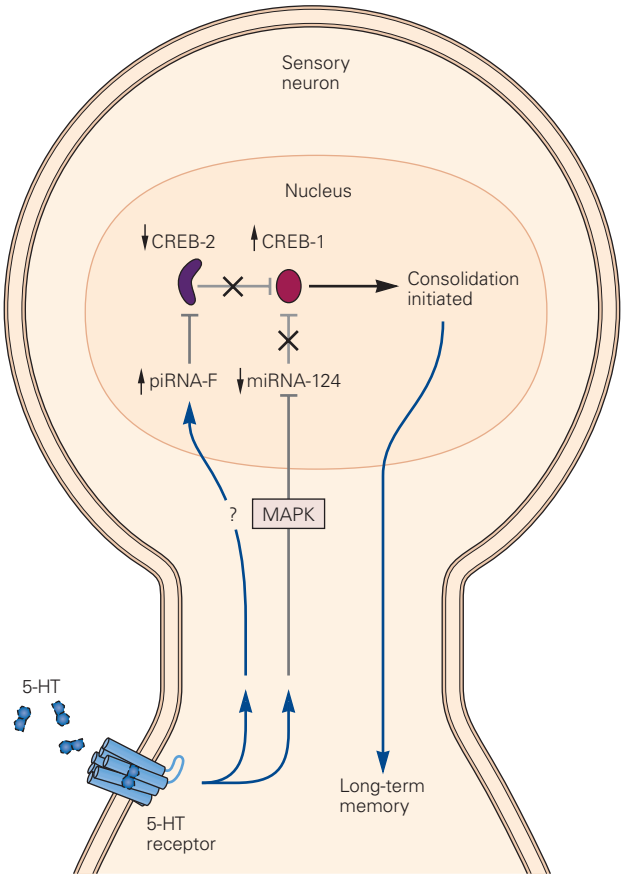

Figure 53–8 Small non-coding RNA molecules contribute to the memory consolidation switch. Long-term facilitation of the sensory to motor neuron synapses is consolidated through the action of two distinct classes of small noncoding RNA molecules. miRNA-124 normally acts to suppress levels of the CREB-1 transcription factor by binding to its mRNA and inhibiting its translation. Serotonin (**5-HT**) downregulates miRNA-124 levels through a mechanism requiring mitogen-activated protein kinase (**MAPK**). This enhances the levels of CREB-1, promoting activation of CREB-1–dependent transcription of gene products necessary for memory consolidation. In a complementary pathway, 5-HT enhances with a delay the synthesis of several piRNAs, including piRNA-F, which bind to the Piwi protein. The piRNA-F/Piwi complex leads to enhanced methylation of the *CREB-2* gene, resulting in long-lasting transcriptional repression of *CREB-2* and decreased levels of CREB-2 protein. Because CREB-2 normally inhibits the action of CREB-1, the increased levels of piRNA-F in response to 5-HT enhance and prolong CREB-1 activity, resulting in more effective memory consolidation.

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 resting level (Figure 53–6B). 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 53–9). 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, as described above. Long-term disuse of functional connections between sensory and motor neurons reduces the number of terminals of each sensory neuron by one-third (Figure 53–9A).

Long-Term Synaptic Facilitation Is Synapse Specific

A typical pyramidal neuron in the mammalian brain makes 10,000 presynaptic connections 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, 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 for 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

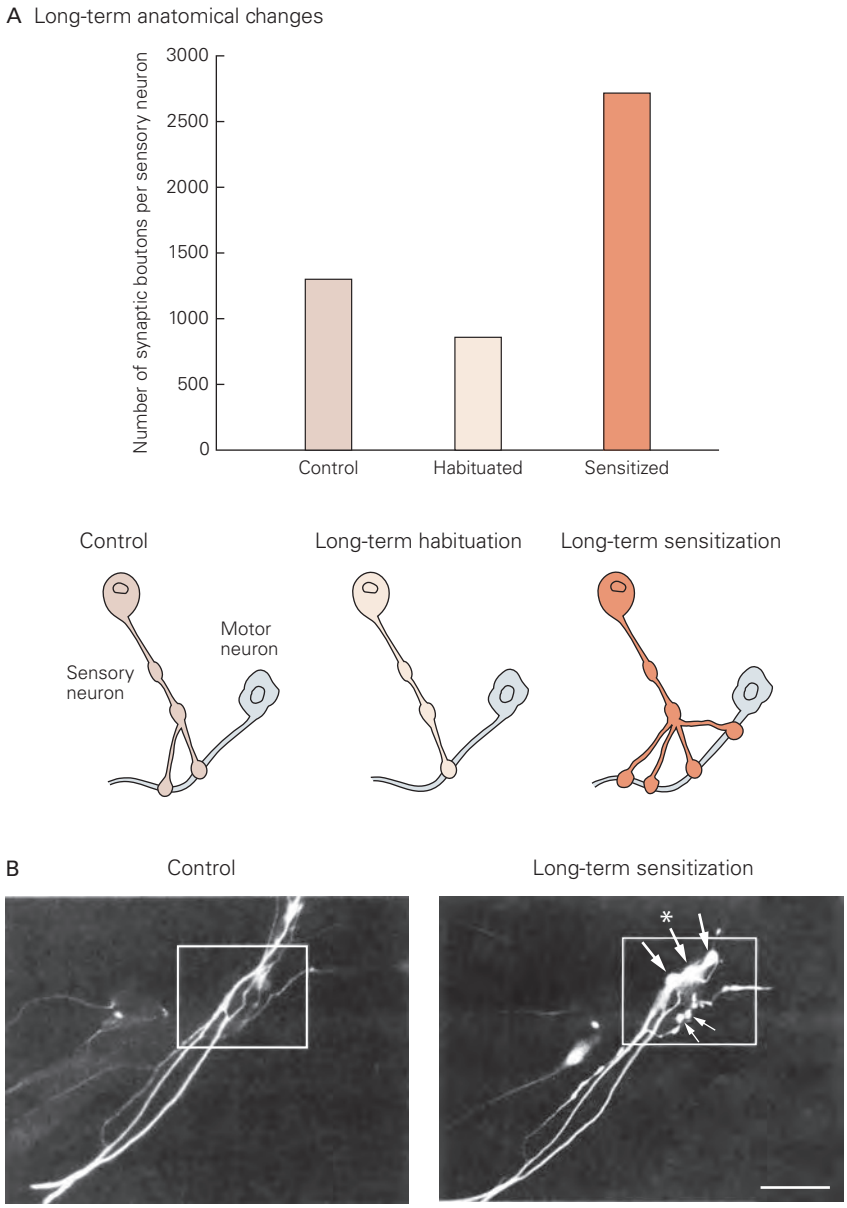

Figure 53–9 Long-term habituation and sensitization involve structural changes in the presynaptic terminals of sensory neurons.

A. Long-term habituation leads to a loss of synapses, and long-term sensitization leads to an increase in the number of synapses. When measured either 1 day (shown here) or 1 week after training, the number of presynaptic terminals relative to control levels is greater in sensitized animals and less in habituated animals. The drawings below the graph illustrate changes in the number of synaptic contacts. The swellings or varicosities on the sensory neuron processes are called synaptic boutons; they contain all the specialized structures necessary for transmitter release. (Adapted, with permission, from Bailey and Chen 1983. Copyright © 1983 AAAS.)

B. Fluorescence images of a sensory neuron axon 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 (*left*). After serotonin, several new boutons are apparent (**arrows**), some of which contain a fully developed active zone (**asterisk**) or have small immature active zones. Scale bar = 50 µm. (Reproduced, with permission, from Glanzman, Kandel, and Schacher 1990.)

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.

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. Although long-term facilitation and synaptic growth require gene transcription and protein synthesis, the synapses that did not receive serotonin showed no enhancement of synaptic transmission (Figure 53–10). 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 only certain synapses and not others of the same neuron? 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 marked by at least a single pulse of serotonin?

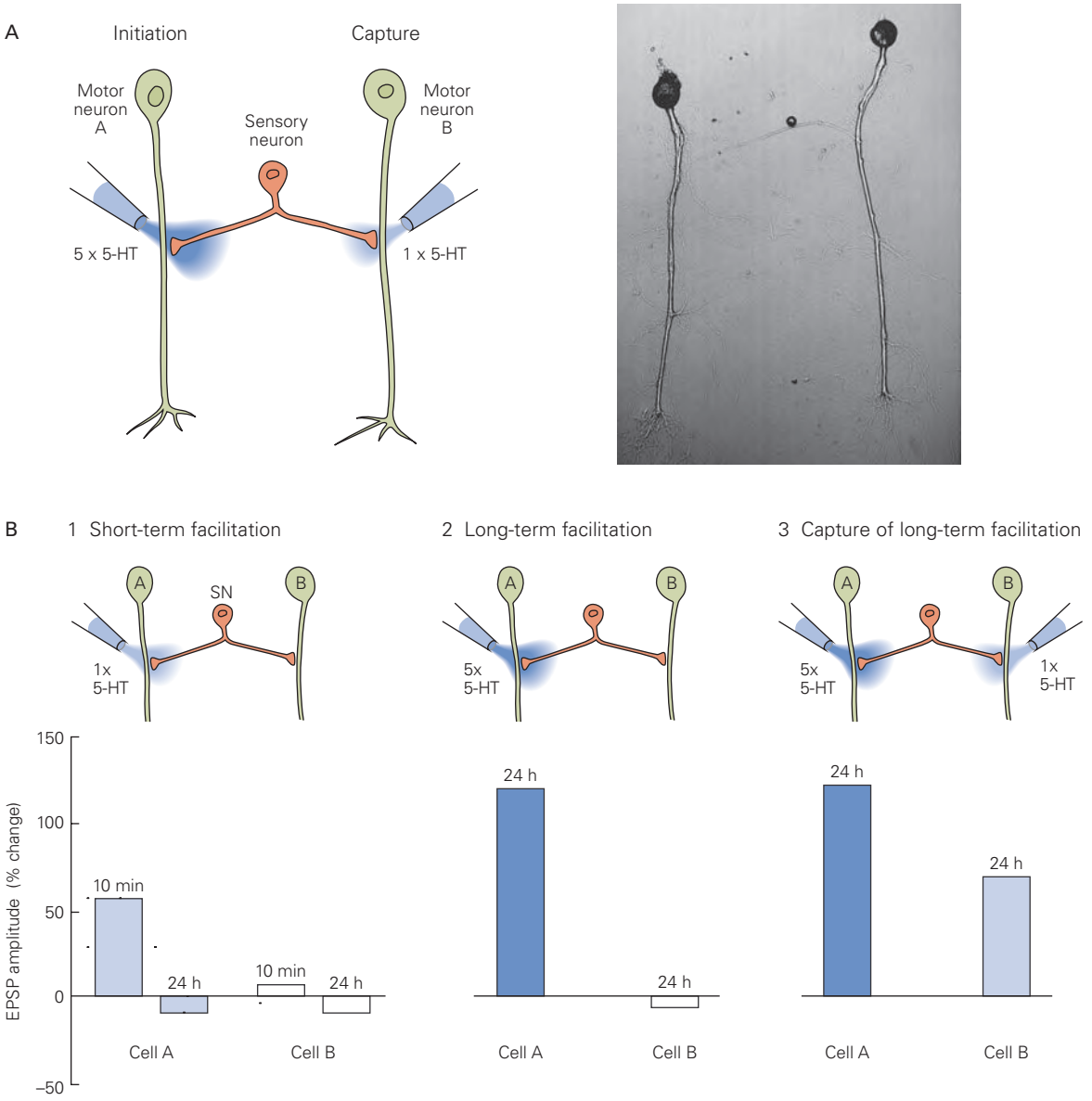

Figure 53–10 **The long-term facilitation of synaptic transmission is synapse specific.** (Adapted, with permission, from Martin et al. 1997.)

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 that synapse. 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 synapse 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 synapse with motor neuron A produces only short-term (10-minute) 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.** When five pulses of 5-HT onto the synapse with cell A are paired with a single pulse of 5-HT onto the synapses with cell B, cell B now displays long-term facilitation and an increase in EPSP size after 24 hours.

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 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 fast axonal transport to all the synapses of a neuron but are functional only at synapses that have been marked by previous synaptic activity, that is, by presynaptic release of 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 mark that synapse, allowing it to make use of new proteins generated in the cell body in response to five pulses of serotonin at another synapse. This idea, developed by Martin and 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 53–11). This indicates that local phosphorylation by PKA is required for synaptic capture.

In the early 1980s, Oswald Steward discovered that ribosomes, the machinery for protein synthesis, are present at synapses as well as 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 a second 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 local protein synthesis inhibitor, synaptic capture still occurred, producing long-term synaptic facilitation at the synapses exposed to only one pulse of serotonin. However, the facilitation only lasted 24 hours. After 24 hours, synaptic growth and facilitation at these synapses collapsed, indicating that the maintenance of learning-induced synaptic growth requires new local protein synthesis at the synapse (Figure 53–11B).

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 54, 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?

Maintaining Long-Term Synaptic Facilitation Requires a Prion-Like Protein Regulator of Local Protein Synthesis

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. Translation of most mRNAs requires that transcripts contain a long tail of adenosine nucleotides at their 3′ end [poly(A) tail]. Joel Richter had earlier found that in *Xenopus* (frog) oocytes the maternal mRNAs only have a short tail of adenine nucleotides and thus are silent until activated by the cytoplasmic polyadenylation element binding protein (CPEB). CPEB 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 terminals. 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 initial 24-hour maintenance.

How might CPEB stabilize the late phase of long-term facilitation? Most biological molecules have a relatively short half-life (hours to days), whereas memory

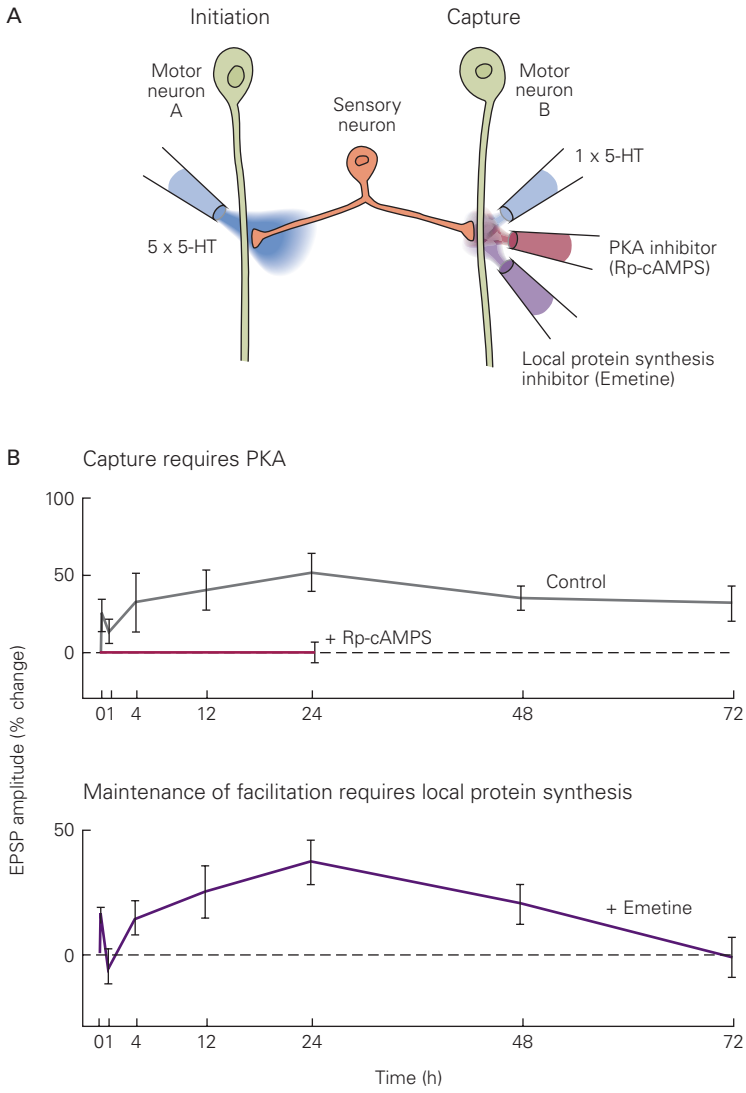

Figure 53–11 Long-term facilitation requires both cyclic adenosine monophosphate (cAMP)-dependent phosphorylation and local protein synthesis. (Adapted, with permission, from Casadio et al. 1999.)

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 (**PKA**; 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. (Abbreviations: **EPSP**, excitatory post-synaptic potential; **Rp-cAMPS**, Rp-diaster-eomer of adenosine cyclic 3′,5′-phosphorothioate.)

lasts days, weeks, or even years. How can learning-induced alterations in the molecular composition of a synapse be maintained for such a long time? Most hypotheses posit some type of self-sustained mechanism that modulates synaptic strength and structure.

Si and his colleagues made the surprising discovery that the neuronal isoform of *Aplysia* CPEB 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 Creutzfeldt-Jakob disease, a devastating neurodegenerative human disease, and mad cow disease. Prion proteins can exist in two forms: 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 depends on 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 resting 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 the aggregates, maintaining its ability to activate the translation of dormant mRNAs. Although dormant mRNAs are made in the cell body and distributed throughout the cell, they are translated only at synapses that have active CPEB aggregates.

Whereas conventional prion mechanisms are pathogenic—the aggregated state of most prion proteins causes cell death—the *Aplysia* CPEB is a new form of a prion-like protein, one whose aggregated state plays an important physiological function. The active self-perpetuating form of *Aplysia* CPEB maintains long-term molecular changes in a synapse that are necessary for the persistence of memory storage (Figure 53–12).

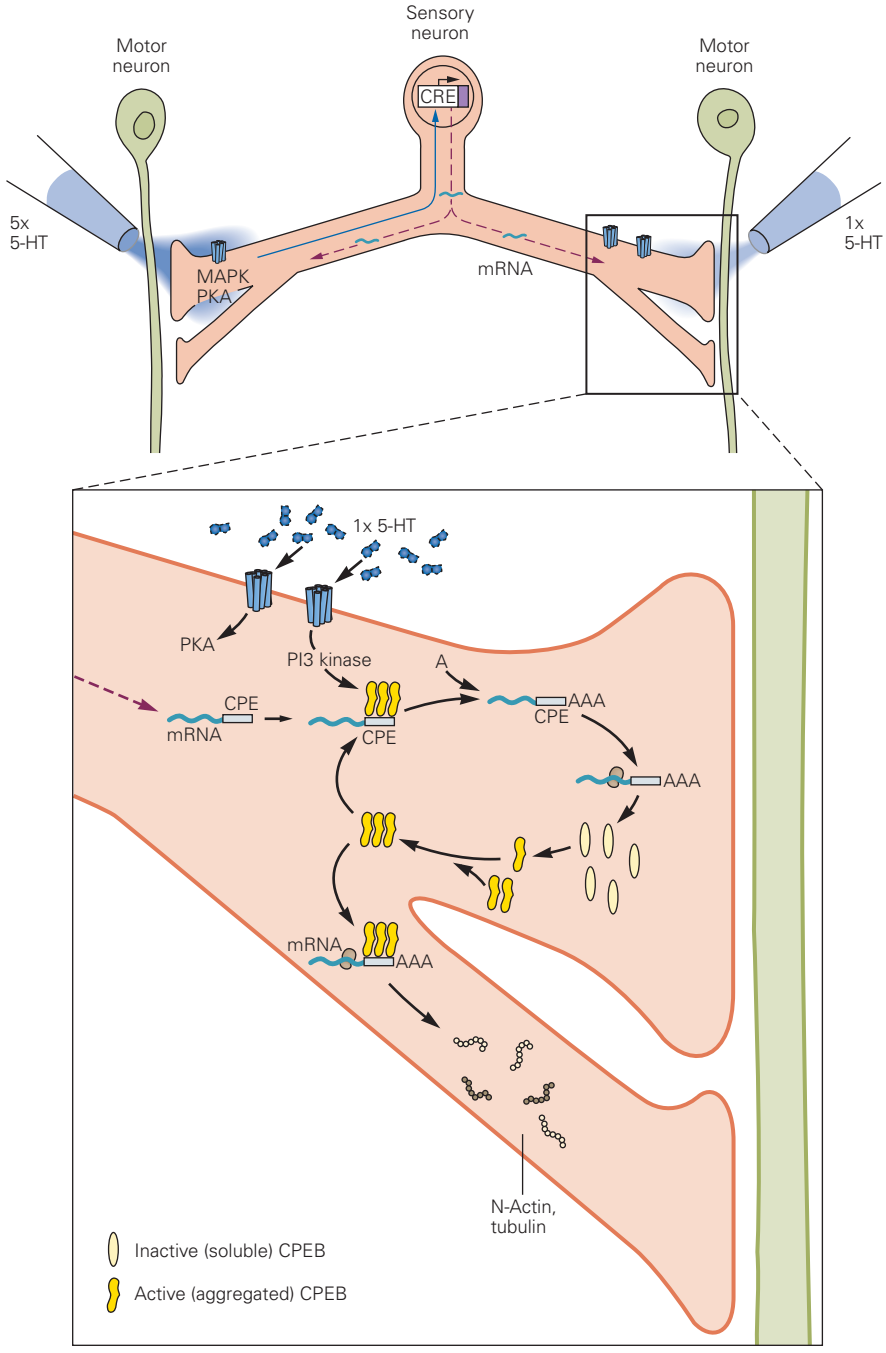

Figure 53–12 A self-perpetuating switch for protein synthesis at axon terminals in *Aplysia* maintains long-term synaptic facilitation. Five pulses of serotonin (**5-HT**) set up a signal that goes back to the nucleus to activate synthesis of mRNA. Newly transcribed mRNAs and newly synthesized proteins in the cell body are then sent to all terminals by fast axonal transport. However, only those terminals that have been marked by at least one pulse of serotonin can use the proteins to grow the new synapses needed for long-term facilitation. The marking of a terminal involves two substances: (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 cytoplasmic polyadenylation element binding protein (**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. (Model based on Bailey, Kandel, and Si 2004.)

Memory Stored in a Sensory-Motor Synapse Becomes Destabilized Following Retrieval but Can Be Restabilized

A variety of studies in mammals by Karim Nader and others have found that in its early stages long-term memory storage is dynamic and can be disrupted. In particular, a memory trace can become labile after retrieval and require an additional round of consolidation (so-called *reconsolidation*).

Until recently, it was unclear whether the same set of synapses involved in storing a memory are destabilized and restabilized following retrieval or whether, after synaptic reactivation following a memory, a new set of synapses is regulated. This question was examined for retrieval of long-term sensitization of the gill- and siphon-withdrawal reflex in *Aplysia*. These experiments revealed that a retrieved memory becomes labile as a result of ubiquitin-mediated protein degradation and is then reconsolidated by means new protein synthesis.

Does a similar reconsolidation mechanism occur at sensory-motor synapses that have undergone long-term facilitation? Indeed, when a synapse that has undergone long-term facilitation is reactivated by a brief burst of presynaptic action potentials, that synapse becomes destabilized through protein degradation and requires protein synthesis for restabilization. Such results suggest that reconsolidation of memory involves restabilization of synaptic facilitation at the same synapses at which the initial memory was stored.

Classical Threat Conditioning of Defensive Responses in Flies Also Uses the cAMP-PKA-CREB Pathway

Do the cellular mechanisms for implicit memory storage found in *Aplysia* have parallels in other animals? Studies on aversive learning indicate that the same mechanisms are also used to store memory in the fruit fly *Drosophila* and in rodents, indicating conserved mechanisms throughout Metazoan 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 shocks to the feet. The extent of learning is then examined by allowing the flies to choose between two arms of a maze, where one arm contains the odor that had been paired with a shock 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 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 dopaminergic neurons that respond 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 adenosine triphosphate [ATP]-gated cation channel) is expressed as a transgene in the dopaminergic neurons. The flies are then injected with a caged derivative of ATP. The dopaminergic neurons can then 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 dopaminergic neurons are activated in this manner in the presence of an odor, the flies undergo aversive conditioning—they learn to avoid the odor. Thus, the unconditioned stimulus activates a dopamine signal that reinforces aversive conditioning, much as serotonin acts as an aversive reinforcement signal for learned defensive responses 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 by elevating the temperature of the chamber housing the flies. 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. Knockout of a CREB activator 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 behavior in this protein. Male flies learn to suppress their courtship behavior after exposure to unreceptive females. When the N-terminal domain of CPEB is deleted genetically, there is a loss of long-term courtship memory; the male fly fails to recognize the unreceptive female. 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 of Threat Learning in Mammals Involves the Amygdala

Research over the past several decades has resulted in a detailed understanding of the neural circuits for both innate and learned defensive responses to threats in mammals, often referred to as "fear learning." In particular, as we have noted in Chapter 42, both types of defensive responses crucially involve the amygdala, which participates in the detection and evaluation of a broad range of significant and potentially dangerous environmental stimuli. The amygdala-based defense system quickly learns about new dangers. It can associate a new neutral stimulus (conditioned stimulus) with a known threat (unconditioned stimulus) after a single paired exposure, and this learned association is often retained throughout life.

The amygdala receives information about threats directly from sensory systems. The input nucleus of the amygdala, the lateral nucleus, is the site of convergence for signals from both unconditioned and conditioned stimuli. Both signals are carried by a rapid pathway that goes directly from the thalamus to the amygdala and a slower indirect pathway that projects from the thalamus to sensory areas of neocortex and from there to the amygdala. These parallel pathways both contribute to conditioning (Figure 53–13). The amygdala also receives higher-order cognitive information by means of connections from cortical associational areas, especially medial cortical regions in the frontal and temporal lobes.

During Pavlovian conditioning, the strength of synaptic transmission is modified 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 to the tone is enhanced by an increase in synaptic transmission, which depends on the

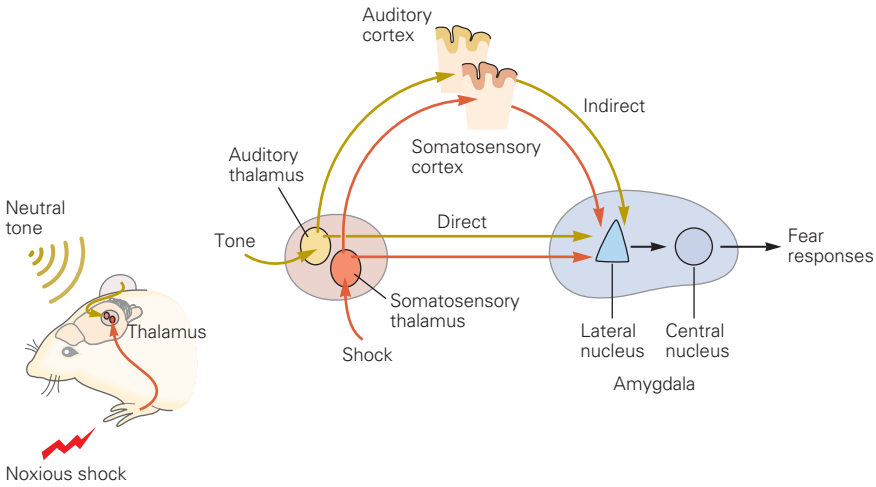

Figure 53–13 Threat learning engages parallel pathways from the thalamus to the amygdala. The signal for the conditioned stimulus, here a neutral tone, is carried by two pathways from the auditory thalamus to the lateral nucleus of the amygdala: by a direct pathway and by an indirect pathway via the auditory cortex. Similarly, the signal for the unconditioned stimulus, here a shock, is conveyed through parallel nociceptive pathways from the somatosensory part of the thalamus to the lateral nucleus, one a direct pathway and one 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 defensive state. (Reproduced, with permission, from Kandel 2006.)

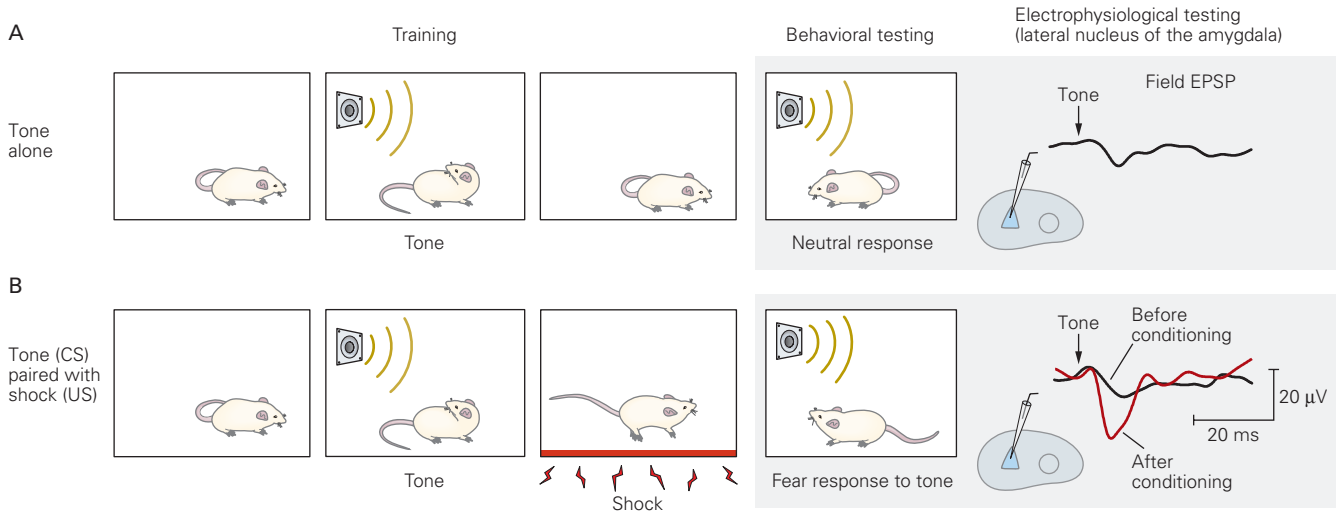

Figure 53–14 Threat learning produces correlated behavioral and electrophysiological changes.

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 excitatory postsynaptic potential (**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, the animal learns to associate the tone with the shock. As a result, the tone alone will elicit what the shock previously elicited: It causes the mouse to freeze, an instinctive defense response. After threat conditioning, the electrophysiological response in the lateral nucleus of the amygdala to the tone is greater than the response prior to conditioning. (Abbreviations: **CS**, conditioned stimulus; **US**, unconditioned stimulus.) (Reproduced, with permission, from Rogan et al. 2005.)

convergence of the tone (conditioned stimulus) and the shock (unconditioned stimulus) onto single neurons in the lateral amygdala (Figure 53–14).

It is generally thought that behavioral learning depends on synaptic plasticity. In an effort to understand how such plasticity might occur during learning in the lateral amygdala, researchers have studied *long-term potentiation* (LTP), a cellular model of plasticity. We initially discussed LTP in connection with excitatory synapse function in Chapter 13 and will examine it in detail in Chapter 54 in connection with explicit memory and the hippocampus. In brain slices that include the lateral amygdala, LTP can be induced by high-frequency tetanic stimulation of either the direct or indirect sensory pathways, which produces a long-lasting increase in the excitatory postsynaptic response to these inputs. This change results from a form of homosynaptic plasticity (Figure 53–15).

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 *N*-methyl-D-aspartate (NMDA)-type glutamate receptors and L-type voltage-gated Ca^{2+} channels in the postsynaptic cell. Because NMDA receptors are normally blocked by extracellular Mg^{2+}, they require a large synaptic input to generate enough postsynaptic depolarization to relieve this blockade (Chapter 13). L-type channels also require a strong depolarization to open. Thus, LTP is only generated in response to coincident synaptic activity. Calcium influx triggers a biochemical cascade that enhances synaptic transmission through both the insertion of additional α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors in the postsynaptic membrane and an increase in transmitter release from the presynaptic terminals. As in *Aplysia*, monoamine neurotransmitters, such as norepinephrine and dopamine, released during tetanic stimulation provide a heterosynaptic modulatory signal that contributes to the induction of LTP.

Studies in awake behaving rodents indicate that similar mechanisms contribute to the acquisition of Pavlovian threat conditioning. This form of learning requires postsynaptic NMDA receptors and voltage-gated calcium channels in the lateral amygdala, and it is enhanced by norepinephrine released in lateral amygdala from the locus ceruleus.

In addition, the size of the LTP elicited by electrical stimulation in slices of the amygdala from animals previously trained is less than that found in slices from untrained animals. Because there is an upper limit to the amount by which synapses can be