Learning and Memory

From Brain to Behavior

THIRD EDITION

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- CS-modulation theories of learning (like Mackintosh's model) presume that limits in attentional capacity cause attention to one stimulus to decrease our ability to attend to other stimuli. In contrast, the Rescorla–Wagner model is a US-modulation theory of learning because it describes the learning of associations as depending on how accurately the US is predicted based on all available information. Current behavioral and biological studies of conditioning now suggest that both CS-modulation and US-modulation mechanisms are likely to be involved in learning.
- Taste is more effective than an audiovisual stimulus for learning to predict illness, while an audiovisual cue is more effective for learning to predict a shock. One interpretation of this difference is the potential causal relationship between eating food and getting sick that is part of the animal's natural ecological environment.

4.2 Brain Substrates

Pavlov was a physiologist. When he discovered associative learning in his dogs in the early 1900s, he was naturally interested in understanding the brain mechanisms responsible for it. He even conducted a few experiments examining how cortical lesions affect conditioning. However, at the beginning of the last century, the technology for observing the brain's inner workings was not highly developed. Only in recent years have scientists gained knowledge and techniques that allow detailed study of the neural circuits for conditioning. We review here two neural systems, one in mammals and the other in invertebrates, that illustrate how studies of the neural bases of conditioning have yielded insights into the circuits, cells, molecules, and genes controlling the formation of new memories.

Mammalian Conditioning of Motor Reflexes

As you saw in Figure 2.4, the **cerebellum** sits just behind and slightly below the rest of the brain and looks like a miniature brain itself. In fact, the name *cerebellum* is Latin for "little brain."

In the early 1980s, Richard Thompson and his coworkers made a startling discovery: small lesions in the cerebellum of rabbits permanently prevented the acquisition of new classically conditioned eyeblink responses and abolished retention of previously learned responses (Thompson, 1986). Thompson and his colleagues have studied the cerebellum and its role in motor-reflex conditioning for more than 25 years. Their work provides an instructive example of how support for a theory can be strengthened by converging evidence from a variety of scientific methods, such as electrophysiological recordings, brain stimulation, experimental lesions, temporary inactivation of brain structures, and genetically mutated animals (Thompson & Steinmetz, 2009).

The cerebellum has two main regions, as diagrammed in Figure 4.18. Lying along its top surface is the *cerebellar cortex*, which contains certain large, drop-shaped, densely branching neurons called **Purkinje cells**. Beneath the cerebellar cortex lies a collection of cells called the *cerebellar deep nuclei*, one of which is the **interpositus nucleus**. There are two major sensory-input pathways to the cerebellum: the CS input pathway and the US input pathway. The CS input pathway is shown in purple in Figure 4.18. (Not all the cells in the cerebellum are shown here, only the cells and pathways critical for understanding the cerebellar circuits for motor-reflex conditioning.) CS pathways from elsewhere in

Purkinje cell. A type of large, drop-shaped, and densely branching neuron in the cerebellar cortex.

interpositus nucleus. One of the cerebellar deep nuclei.

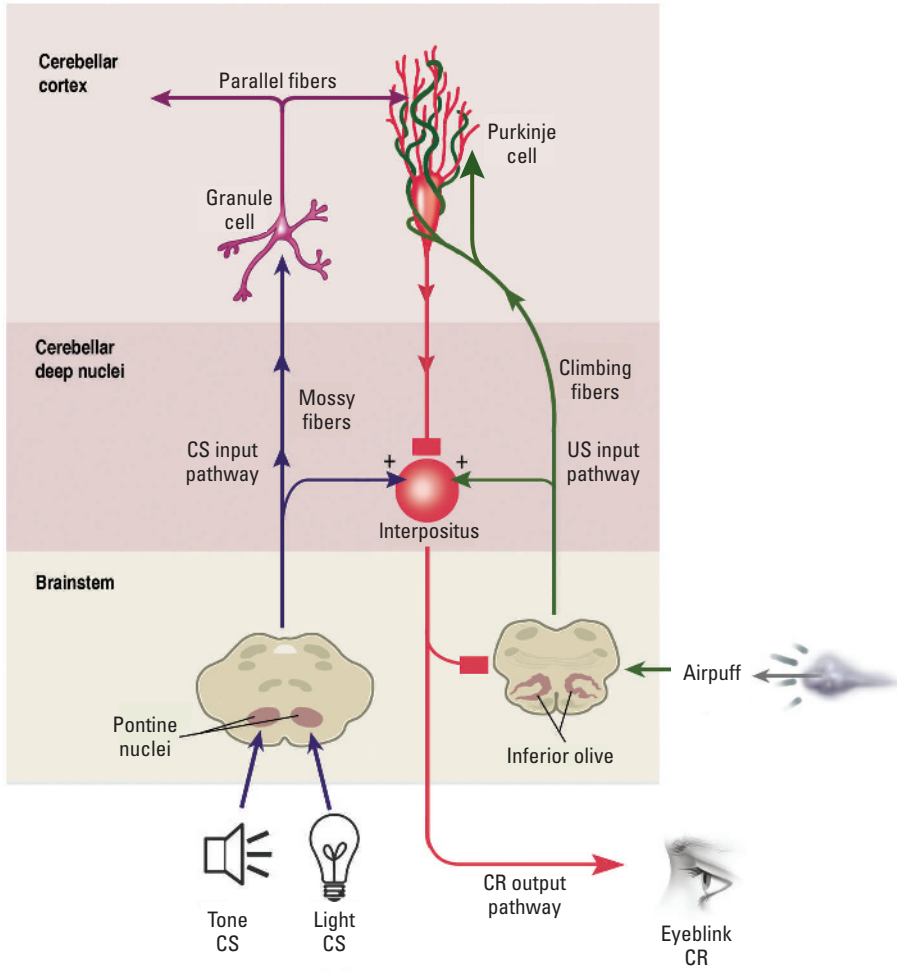

Figure 4.18 Cerebellar circuits for motor-reflex conditioning in mammals A schematic diagram of the cerebellar circuits for conditioning. The CS input pathway is purple, the CR output pathway is red, and the US input pathway is green. Excitatory synapses are shown as arrows, and inhibitory synapses terminate with a rectangle.

the brain project first to an area in the brainstem called the pontine nuclei. The pontine nuclei have different subregions for each kind of sensory stimulation. Thus, a tone CS would travel to one area of the pontine nuclei and a light CS to another. This CS information then travels up to the deep nuclei of the cerebellum along axon tracts called the mossy fibers, which branch in two directions. One branch makes contact with the interpositus nucleus. The other branch projects up toward the cerebellar cortex (by way of the granule cells and other cells not shown) and across the parallel fibers, and connects to the dendrites of the Purkinje cells.

inferior olive. A nucleus of cells with connections to the thalamus, cerebellum, and spinal cord.

The second sensory-input pathway, shown in green, is the US pathway. An airpuff US to the eye activates neurons in the **inferior olive**—a structure in the lower part of the brainstem—which in turn activates the interpositus nucleus. In addition, a second branch of the pathway from the inferior olive projects up to the cerebellar cortex by means of the climbing fibers (Figure 4.18). Each climbing fiber extends to and wraps around a Purkinje cell. The climbing fibers have a very strong excitatory effect on the Purkinje cells, indicated in Figure 4.18 by the large arrowhead at this synaptic junction.

Complementing these two converging input pathways is a single output pathway for the CR, shown in red, which starts from the Purkinje cells. The Purkinje cells project down from the cerebellar cortex into the deep nuclei, where they

form an inhibitory synapse (shown as a red rectangle) with the interpositus nucleus. To produce an eyeblink response, output from the interpositus nucleus travels (via several other intermediary cells) to the muscles in the eye to generate the eyeblink CR. You may notice that Figure 4.18 also includes an inhibitory pathway from the interpositus to the inferior olive, but we will postpone discussion of this pathway until later in the chapter. The unconditioned response (UR) pathway is not shown in Figure 4.18 because that is an innate response; it is not learned and does not originate in, or require, the cerebellum. Instead, it is a reflex circuit, similar in principle to the spinal reflexes you read about in Chapter 2.

The most important thing to note about this circuit (as diagrammed in Figure 4.18) is that there are two sites in the cerebellum where CS and US information converge and, thus, where information about the CS–US association might be stored: (1) the Purkinje cells in the cerebellar cortex and (2) the interpositus nucleus. These two sites of convergence are intimately interconnected in the output pathway: the Purkinje cells project down to the interpositus nucleus with strong inhibitory synapses.

Electrophysiological Recording in the Cerebellum

When an electrode is inserted into the interpositus nucleus (one of the two sites where CS and US information converge and the final exit point of CR information from the cerebellum), the recordings of spiking neurons during conditioned eyeblink responses display a pattern that corresponds very closely to the pattern of the eyeblinks themselves, as seen in Figure 4.19a, taken from a rabbit after one day of tone CS–US training (McCormick & Thompson, 1984). The main difference between the two patterns is that the neural activity occurs just a few milliseconds before the actual behavior. The upper blue line shows the eyeblink behavior (the extent of eyelid closure over time), while the lower graph shows

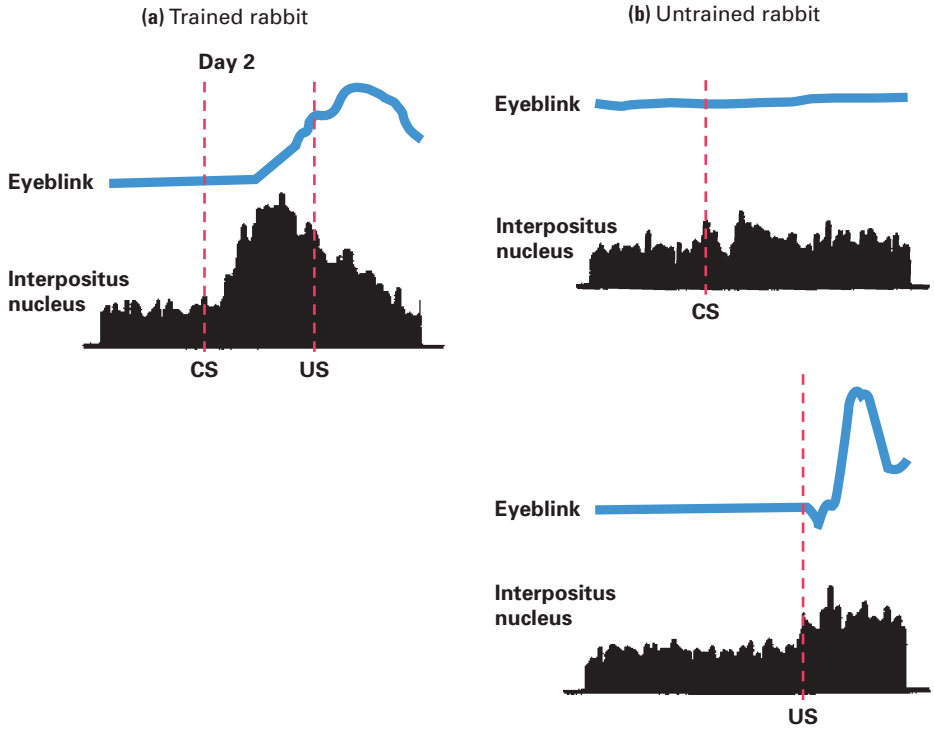

Figure 4.19 Electrophysiological recordings in the rabbit cerebellum during classical conditioning (a) Response of a trained rabbit to the CS. (b) Response of an untrained, naive rabbit to the CS alone (top) and to the US alone (bottom). The blue lines show the eyeblink behavior (the extent of eyelid closure over time), while the graphs below them show the frequency of neuronal firing in the interpositus nucleus.

Data from McCormick and Thompson, 1984.

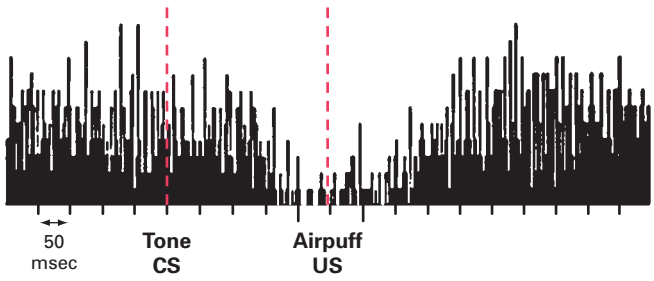

Figure 4.20 Purkinje cell activity in a well-trained rabbit The Purkinje cell's normal high rate of firing is halted in response to the CS and resumes after the US has occurred.
Data from R. F. Thompson.

the frequency of neuron firing in the interpositus nucleus, averaged over several rabbits and several trials.

Researchers have also recorded unpaired CS- or US-alone trials in naive rabbits. In both cases, where there is no CR (eyeblink), there is no activity in the interpositus nucleus, as seen in Figure 4.19b. The lack of substantial interpositus activity in a US-alone trial (despite a strong eyeblink UR) confirms that the cerebellum is responsible for conditioned eyeblink CRs only and not for the unconditioned eyeblink URs.

Figure 4.20 shows the firing rates recorded for a single Purkinje cell in a well-trained rabbit, with the time of the CS onset and the US indicated below. Purkinje cells spontaneously fire all the time, even when nothing is happening. However, in a well-trained animal, many of these cells *decrease* their firing in response to the tone CS, as shown in Figure 4.20. Why would the Purkinje cells turn off in response to a CS? Looking back at the diagram of cerebellar circuitry in Figure 4.18, note that Purkinje cells *inhibit* the interpositus nucleus, the major output pathway driving the conditioned motor response. Shutting off the Purkinje cells removes inhibition from the interpositus, freeing the interpositus to fire (as in Figure 4.19a).

Brain Stimulation as a Substitute for Behavioral Training

What if we knew exactly which pathways in your brain would change as a result of reading the words on this page? If so, we might be able to put electrodes in your brain and electrically stimulate those pathways in just the right pattern, at just the right time, to mimic the effect of reading this text. If that were possible, you wouldn't have to bother reading this book any further or studying for the final exam. Instead, you could stimulate a few neural pathways, create a little synaptic change, and then take the final exam and score an A+, even if you had never opened the textbook or sat through your professor's lectures! Science fiction, right? Unfortunately, it is still a fantasy because we don't yet know exactly where or in what way complex learning is stored in the brain. However, for simpler forms of learning, like eyeblink conditioning, this scenario is not only possible, it's been done.

Through electrical brain stimulation of the CS and US pathways shown in Figure 4.18, an experimenter can create conditioned eyeblink responses in the rabbit that are indistinguishable from those arising from behavioral training.

Recall that different parts of the pontine nuclei respond to different kinds of sensory input, such as auditory tones or visual signals, as illustrated in Figure 4.18. It is even possible to find a specific region in the pontine nuclei that responds to a *particular* tone. As a result, it is possible to condition rabbits merely by pairing electrical stimulation of the pontine nuclei (CS) with electrical stimulation of the inferior olive (US), that is, without presenting any external stimuli (airpuff or tone). After training with this type of brain stimulation, rabbits give precisely timed, reliable eyeblink responses the very first time they hear an actual tone corresponding to the pontine nuclear region that was stimulated, just as if they had been trained all along with tones and airpuffs (Steinmetz et al., 1989).

In these studies, direct stimulation of the inferior olive causes the rabbit to blink and can be substituted for an airpuff US, as shown in Figure 4.21. Similar conditioning over 4 days of training is seen whether an airpuff US (dashed line)

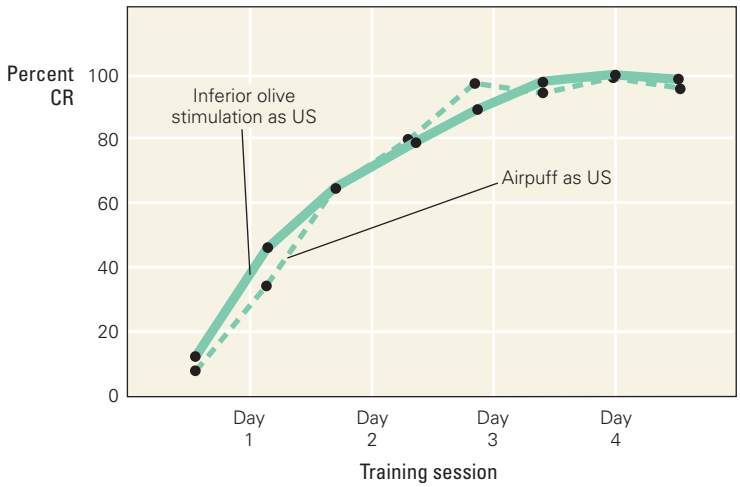

Figure 4.21 Substituting stimulation of the inferior olive for a US Four days of training using stimulation of the inferior olive as the US (solid line) produces the same amount of conditioned eyeblink response as four days of training with an airpuff US (dotted line).
Data from Steinmetz et al., 1989.

or a stimulation of the inferior olive (solid line) is used (Steinmetz, Lavond, & Thompson, 1989).

Thus, rabbits that have had their inferior olives and pontine nuclei electrically stimulated will "pass the eyeblink test" much as if they had gone through days of tone–airpuff training. Like the science fiction fantasy alluded to earlier, stimulating the correct pathways creates learning that seems indistinguishable from conditioning in a rabbit that has gone through the usual training with tones and airpuffs.

Impaired Conditioning Following Cerebellar Damage

Another experimental approach for investigating the neural bases of classical conditioning is to introduce brain lesions—that is, to selectively remove small areas of the brain—and observe the consequences. Recall that the interpositus nucleus (see Figure 4.18) projects information about the CR out of the cerebellum. Thus, without the interpositus nucleus, you would expect that there could be no CR. This is exactly what Thompson and colleagues found: removing even 1 cubic millimeter of tissue from the interpositus nucleus completely and permanently abolished all previously learned conditioned responses and prevented all future eyeblink learning.

In contrast to lesions of the interpositus, which totally abolish learned eyeblink CRs, lesions of the cerebellar cortex (including the Purkinje cells) disrupt, but do not eliminate, eyeblink conditioning. Animals with lesions of the cerebellar cortex show small, poorly timed conditioned CRs (Perret, Ruiz, & Mauk, 1993). Recently, researchers have developed mutant mice with a genetic variation that causes selective degeneration of Purkinje cells. These mutant mice are slow at learning eyeblink conditioning, much like animals that have their cerebellar cortex physically removed (Chen, Bao, Lockard, Kim, & Thompson, 1996). Together, these lesion and mutant studies provide strong converging evidence that the interpositus nucleus is involved in the formation and execution of the conditioned response, while the cerebellar cortex is involved in response timing.

Given the critical role of the cerebellum in motor-reflex conditioning, it is not surprising that patients with cerebellar damage display significant deficits in acquiring the eyeblink conditioning. Such patients are slower to learn the CR and show low overall frequency and abnormal timing of CRs (Daum et al., 1993). Interestingly, patients who have undergone surgery that spares the

deep nuclei are able to acquire a little conditioning, while patients with more extensive cerebellar damage show no conditioning at all. It is important to note that cerebellar damage does not impair all forms of associative learning. For example, cerebellar patients perform within the normal range on learning verbal associations, such as matching names with faces, which suggests that other areas of the brain play a role in these more abstract tasks (Daum et al., 1993). There is also a clear lateralization of cerebellar involvement in eyeblink conditioning: damage to the left cerebellum interferes only with conditioning to the left eye, while damage to the right cerebellum interferes only with conditioning to the right eye; this is true in both rabbits and humans (Thompson & Krupa, 1994; Woodruff-Pak & Lemieux, 2001).

Genetics offers additional insights into human eyeblink conditioning. Irene Daum and colleagues have studied several groups of patients in whom chromosomal irregularities cause abnormalities and degeneration in either the cortical Purkinje cells or the deep nuclei (Daum et al., 1993). They found that patients with genetic abnormalities of the deep nuclei are severely impaired at acquiring the eyeblink CRs, while those with abnormalities in the Purkinje cells show more mixed results. These genetic studies provide additional evidence that the deep cerebellar nuclei are essential for learning the CR, while the Purkinje cells in the cerebellar cortex exert some modulating but nonessential influence on this learning.

Error Correction through Inhibitory Feedback

As described in Chapter 2, long-term potentiation (LTP) of a synapse occurs when simultaneous activity in two adjoining neurons leads to a strengthening of the connecting synapse. LTP is a mechanism for synaptic change that occurs whenever two adjoining neurons fire at the same time and is thus much simpler than the error-correcting rule of the Rescorla–Wagner model, in which associative changes depend on many inputs (such as all the CSs present on a trial). Given its complexity, the Rescorla–Wagner model of learning probably does not describe what takes place in a learning brain at the cellular level, but the error-correction mechanisms the model predicts do appear to emerge from brain circuits.

If you look again at the cerebellar network in Figure 4.18, you will see an additional pathway within the cerebellum we have not yet discussed. This inhibitory feedback pathway projects from the interpositus nucleus to the inferior olive. In a well-trained animal, the production of a CR, through activation of the interpositus nucleus, will in turn inhibit the inferior olive from sending US information to the Purkinje cells in the cerebellar cortex (Sears & Steinmetz, 1991). This means that activity in the inferior olive will reflect the actual US minus (due to inhibition) the expected US, where the expected US is measured by the interpositus activity that drives the CR. Actual US minus expected US: sound familiar? It should. This is the same difference (actual US minus expected US) that the Rescorla–Wagner model uses to calculate the prediction error on a trial, which is then used to determine how much weight should accrue to the CS association.

If the inferior olive is where the brain codes the prediction error during conditioning, then we should be able to predict changes in the firing of the inferior olive based on the Rescorla–Wagner model (Gluck, Reifsnider, & Thompson, 1990; Gluck, Allen, Myers, & Thompson, 2001). During CS–US acquisition training, the prediction error diminishes on each successive learning trial. Thus, we should expect to see inferior olive activity in response to the US diminish the more the US is predicted by the trained CS. Eventually, when the CR is well learned, there should be very little activity in the inferior olive (that is, when

error in the Rescorla–Wagner model is close to zero). What happens matches the predictions exactly: inferior olive activity starts off high early in training and then gradually diminishes as the conditioned response is acquired (Sears & Steinmetz, 1991).

This interpretation of how the cerebellar circuits compute the changes in association weight called for in the Rescorla–Wagner model implies that Kamin's blocking effect (the clearest experimental evidence for error-correction learning) should depend on the inhibitory pathway from the interpositus to the inferior olive. This prediction was confirmed in a study by Thompson and colleagues. The researchers first trained rabbits to give reliable eyeblink responses to a tone CS and then injected a drug into the interpositus that temporarily disabled the inhibitory connection from the interpositus to the inferior olive. With this pathway disabled, they predicted, the inferior olive's activity would reflect the presence of the actual US and no longer the expected US.

The rabbits were then given phase 2 blocking training, in which a compound tone-and-light CS was paired with the US. The rabbits showed high inferior olive activity whenever the US was presented, whether or not a conditioned response was generated. As a result, in phase 3, the rabbits gave a strong response to the light CS. In other words, by disabling that one inhibitory pathway which is essential for the actual US minus expected US computation, Thompson and colleagues were able to "*block* blocking" (Kim, Krupa, & Thompson, 1998). These and related results suggest that the cerebellar–inferior olive circuit plays a role in the execution of Rescorla and Wagner's error-correction rule.

Test Your Knowledge

The Cerebellum in Motor Reflex Conditioning

1. What is the role of the Purkinje cells in the cerebellar cortex? Discuss the evidence that suggests this.
2. What are the two main cerebellar regions and the major sensory-input pathways to the cerebellum? Where do these two pathways in the cerebellum converge?
3. How do electrophysiological recordings in the rabbit cerebellum during classical conditioning demonstrate that the cerebellum is responsible for conditioned responses and not for unconditioned responses?

(Answers appear in the back of the book.)

The Hippocampus in CS Modulation

Error correction as explained by the Rescorla–Wagner model is only one mechanism at work in classical conditioning. Another, CS modulation, was suggested, as noted above, by the theories of Mackintosh and of Pearce and Hall. Here we briefly discuss some of the brain systems that appear to govern these mechanisms for modulating the processing of CS cues.

As you learned in Chapter 2, the hippocampus is a string-bean–shaped structure that lies, in humans, just inward from the ears. Figure 4.22 shows the hippocampus in various species.

The hippocampus is not necessary for learning new conditioned responses. For example, animals or humans with hippocampal damage are able to learn a basic conditioned eyeblink response quite normally. Nevertheless, electrophysiological recordings of animals show that the hippocampus is very active during conditioning, especially early in training. What role does the hippocampus

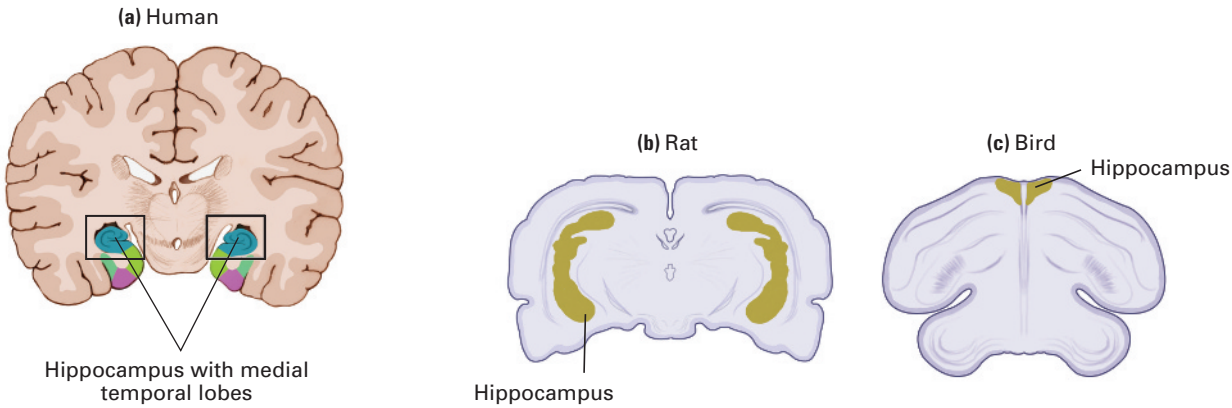

Figure 4.22 The hippocampus in different species of animals, including humans The medial (inner) part of the temporal lobes contains the hippocampus, the amygdala, and several nearby cortical areas.

play in conditioning? One way to find possible clues to its role is to look at more complex conditioning paradigms, such as latent inhibition (described in Table 4.7). As you learned in Section 4.1, latent inhibition is demonstrated when, before training, an organism is exposed to a cue unassociated with a US; later, during conditioning, the organism is then slow to learn that the cue does predict a US.

As you also learned, the Rescorla–Wagner model is *not* able to explain the phenomenon of latent inhibition. If the Rescorla–Wagner model's error-correction process cannot explain latent inhibition and if the cerebellum implements the error-correction principle, then perhaps other brain regions involved in classical conditioning besides the cerebellum are responsible for latent inhibition. Might the hippocampus be such a region? If so, then the animal learning theories that capture behavioral phenomena other than error-correction learning might provide us with some ideas of what the hippocampus may do during classical conditioning.

The CS modulation theories of Mackintosh and of Pearce and Hall, discussed earlier in this chapter, suggest that to find the system responsible for latent inhibition and related phenomena, we should look for a system involved in determining the salience of sensory cues. If the hippocampus is needed for CS modulation effects in classical conditioning, then an animal *without* a hippocampus should *not* exhibit CS modulation effects such as latent inhibition. In fact, this is exactly what researchers have found: removing the hippocampus (and associated cortical input regions) eliminates the latent inhibition effect in classical conditioning of the rabbit eyeblink reflex (Solomon & Moore, 1975; Shohamy, Allen, & Gluck, 2000).

Many other behavioral phenomena that cannot be explained by the Rescorla–Wagner model are also found to disappear in animals that have lesions to the hippocampus and surrounding brain regions. This suggests that the Rescorla–Wagner model may be better described as a model of the cerebellar contributions to motor-reflex conditioning in hippocampal-lesioned animals than as a model of conditioning in healthy, intact animals. That is to say, the model applies best to the brain regions responsible for error-correction learning, such as the cerebellum, but does not explain the additional contributions of the hippocampus.

What functional role, then, does the hippocampus play in classical conditioning of motor reflexes such as the eyeblink response? If the hippocampus is necessary for latent inhibition and other forms of CS modulation, we might infer that the hippocampus plays a role in determining how sensory cues are processed before they are used by the cerebellum to form long-term memory traces.

Further discussion of the role of the hippocampus in processing sensory relationships while remembering new facts and events will be discussed in Chapter 6. Later, in Chapter 9, we describe some specific theories about how the hippocampus modulates sensory processing in various forms of learning and memory.

Invertebrates and the Cellular Basis of Learning

Chapter 3 introduced you to the sea snail *Aplysia* and studies by Eric Kandel and colleagues on the neural substrates of two forms of non-associative learning: habituation and sensitization. To briefly recap, habituation occurs when *Aplysia's* siphon (see Figure 3.9) is repeatedly but lightly touched. Initially this results in a gill-withdrawal reflex. However, each subsequent stimulation of the siphon elicits a progressively smaller response. The circuit for this learned response includes a sensory neuron (activated by touching the siphon) that makes an excitatory synapse with a motor neuron that controls the gill withdrawal (see Figure 3.10). The neural mechanism for habituation is thought to be a progressive decrease in the number of neurotransmitter (in this case, glutamate) vesicles available in the sensory neuron's axon for each successive stimulation of the siphon. In contrast, sensitization is a global increase in responding to all or most stimuli following an unpleasant stimulus, such as an electric shock to *Aplysia's* tail. The tail shock activates modulatory interneurons that release serotonin onto the axon terminals of all the sensory neurons that project to the gill-withdrawal motor neuron. Serotonin increases the number of glutamate vesicles released when the sensory neuron is stimulated. This results in the generalized (non–stimulus-specific) increase in gill withdrawal elicited by all future stimuli, including touches on either the siphon or the mantle. The top two entries in Table 4.9 summarize the key differences between these two forms of non-associative learning.

What do you think would happen if both kinds of stimuli—touching the siphon and shocking the tail—were repeatedly paired? Tom Carew, in collaboration with Kandel and other colleagues, showed that *Aplysia's* siphon-withdrawal reflex can be classically conditioned, as illustrated in Figure 4.23a. When touching the siphon (a potential CS) is repeatedly paired with shocking the tail (the US), an enhanced siphon withdrawal (CR) results in response to subsequent touches of the siphon (Carew, Hawkins, & Kandel, 1983). The enhanced siphon-withdrawal response to the siphon-touch CS following paired training is considerably greater than the generalized sensitization that occurs from presentation of the tail shock alone. Moreover, this classically conditioned

Table 4.9 Varieties of learning in *Aplysia*

Type of learning	Associative	Stimulus specific	Mechanism(s)	Locus of effect
Habituation	No	Yes	Decrease in glutamate	Cellular process
Sensitization	No	No	Serotonin-induced increase in glutamate	Cellular process
Classical conditioning	Yes	Yes	1. Presynaptic activity–dependent enhancement of glutamate release from sensory neuron	Cellular process
			2. Postsynaptic change in receptors of motor neuron	Structural change
			3. A cascade of intracellular molecular events that activate genes in the neuron's nucleus, causing an increase in the number of sensory-motor synapses	Structural change

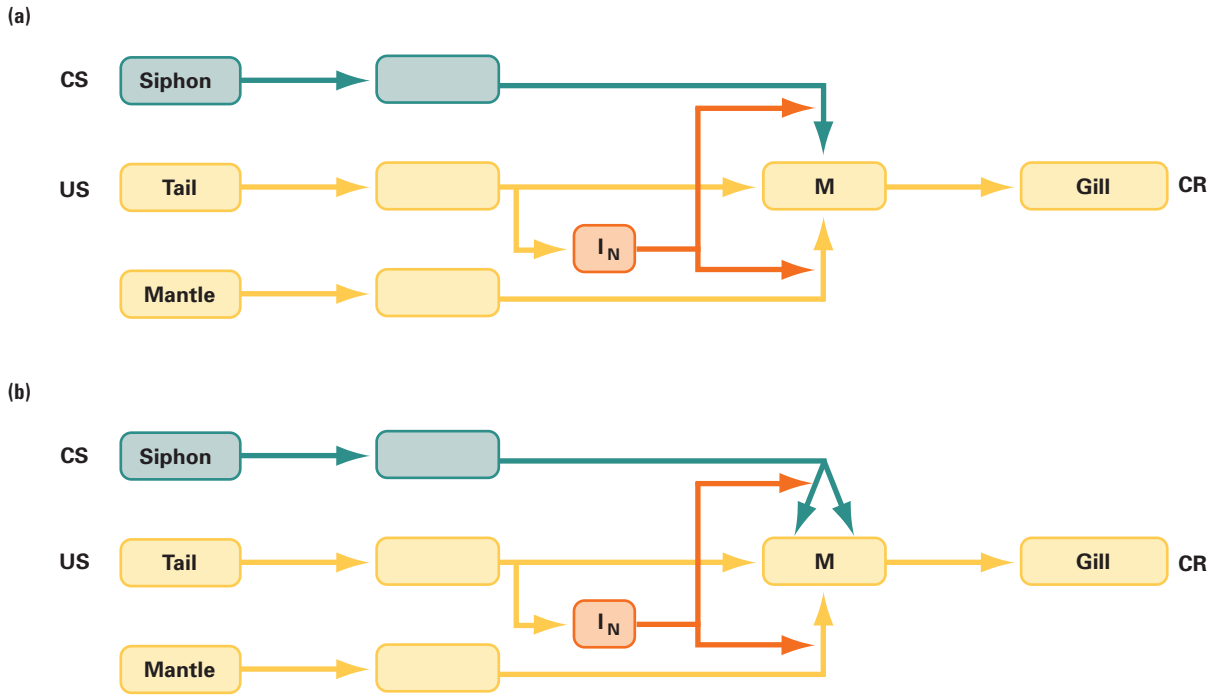

Figure 4.23 Classical conditioning in *Aplysia* (a) As with habituation and sensitization, classical conditioning (a CR) in *Aplysia* results when three sensory pathways—the siphon (CS), the tail (US), and the mantle—converge on the gill-withdrawal motor neuron (M). The tail pathway includes a secondary pathway through an interneuron (I_N), and this releases serotonin onto the other sensory synapses when the tail is shocked. (b) Long-lasting forms of classical conditioning require the formation of new synapses (the dark green arrow heads) between the sensory neurons of the siphon and the motor neuron. The new synapses are created through a molecular cascade set in motion by the serotonin released by the interneuron.

siphon-withdrawal CR is also specific to the siphon and does not generalize to other stimuli, such as a touch on the mantle.

What happens inside the nervous system of *Aplysia* when these two stimuli are paired? Kandel and colleagues demonstrated that paired training produces an increase in the glutamate vesicles that are released in the siphon's synapse on the motor neuron, much like an exaggerated form of the mechanism for sensitization described in Chapter 3 (Hawkins, Abrams, Carew, & Kandel, 1983). This implies that a cellular mechanism for classical conditioning can be understood as an elaboration of the same cellular mechanism used for sensitization.

activity-dependent enhancement. Paired training of CS and US that produces an increase in the glutamate vesicles released from sensory to motor neurons.

The pairing-specific enhancement of glutamate release in the sensory neuron synapse is called an **activity-dependent enhancement** because it depends on activation of the sensory neuron prior to the administration of the US. Earlier in this chapter, we discussed how classical conditioning of the rabbit eyeblink response is sensitive to the order and timing of the tone CS and the airpuff US. The same holds true for conditioning of *Aplysia's* siphon withdrawal: conditioning occurs only if the siphon-touch CS is presented about half a second before the tail-shock US. If the US occurs much later (more than 2 seconds after the CS) or before the CS, nothing other than nonspecific sensitization will occur. Thus, after sensory stimulation, whatever process occurs within the neuron to prime the neuron for an increase in glutamate release has a time course of about a half a second.

To summarize, Kandel and colleagues demonstrated that activation of *Aplysia's* sensory neuron has at least three consequences. First, it causes the motor neuron to fire, by the release of the neurotransmitter glutamate into

the synapse. Second, it causes a decrease in glutamate vesicles available for any subsequent stimulation of the sensory neuron, resulting in habituation. Third, it primes the synapse, through a series of intracellular events lasting about half a second, so that a subsequent presentation of the neurotransmitter serotonin (released following activation of an aversive tail shock) creates an increase in future glutamate release—resulting in a classically conditioned increase in gill withdrawal following pairing of the sensory stimulus (the CS) and the tail shock (the US).

Presynaptic versus Postsynaptic Changes during Learning

This activity-dependent enhancement of the sensory neuron's release of glutamate onto the motor neuron is a presynaptic form of synaptic plasticity, because like the mechanism for sensitization discussed in Chapter 3, it involves a change in the sensory neuron. However, the story is actually more complicated. Later studies demonstrated that there is also a postsynaptic mechanism for conditioning that involves changes in neurotransmitter receptors on the motor neuron (Bao, Kandel, & Hawkins, 1998). Thus, the mechanisms for classical conditioning in *Aplysia* involve both presynaptic and postsynaptic changes in the circuits connecting the CS and the CR, as summarized in Table 4.9.

One advantage of *Aplysia* as a model system for studying the intracellular molecular pathways of learning is that it is possible to identify key neurons (such as entire memory-trace circuits), remove them from the animals, and keep those neurons functioning in a culture dish. By isolating the key circuits for learning and studying them outside the animal, Kandel and colleagues were able to explore the question, What long-term changes in *Aplysia* circuitry could account for long-lasting forms of classical conditioning? The search for the answer to this question took scientists back to the very origins of who we are—our genes—and has given rise to an important new field, the molecular genetics of memory (and also won Kandel the Nobel Prize for Physiology or Medicine in 2001). As we review in more detail in Chapter 12, genes are stretches of DNA molecules (deoxyribonucleic acid), found in the nucleus of every cell, that encode information needed to produce protein molecules. Most people are aware of the role that genes play in determining how our bodies and brains develop during gestation in the uterus. However, our genes don't stop working after birth; rather, they play a critical role throughout our lives, continuing to maintain and guide further growth and development of our bodies and brains, including the changes that result in long-lasting forms of memory.

Long-Term Structural Changes and the Creation of New Synapses

Using recent advances in molecular biology techniques, Kandel and colleagues were able to show that the serotonin released by *Aplysia's* interneurons following a tail-shock US does more than cause a short-term increase in the sensory neuron's release of glutamate; it also launches a cascade of intracellular molecular events that set the stage for long-term structural changes in the neuron. Following multiple pairings of the CS and US, protein molecules in the sensory neuron's synapse travel back up the axon of the sensory neuron all the way to the cell body. There they switch on genes inside the nucleus of the neuron that in turn set in motion the growth of new synapses (Figure 4.23b).

More recent work by Kandel and others has identified two proteins that are found inside neurons and that play critical regulatory roles in this synapse-creation process. The first protein, CREB-1, activates genes in the neuron's nucleus that initiate the growth of new synapses. The second protein, CREB-2,

plays an opponent role, inhibiting the actions of CREB-1. The creation of new synapses during learning requires a cascade of processes inside the cell that activate CREB-1 and suppress CREB-2.

What do you think would happen if functioning of the CREB-1 protein was impaired? Kandel and colleagues demonstrated that if CREB-1 is rendered inactive by injection of molecules into the neuron that compete with CREB-1's ability to activate genes for new synapses, the circuits subsequently fail to show long-lasting forms of associative learning (Dash, Hochner, & Kandel, 1990). Most important, the inactivation of CREB-1 does not affect the short-lasting forms of learning that depend only on increased glutamate release. This study provided critical evidence for a dissociation between short-lasting forms of learning, which do not require the CREB-1 protein, and long-lasting forms, which do.

In a related study, Kandel and colleagues showed that removing the influence of the opponent protein, CREB-2, had the opposite effect: with the CREB-2 inactivated, long-lasting learning occurs rapidly at the sensory neurons, after even a single exposure to serotonin (Bartsch et al., 1995). The role of CREB molecules in modulating long-lasting forms of memory is not limited to *Aplysia*; increasing CREB-1 in fruit flies (*Drosophila*) allows them to learn much more rapidly than usual, while increasing their CREB-2 blocks the formation of long-term memories, such as those produced in the odor-conditioning task described earlier in this chapter (Yin et al., 1994). The CREB molecules also play a critical role in mammals' learning; studies in mice have shown that activity of CREB-1 in the hippocampus is critical to long-lasting but not short-term increases in neuron-to-neuron associations based on LTP (Bourtchuladze et al., 1994).

Studies of classical conditioning in *Aplysia* have demonstrated that anatomical changes in neural circuits, including the growth or deletion of synapses, are characteristic of long-lasting forms of memory. In contrast, short-term, labile forms of memory are associated with temporary intracellular changes within existing anatomical pathways, including shifts in the location, size, or number of neurotransmitter vesicles, which alter synaptic transmission efficacy. Thus, as was also discussed in Chapter 3, the transition from short-term to long-term learning may be characterized as a shift from transmission-process–based changes within the neuron to structural changes within the neural circuits (see Table 4.9).

Interim Summary

- There are two sites in the cerebellum where CS and US information converges and that might potentially be locations for the storage of the CS–US association: (1) the Purkinje cells in the cerebellar cortex and (2) the interpositus nucleus. The interpositus nucleus is the only output pathway from the cerebellum; it is the route through which the learned response travels to the motor systems that control behavior, such as an eyeblink CR.
- The inferior olive is believed to compute the degree to which a US is unexpected, providing the information necessary to implement Rescorla and Wagner's principle of error-correction learning in the cerebellum.
- The hippocampus is a structure underlying some of the CS-modulation effects in conditioning. This is consistent with data showing that an animal without a hippocampus does not exhibit CS-modulation effects such as latent inhibition.
- Kandel and colleagues demonstrated that activation of *Aplysia's* sensory neuron by an external stimulation (such as presentation of a stimulus cue)

primes the synapse, through a series of intracellular events lasting about half a second, so that a subsequent presentation of serotonin (released following activation of an aversive tail shock) creates an increase in future glutamate release, resulting in a classically conditioned increase in gill withdrawal.

- After multiple pairings of the CS and US in *Aplysia*, protein molecules in the sensory neuron's synapse travel back up the axon of the sensory neuron all the way to the cell body. There they activate genes inside the nucleus of the neuron that in turn set in motion the growth of new synapses.

4.3 Clinical Perspectives

In this final section of the chapter, we focus on two clinical applications of classical conditioning. The first involves recognition of the ways drug addiction and drug abuse are intimately linked to classical conditioning, the other harnesses classical conditioning to reduce the amount of medication needed for treating a chronic disease.

Classical Conditioning in Tolerance to Addictive Drugs

The role of learning and memory in drug addiction is a fascinating topic that we consider from several viewpoints in this textbook. In Chapter 5, we explore the neural mechanisms of reward that are impaired by most drugs of abuse. Chapter 8 discusses the role of the frontal lobes as the brain's executive controller, their importance in inhibiting inappropriate behaviors, and how this role is compromised in drug addicts. In the following discussion of drug tolerance, we see how the behavioral and biological mechanisms of classical conditioning influence another aspect of drug addiction and abuse.

Early in this chapter, we discussed how automatic compensatory responses occur in body systems that have a mechanism for **homeostasis**, the tendency of the body (including the brain) to gravitate toward a state of equilibrium or balance. An addict's tolerance to drugs of abuse such as alcohol, cocaine, or ecstasy develops in the same way. As the addict's body adjusts to the drug effects (through expectation of the forthcoming "high"), larger and larger doses are required to produce the same high the addict experienced on first taking the drug. One way this happens is through conditioning: environmental cues that accompany drug use can classically condition the user to expect to receive the drug. In other words, the environmental cues (people, places, and so on) act like CSs associated with the drug (the US). The intense craving an addict feels in response to these cues is the CR and results from the body's conditioned compensatory response of lowering the levels of the brain chemicals enhanced by the drug in anticipation of the drug's arrival (more on the role of conditioning in drug addiction in Chapter 5).

A potential consequence of such conditioned tolerance is that victims of heroin who overdose are rarely novice users (Siegel, 2001). Rather, they tend to be long-time heroin addicts who have developed a high degree of tolerance to the drug but make the mistake of taking their usual dose in an unusual setting. For example, the situational cues that result in conditioned drug tolerance can include the room in which the drug is usually taken. You may recall reports of rock stars and others dying of heroin overdoses in hotel bathrooms, which were most likely far different from the settings in which they were used to taking their drug. What might have happened is that they overdosed on what was