral tone, is carried directly from the auditory thalamus to the lateral nucleus of the amygdala and by an indirect pathway via the auditory cortex. Similarly the signal for the unconditioned stimulus, here a shock, is conveyed through nociceptive pathways directly from the somatosensory part of the thalamus to the lateral nucleus and by an indirect pathway via the somatosensory cortex. The lateral nucleus in turn projects to the central nucleus, the output nucleus of the amygdala, which activates neural circuits that increase heart rate, produce other autonomic changes, and elicit defensive behaviors that constitute the fear state. (Reproduced, with permission, from Kandel 2006.)

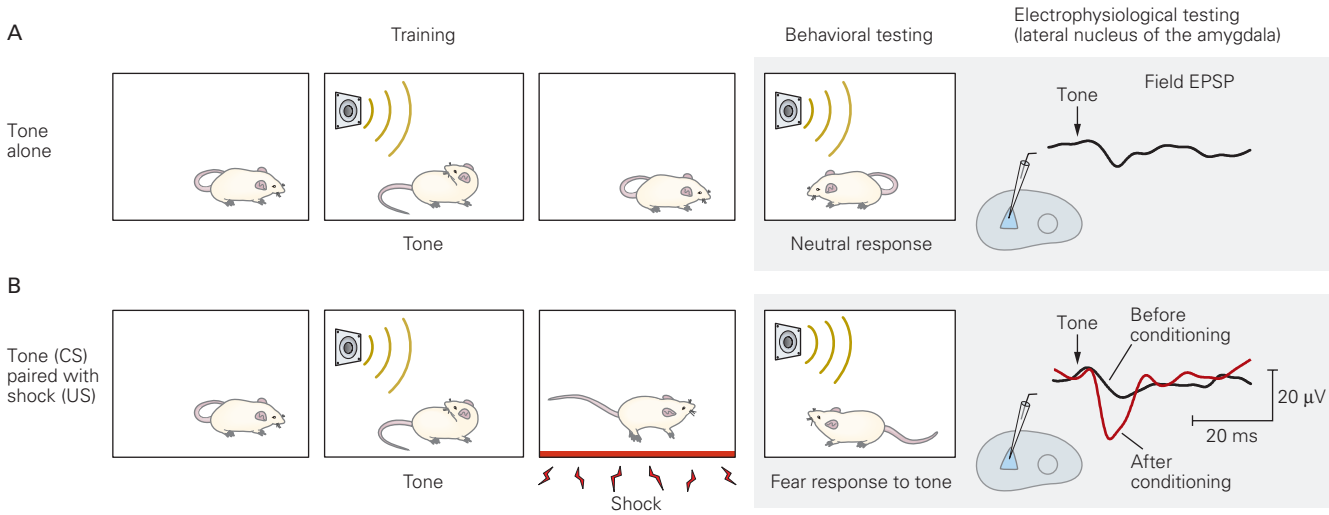


Figure 66–13 Learned fear produces parallel and correlated behavioral and electrophysiological changes.

A. An animal ordinarily ignores a neutral tone. The tone produces a small synaptic response in the amygdala recorded by an extracellular field electrode. This field EPSP is generated by the small voltage drop between the recording electrode in the amygdala and a second electrode on the exterior of the brain as excitatory synaptic current enters the dendrites of a large population of amygdala neurons.

B. When the tone is presented immediately before a foot shock (US), the animal learns to associate the tone (CS) with the shock. Now the tone alone will elicit what the shock previously elicited. Thus the tone causes the mouse to freeze, an instinctive fear response. After fear conditioning the electrophysiological response in the lateral nucleus of the amygdala to the tone is greater than the response prior to conditioning. (Reproduced, with permission, from Rogan et al. 2005.)

the induction of LTP in pathways that transmit the conditioned stimulus (tone) signal to the lateral amygdala. Moreover, this mutation affected only learned fear; it did not affect instinctive fear responses or routine synaptic transmission. Conversely, overexpression of the GluN2B subunit facilitated fear learning. Similarly, disruption of CREB signaling, a step downstream from Ca^{2+} influx, interfered with fear conditioning whereas enhancement of CREB activity facilitated learning.

Convincing evidence that LTP is important for learned fear comes from the finding that the size of the LTP elicited by electrical stimulation in slices of the amygdala isolated from animals previously trained for fear is reduced compared to the size of LTP in slices from animals that did not undergo prior fear training. This result is taken as evidence that fear learning recruits LTP: Because there is an upper limit to the amount by which synapses can be potentiated, the LTP induced by fear conditioning precludes further LTP in response to electrical stimulation. These results also suggest that artificially induced LTP and fear-induced LTP are related and are mutually exclusive.

A second line of experiments suggests that memory for a single emotional event requires the induction of LTP, and that a significant fraction of the total population of pyramidal cells in the lateral nucleus must express LTP to generate fear memory. In these experiments pyramidal neurons in the lateral nucleus were infected with a genetically engineered virus that did not damage the neurons but caused them to express AMPA receptors tagged with a fluorescent label. Fear conditioning led to an increase in insertion of the tagged AMPA receptors into the cell membrane, similar to what is seen during experimentally induced LTP in brain slices. When a different virus was used to express a C-terminal portion of the AMPA receptor that competes with and prevents the insertion of endogenous AMPA receptors, memory for learned fear was substantially reduced, even though the virus infected only 10% to 20% of the neurons in the lateral nucleus.

Habit Learning and Memory Require the Striatum

Habits are routines that are acquired gradually by repetition and are the result of a distinct form of implicit learning. A habit is a stimulus-response association, a behavior that is triggered simply by particular stimuli rather than by desire for (or fear of) some outcome.

In his classic book *The Principles of Psychology,* the great American psychologist William James characterized habit as the driving force of our daily operations—we are creatures of habit. As with all forms of implicit learning, habits are expressed in action alone, without conscious control, and not in verbal reports.

Many habits are learned early and retained throughout life. We learn to navigate through the world without conscious thought. Learned motor skills allow us to avoid objects in our path or to avoid bumping into people in a crowd. We learn through imitation, trial and error, practice and experience to dry ourselves and comb our hair after a shower, put on our clothes and even drive to work, all in a sequence that requires minimal attention. Much as proposed by Pavlov and the American psychologist Edward Thorndike, we can

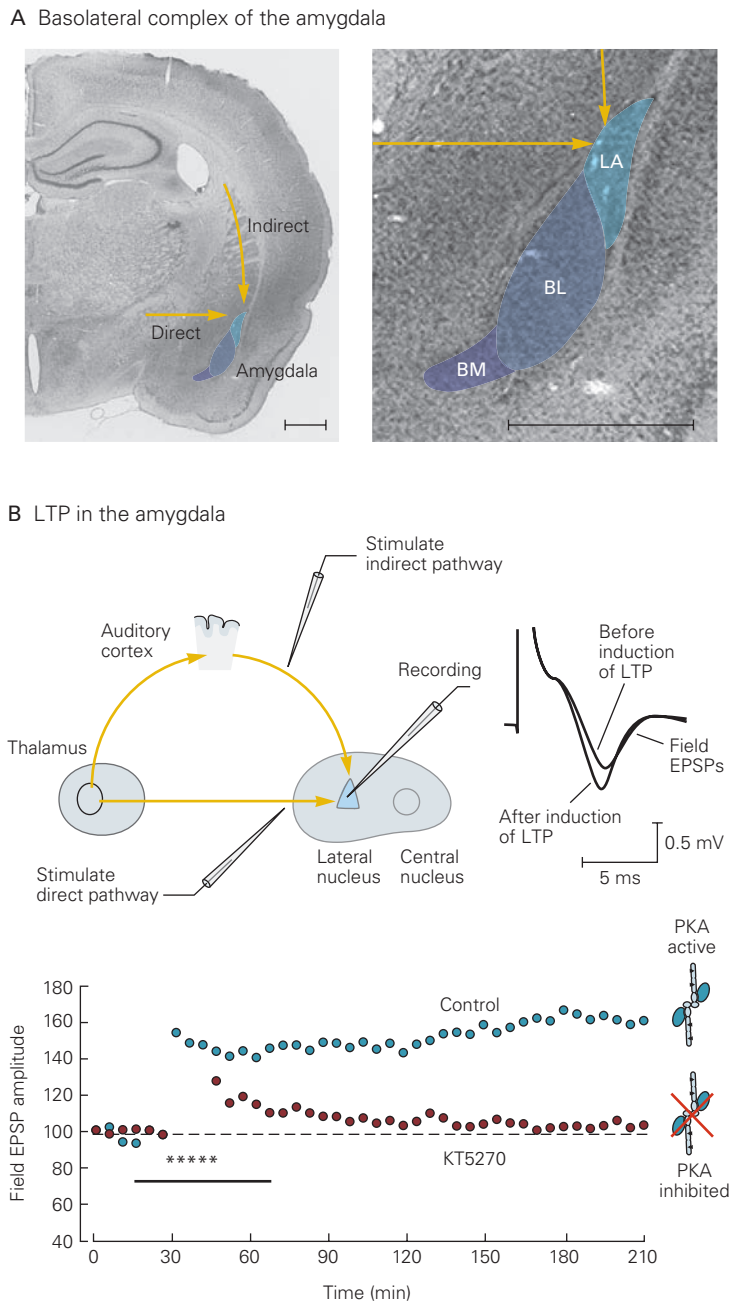


Figure 66–14 Long-term synaptic change in the amygdala may mediate fear conditioning.

A. A coronal brain slice from a mouse shows the position of the amygdala. The enlargement shows three key input nuclei of the amygdala—the lateral (**LA**), basolateral (**BL**), and basomedial (**BM**) nuclei—which together form the basolateral complex. These nuclei project to the central nucleus, which projects to the hypothalamus and brain stem. (Adapted, with permission, from Maren 1999.)

B. High-frequency tetanic stimulation of the direct or indirect pathway to the lateral nucleus initiates long-term potentiation (**LTP**). The drawing shows the position of the extracellular voltage recording electrode in the lateral nucleus, and the positions of two stimulating electrodes used to activate either the direct pathway (from the thalamus) or the indirect pathway (via the auditory cortex). The plot shows the amplitude of the extracellular field EPSP in response to stimulation of the indirect cortical pathway during the time course of the experiment. When a pathway is stimulated at a low frequency (once every 30 seconds), the field EPSP is stable. However, when five trains of high-frequency tetanic stimulation are applied (**asterisks**) the response is enhanced for a period of hours. The facilitation depends on PKA and is compromised when the PKA inhibitor KT5720 is applied (the **bar** shows period of drug application). Field EPSPs before and after induction of LTP are also shown. (Adapted, with permission, from Huang and Kandel 1998; Huang, Martin, and Kandel 2000.)

build up complex behaviors by combining simpler behaviors learned through repeated stimulus-response conditioning.

Many forms of habit learning depend on the four nuclei of the basal ganglia: the striatum, globus pallidus, substantia nigra, and subthalamic nucleus (see Chapter 43). The striatum, the input nucleus, has three subdivisions (at least in humans and other primates): the caudate nucleus, the putamen, and the ventral striatum. The caudate nucleus is involved in certain forms of procedural learning, including stimulus-response associations and some forms of skill learning. It and the ventral striatum malfunction in a variety of diseases in which habit learning is disordered, including obsessive-compulsive disorder and addiction.

Striatum-based implicit memory differs from hippocampus-based explicit memory in interesting ways. Mark Packard and his colleagues demonstrated fundamental differences in the neural structure underlying the two types of memory. They tested both types of memory using the same eight-arm maze (Figure 66–15). Explicit memory was tested with a "win-shift" foraging task. A rat was placed in the maze daily and removed after it had collected food from every arm, and this was repeated over several consecutive days. The rat's task was to minimize wasted effort by remembering where it had already found food (a win); to avoid revisiting those arms the rat had to shift its focus to the unvisited arms. In performing this task the animal has to acquire and use information about single events. It must remember the specific locations it has visited on a given day. This type of learning requires the hippocampus and is impaired by its lesion. Damage to the caudate nucleus has no effect on this behavior.

The same maze was then used to teach the rat a "win-stay" strategy, an example of implicit learning. With this task the animal needs to learn to visit four of the eight arms of the maze that are identified by a light at the entrance. Only these four arms contained a food reward. Over two weeks of training the animals learned to revisit only the arms that were lit. The win-stay task, in contrast to the win-shift task, is disrupted by damage to the caudate nucleus but not by damage to the hippocampus. The two tasks are superficially similar, but the win-stay task requires the animal to learn about regularities that are constant from day to day (lit arms always contain food) rather than to remember specific events on a given day (which unmarked arms it has already visited).

Examination of such striatum-dependent learning in mice has begun to shed light on the molecular mechanisms underlying habit learning. As is the case with many forms of implicit memory, striatum-based habit memory also requires CREB. Animals with selective impairment of CREB function in the striatum show impaired striatum-dependent learning.

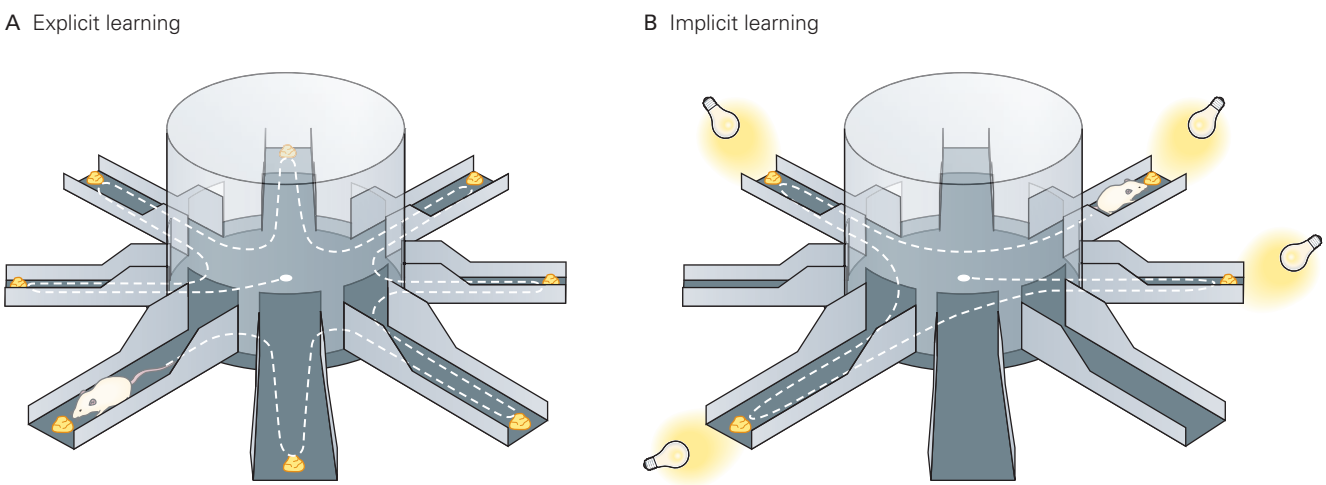


Figure 66–15 An eight-arm maze is used to demonstrate the difference between explicit and implicit learning. (Adapted, with permission, from Packard, Hirsh, and White 1989; Squire and Kandel 2008.)

A. In an explicit learning task a rat finds that food is available at the end of each arm. Initially a rat enters arms at random; with practice the animal will learn to find all of the eight morsels by entering each arm only once (by following a path similar to the one shown by the **dashed line**).

B. In an implicit learning task food is available only in four arms that are illuminated. The animal learns to visit each of these arms by associating the light with the food.

In the striatal learning system one likely locus of synaptic plasticity is the excitatory projection from the cortex to the striatum. This pathway undergoes a form of LTP that is mediated by NMDA receptors, much like that at the synaptic sites in the amygdala involved in learned fear. Like LTP in the amygdala, the persistence of LTP at corticostriatal synapses also requires CREB and is impaired in mice when CREB function in the striatum is selectively inhibited. The parallel impairment of synaptic plasticity and learning in the striatum is similar to what has been seen in *Drosophila* and *Aplysia* as well as in the mammalian amygdala, and supports the view that transcriptional-dependent alterations in synaptic strength provide a general mechanism of implicit memory.

Learning-Induced Changes in the Structure of the Brain Contribute to the Biological Basis of Individuality

To what extent do the anatomical alterations in synapses required for long-term memory storage alter the large-scale functional architecture of the mature brain? The answer is well illustrated by the fact that the maps of the body surface in the primary somatic sensory cortex differ among individuals in a manner that reflects the use of specific sensory pathways. This remarkable finding results from the expansion or retraction of the connections of sensory pathways in the cortex according to the specific experience of the individual (see Chapter 17).

The reorganization of afferent inputs as a result of behavior is also evident at lower levels in the brain, specifically at the level of the dorsal column nuclei, which contain the first synapses of the somatic sensory system. Therefore organizational changes probably occur throughout the somatic afferent pathway.

The process by which experience alters the somatosensory maps in the cortex is illustrated in an experiment in which adult monkeys were trained to use their middle three fingers at the expense of other fingers to obtain food. After several thousand trials of this behavior, the area of cortex devoted to the middle fingers expanded greatly (see Figure 66–16A). Thus practice may expand synaptic connections by strengthening the effectiveness of existing connections.

The normal development of the afferent input to cortical neurons in the somatosensory system may depend on different levels of activity in neighboring afferent axons. When the skin surfaces of two adjacent fingers in monkeys were surgically connected so that the connected fingers were always used together, thus ensuring that their afferent somatosensory axons were normally coactivated, the normally sharp discontinuity between the zones in the somatosensory cortex that receive inputs from these digits was abolished. Thus normal development of the boundaries of representation of adjacent fingers in the cortex may be guided not only genetically but also through experience. Fine tuning of cortical connections may depend on associative mechanisms such as LTP, similar to the role of cooperative activity in shaping the development of ocular dominance columns in the visual system (see Chapter 56).

This plasticity is evident in humans as well. Thomas Elbert explored the hand representation in the motor cortex of string instrument players. These musicians use their left hand for fingering the strings, manipulating the fingers in a highly individuated way. By contrast, the right hand, used for bowing, is used almost like a fist. The representation of the right hand in the cortex of string instrument players is the same as that of nonmusicians. But the representation of the left hand is greater than in nonmusicians and substantially more prominent in players who started to play their instrument prior to age 13 years (Figure 66–16B).

Because each of us is brought up in a somewhat different environment, experiencing different combinations of stimuli and developing motor skills in different ways, each individual's brain is uniquely modified. This distinctive modification of brain architecture, along with a unique genetic makeup, constitutes a biological basis for individuality.

An Overall View

A striking feature of implicit or procedural memory storage is that the recall of this memory is accomplished without recourse to conscious thought. Many aspects of personality, much of what we do in our daily life, is guided by implicit memory. These principles are consistent with a central tenet of psychoanalytic theory, the idea that we are unaware of much of our mental life. A great deal of what we experience—what we perceive, think, fantasize—cannot be directly accessed by conscious thought. Nor can we explain what often motivates our actions. The idea of unconscious mental processes not only is important in its own right but it is critical in the approach to neuroscientific studies of implicit memory storage and the resulting consequences for our individuality.

As we learned in Chapter 65, Brenda Milner made the remarkable discovery in 1954 that the medial temporal lobe, especially the hippocampus, mediates storage of what we now call explicit memory, the memory

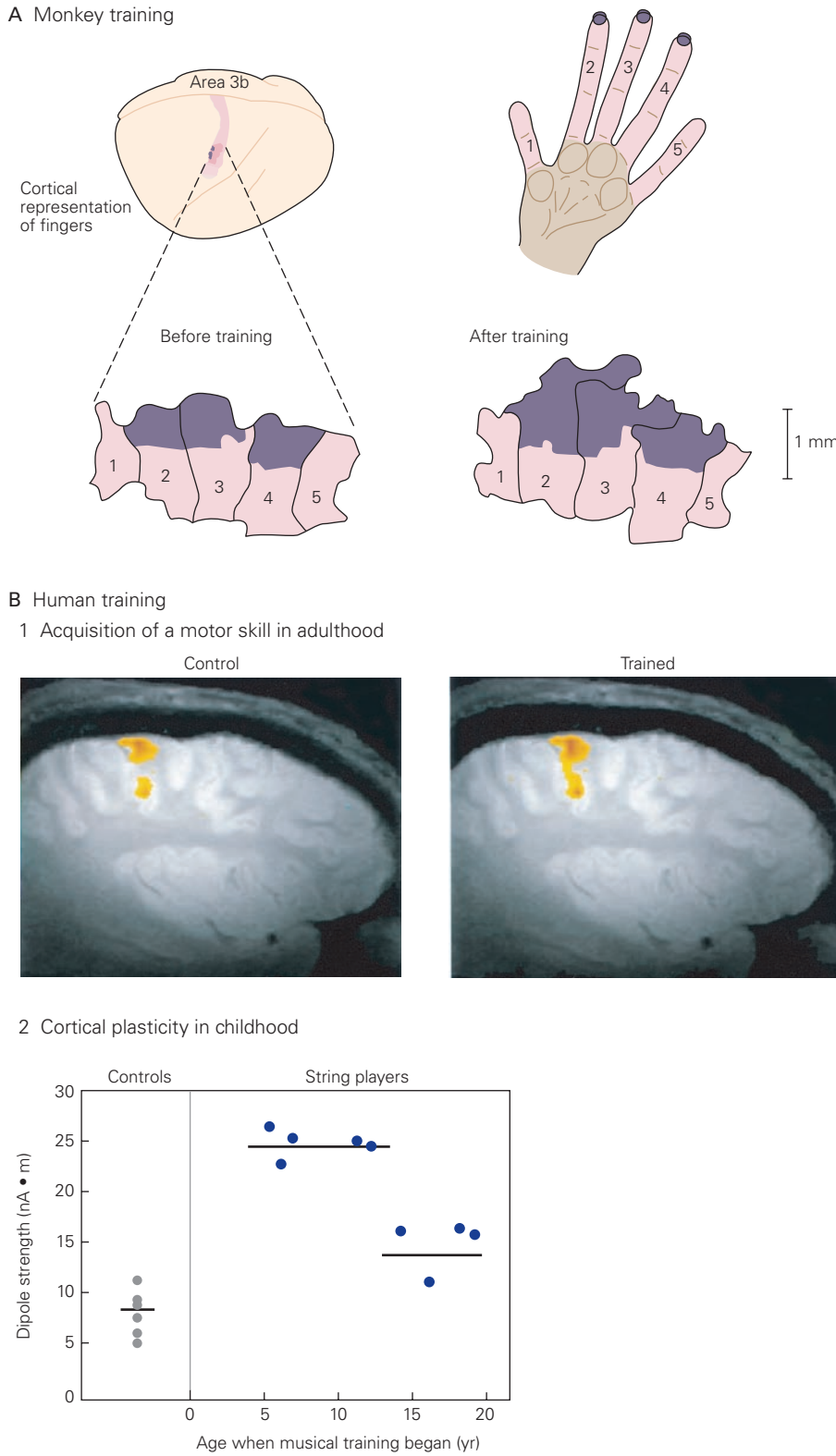


Figure 66–16 Training expands existing representation in the cortex of inputs from the fingers.

A. A monkey was trained for 1 hour per day to perform a task that required repeated use of the tips of fingers 2, 3, and occasionally 4. After training the portion of area 3b of the somatosensory cortex representing the tips of the stimulated fingers (**dark color**) is substantially greater than normal (measured 3 months prior to training). (Adapted, with permission, from Jenkins et al. 1990.)

B. 1. A human subject trained to do a rapid sequence of finger movements will improve in accuracy and speed after 3 weeks of daily training (10–20 min each day). Functional magnetic resonance imaging scans of the primary motor cortex (based on local blood oxygenation level-dependent signals) show that after 3 weeks of training the region activated in trained subjects (**orange region**) is larger than the region activated in control subjects who performed unlearned finger movements in the same hand. The change in cortical representation in trained subjects persisted for several months. (Reproduced, with permission, from Karni et al. 1998.)

2. The size of the cortical representation of the fifth finger of the left hand is greater in string players than in nonmusicians. The graph plots the dipole strength obtained from magnetoencephalography, a measure of neural activity. The increase is most pronounced in musicians that began musical training before age 13. (Reproduced, with permission, from Elbert et al. 1995.)

for people, objects, and places that is consciously recalled. In 1962 she made the further discovery that even though the patient H.M. had no conscious recall of new experiences with people, places, and objects, he was nonetheless fully capable of learning new perceptual and motor skills. This learning is stored in what we now call implicit memory, which is "recalled" only in performance and not typically reached through conscious recall.

Using the two memory systems together is the rule rather than the exception. The two systems overlap and are commonly used together in many learning experiences. Indeed, constant repetition often can transform explicit learning into implicit learning. For example, learning to drive an automobile at first involves conscious recollection; many aspects of driving eventually become an automatic and nonconscious motor activity.

Implicit memory itself comprises several processes that involve different brain systems. Acquisition of emotional states involves the amygdala; formation of new motor (and perhaps cognitive) habits requires the neostriatum; learning new motor behavior depends on the cerebellum; and simple reflex learning occurs directly in sensory and motor pathways. In different situations and learning experiences implicit memory formation depends on different combinations of these components of the nervous system. These implicit memory systems also work in parallel with the explicit memory system of the hippocampus so that with extensive experience explicit memory can essentially be carried forward by implicit memory systems.

In implicit memory, then, we have a biological manifestation of one component of unconscious mental life. How does this biologically delineated unconscious process relate to Freud's concept of the unconscious? In his later writings Freud used the term "unconscious" in different ways. Sometimes he used it in a strict way to refer to the *repressed* or *dynamic unconscious.* In this dynamic unconscious information about conflict and drive is prevented from reaching consciousness by powerful defensive mechanisms such as repression. This dynamic unconscious is what the classical psychoanalytic literature refers to simply as the unconscious.

At the same time, Freud proposed another component of unconscious activity, one concerned with habits and with perceptual and motor skills. This component fits our current understanding of implicit memory. According to Freud, an individual is not aware of most of the mental processes underlying our habits and as a result underlying these aspects of our personality. This idea is consistent with current neurological thinking that much of mental life is unconscious.

As these arguments make clear, the empirical study of unconscious psychic processes was severely limited for many years by the lack of suitable experimental methods. Today, however, biology has a wide range of empirical methods that are providing cellular and molecular insights that are expanding our understanding of a very wide range of mental activity.

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