Cellular Mechanisms of Learning and the Biological Basis of Individuality

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THROUGHOUT THIS BOOK we have emphasized that all behavior is a function of the brain and that malfunctions of the brain give rise to characteristic disturbances of behavior. Behavior, in turn, is shaped by learning. How does learning act on the brain to change behavior? How is new information acquired and, once acquired, how is it retained? In the preceding chapter we saw that memory—the outcome of learning—is not a single process but has at least two forms. Implicit (declarative) memory is unconscious memory for perceptual and motor skills, whereas explicit (nondeclarative) memory is a memory for people, places, and objects that requires conscious recall.

In this chapter we examine the cellular and molecular mechanisms that contribute to these two forms of memory by exploring the mechanisms that underlie simple implicit forms of memory storage in invertebrates and the more complex explicit forms in vertebrates. We shall see that the molecular mechanisms of memory storage are highly conserved throughout evolution, and that the more complex forms of learning and memory depend on many of the same molecular mechanisms used in the simplest forms. Finally, we shall consider the idea that these mechanisms contribute to individuality by changing the connectivity of neurons in our brains.

Figure 63-1 The cellular mechanisms of habituation have been investigated in the gill-withdrawal reflex of the marine snail Aplysia.

A. A dorsal view of Aplysia illustrates the respiratory organ (gill), which is normally covered by the mantle shelf. The mantle shelf ends in the siphon, a fleshy spout used to expel seawater and waste. A tactile stimulus to the siphon elicits the gill-withdrawal reflex. Repeated stimuli lead to habituation.

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

Short-Term Storage of Implicit Memory for Simple Forms of Learning Results From Changes in the Effectiveness of Synaptic Transmission

Much progress in the cellular study of memory storage has come from examining elementary forms of learning: habituation, sensitization, and classical conditioning. These cellular modifications have been analyzed in the behavior of simple invertebrates and in a variety of vertebrate reflexes, such as flexion reflexes, fear responses, and the eyelid. Most simple forms of implicit learning change the effectiveness of the synaptic connections that make up the pathway mediating the behavior.
Habituation Involves an Activity-Dependent Presynaptic Depression of Synaptic Transmission

In habituation, the simplest form of implicit learning, an animal learns about the properties of a novel stimulus that is harmless. An animal first responds to a new stimulus by attending to it with a series of orienting responses. If the stimulus is neither beneficial nor harmful, the animal learns, after repeated exposure, to ignore it.

Habituation was first investigated by Ivan Pavlov and Charles Sherrington. While studying posture and locomotion, Sherrington observed a decrease in the intensity of certain reflexes, such as the withdrawal of a limb, in response to repeated stimulation. The reflex response returned only after many seconds of rest. He suggested that this decrease, which he called habituation, results from diminished synaptic effectiveness within the pathways to the motor neurons that had been repeatedly activated.

This problem was later investigated at the cellular level by Alden Spencer and Richard Thompson. They found close cellular and behavioral parallels between habituation of the spinal flexion reflex in the cat and habituation of more complex behavioral responses in humans. They showed, through intracellular recordings from spinal motor neurons in cats, that habituation leads to a decrease in the strength of the synaptic connections between excitatory interneurons and motor neurons. The connections between the sensory neurons innervating the skin and the interneurons were unaffected.

Since the organization of interneurons in the spinal cord of vertebrates is quite complex, further analysis of the cellular mechanisms of habituation in the flexion reflex proved difficult. Progress in this effort required a simpler system. The marine sea slug Aplysia californica, which has a simple nervous system containing only about 20,000 central nerve cells, is an excellent simple system for studying implicit forms of memory. Aplysia

has a repertoire of defensive reflexes for withdrawing its gill and its siphon, a small fleshy spout above the gill used to expel seawater and waste (Figure 63-1A). These reflexes are similar to the leg withdrawal reflex studied by Spencer and Thompson. For example, a mild tactile stimulus delivered to the siphon elicits reflex withdrawal of both siphon and gill. With repeated stimulation these reflexes habituate. They can also be sensitized and classically conditioned, as we shall see later.

Gill withdrawal in Aplysia has been studied in detail. In response to a novel tactile stimulus to the siphon, firing in the sensory neurons innervating the siphon generates excitatory synaptic potentials in interneurons and motor cells (Figure 63-1B). The synaptic potentials from the sensory neurons and interneurons summate both temporally and spatially to cause the motor cells to discharge repeatedly, leading to strong reflexive withdrawal of the gill. If the stimulus is repeated, the direct monosynaptic excitatory synaptic potentials produced by sensory neurons in both the interneurons and the motor cells become progressively smaller. Thus, with repeated stimulation, several of the excitatory interneurons also produce weaker synaptic potentials in the motor neurons, with the net result that the motor neuron fires much less briskly and consequently the reflex response diminishes.

What reduces the effectiveness of synaptic transmission by the sensory neurons? Quantal analysis revealed that the decrease in synaptic strength results from a decrease in the number of transmitter vesicles released from presynaptic terminals of sensory neurons. These sensory neurons use glutamate as their transmitter. Glutamate interacts with two types of receptors in motor cells: one similar to the N-methyl-d-aspartate (NMDA) type of glutamate receptors of vertebrates and the other to a non-NMDA-type (Chapter 12). There is no change in the sensitivity of these receptors with habituation. How this decrease in transmitter release occurs is not yet understood; it is thought to be due in part to a reduced mobilization of transmitter vesicles to the active zone (see Chapter 14). This reduction lasts many minutes.

These enduring plastic changes in the functional strength of synaptic connections thus constitute the cellular mechanisms mediating the short-term memory for habituation. Since these changes occur at several sites in the reflex circuit, memory in this instance is distributed and stored throughout the circuit, not at one specialized site. Synaptic depression of the connections made by sensory neurons, interneurons, or both is a common mechanism for habituation and explains habituation of the several well-studied escape responses of crayfish and cockroaches as well as startle reflexes of vertebrates.
The synaptic mechanisms underlying habituation can vary in two ways. First, the locus of the depression can be situated at any of several synaptic sites. For example, in the flexion reflex of the spinal cord there is no depression of synaptic transmission at the direct connections made by the sensory neurons on interneurons. Rather, depression is thought to occur at downstream sites: at the synapses made by certain classes of interneurons on the motor neurons of the reflex. Second, mechanisms other than homosynaptic depression, such as enhancement of synaptic inhibition, can contribute to habituation.

These studies illustrate that learning can lead to changes in synaptic strength and that the duration of the short-term memory storage is determined by the duration of the synaptic change. For example, in the flexion reflex of the spinal cord there is no depression of synaptic transmission at the direct connections made by the sensory neurons on interneurons. Rather, depression is thought to occur at downstream sites: at the synapses made by certain classes of interneurons on the motor neurons of the reflex. Second, mechanisms other than homosynaptic depression, such as enhancement of synaptic inhibition, can contribute to habituation.

Not all synapses are equally adaptable. The strength of some synapses in Aplysia rarely changes, even with repeated activation. However, at synapses specifically involved in learning and memory storage, such as the connections between sensory and motor neurons and some interneurons in the withdrawal-reflex circuit, a relatively small amount of training, especially if it is appropriately spaced over many minutes or hours, can produce large and enduring changes in synaptic strength. Massed training, whereby the habituating stimuli are given one after the other without rest between training sessions, produces a robust short-term memory but long-term memory is seriously compromised. This illustrates a general principle of learning psychology: Spaced training is usually much more effective than massed training in producing long-term memory.

Sensitization Involves Presynaptic Facilitation of Synaptic Transmission

When an animal repeatedly encounters a harmless stimulus it learns to habituate to it. In contrast, with a harmful stimulus the animal typically learns to respond more vigorously not only to that stimulus but also to other stimuli, even harmless ones. Defensive reflexes for withdrawal and escape become heightened. This enhancement of reflex responses, called sensitization, is more complex than habituation: a stimulus applied to one pathway produces a change in the reflex strength in another pathway. Like habituation, sensitization has both a short-term and a long-term form. Thus, whereas a single shock to an animal's tail produces a short-term sensitization that lasts minutes, five or more shocks to the tail produce sensitization lasting days to weeks.

A noxious stimulus to the tail enhances 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. Thus a synapse can participate in more than one type of learning and store more than one type of memory. However, habituation and sensitization use different cellular mechanisms to produce synaptic change. Short-term habituation in Aplysia is a homosynaptic process; the decrease in synaptic strength is a direct result of activity in the sensory neurons and their central connections in the reflex pathway. In contrast, sensitization is a heterosynaptic process; the enhancement of synaptic strength is induced by modulatory interneurons activated by stimulation of the tail.
Classical Conditioning Involves Presynaptic Facilitation of Synaptic Transmission That Is Dependent on Activity in Both the Presynaptic and the Postsynaptic Cell

Classical conditioning is a more complex form of learning than sensitization. Rather than learning only about one stimulus, the organism learns to associate one type of stimulus with another. As we have learned in Chapter 62, an initially weak conditioned stimulus can become highly effective in producing a response when paired with a strong unconditioned stimulus. In reflexes that can be enhanced by both classical conditioning and sensitization, classical conditioning results in a greater and longer-lasting enhancement.

The siphon- and gill-withdrawal reflexes of Aplysia are examples of reflexes that can be enhanced by both classical conditioning and sensitization. The gill-withdrawal reflex can be elicited in one of two ways: by stimulating either the siphon or a nearby structure called the mantle shelf. The siphon and the mantle shelf are separately innervated by distinct populations of sensory neurons. Thus, each reflex pathway can be conditioned independently by pairing a conditioned stimulus to the appropriate area (either the siphon or the mantle shelf) with an unconditioned stimulus (a strong shock to the tail). After such paired or associative training, the response of the reflex can be elicited in one of two ways: by stimulating either the siphon or a nearby structure called the mantle shelf.

In classical conditioning the timing of the conditioned and unconditioned stimuli is critical. The conditioned stimulus must precede the unconditioned stimulus, often within an interval of about 0.5 s. What cellular mechanisms are responsible for this requirement for temporal pairing of stimuli? In classical conditioning of the gill-withdrawal reflex of Aplysia one important feature is the timing of the convergence in individual sensory neurons of the conditioned stimulus (siphon touch) and the unconditioned stimulus (tail shock).

As we have seen, an unconditioned stimulus to the tail activates interneurons that make axo-axonic connections with the presynaptic terminals of the sensory neurons that carry information from the siphon and the mantle shelf (Figure 63-4A). The resulting presynaptic facilitation ordinarily gives rise to behavioral sensitization. However, if the unconditioned stimulus (to the tail) and the conditioned stimulus (to the siphon or mantle shelf) are timed so that the conditioned stimulus just precedes the unconditioned stimulus, then the modulatory interneurons engaged by the unconditional stimulus will activate the sensory neurons immediately after the conditioned stimulus has activated the sensory neurons. This sequential activation of the sensory neurons during a critical interval by the CS and US leads to greater presynaptic facilitation than when the two stimuli are not appropriately paired (Figure 63-4B). This novel feature unique to classical conditioning is called activity dependence.
There are presynaptic and postsynaptic components to activity-dependent facilitation. Activity in the conditioned stimulus pathway leads to Ca\(^{2+}\) influx into the presynaptic sensory neuron with each action potential, and this influx activates the Ca\(^{2+}\)-binding protein calmodulin. The activated Ca\(^{2+}\)/calmodulin binds to adenylyl cyclase, potentiating its response to serotonin and enhancing its production of cAMP. Thus, the presynaptic cellular mechanism of classical conditioning in the monosynaptic pathway of the withdrawal reflex in *Aplysia* is in part an elaboration of the mechanism of sensitization in this same pathway. This is because adenylyl cyclase acts as a coincidence detector. That is, it recognizes the molecular representation of both the conditioned stimulus (spike activity in the sensory neuron and the consequent Ca\(^{2+}\) influx) and the unconditioned stimulus (serotonin released by tail stimuli), and it responds both to the conditioned stimulus (binding to the Ca\(^{2+}\)/calmodulin activated by the Ca\(^{2+}\)/influx following action potentials) and the unconditioned stimulus (binding to the G\(_\text{G}\)) activated by the binding of serotonin to a receptor).

The postsynaptic component of classical conditioning is a retrograde signal to the sensory neuron. In the withdrawal reflex pathway in *Aplysia* the postsynaptic motor cell has two types of receptors to glutamate: non-NMDA and NMDA-type receptors. As we have learned in Chapter 11, the extracellular mouth of the NMDA-type receptor-channel is plugged by Mg\(^{2+}\) at the resting membrane potential. Thus, under normal circumstances and during habituation and sensitization only the non-NMDA receptor is activated because the NMDA receptor is blocked by Mg\(^{2+}\). However, when the conditioned stimulus and unconditioned stimulus are paired appropriately during classical conditioning, the motor neuron fires a whole train of action potentials. The depolarization of the postsynaptic cell expels Mg\(^{2+}\) from the NMDA-type receptor-channel and Ca\(^{2+}\) flows into the cell. The Ca\(^{2+}\) influx is thought to activate signaling pathways in the motor cell that give rise to a retrograde messenger that is taken up in the presynaptic terminals of the sensory cell, where it acts to enhance transmitter release even further.

**Figure 63-4 Classical conditioning of the gill-withdrawal reflex in *Aplysia.* A conditioned stimulus (CS) applied to the mantle shelf is paired with an unconditioned stimulus (US) applied to the tail. As a control, a CS applied to the siphon is not paired with the US. (Adapted from Hawkins et al. 1983.)

A. A shock to the tail (US) excites facilitating interneurons that form synapses on the presynaptic terminals of sensory neurons innervating the mantle shelf and siphon. This is the mechanism of sensitization (A1).

B. When the mantle pathway is activated by a CS just before the US, the action potentials in the mantle sensory neurons prime them so that they are more responsive to subsequent stimulation from the (serotonergic) facilitating interneurons in the US pathway. This is the mechanism of classical conditioning; it both amplifies the response of the CS pathway and restricts the amplification to that pathway (B1).

Recordings of the excitatory postsynaptic potentials produced in an identified motor neuron by the mantle and siphon sensory neurons were made before training (Pre) and 1 hour after training (Post). After training the excitatory postsynaptic potential due to input from the mantle (paired) sensory neuron (B2) is considerably greater than the one due to the siphon (unpaired) neuron (A2).

C. The experimental protocol for classical conditioning compares the responses of paired and unpaired stimuli mediated by siphon and mantle sensory neurons. In the mantle sensory neurons the action potentials produced by the CS are paired with those produced by the US (tail stimulus). In a siphon sensory neuron the action potentials produced by the CS are not paired with the same US.

Thus, three signals in a siphon sensory neuron must converge to produce the large increase in transmitter release that occurs with classical conditioning: (1) activation...
of adenylate cyclase by Ca\(^{2+}\) influx, representing the conditioned stimulus; (2) activation of serotonergic receptors coupled to adenylate cyclase, representing the unconditioned stimulus; and (3) a retrograde signal indicating that the postsynaptic cell has been adequately activated by the unconditioned stimulus.

**Long-Term Storage of Implicit Memory for Sensitization and Classical Conditioning Involves the cAMP-PKA-MAPK-CREB Pathway**

**Molecular Biological Analysis of Long-Term Sensitization Reveals a Role for cAMP Signaling in Long-Term Memory**

As with habituation and most other forms of learning, practice makes perfect. Repeated experience consolidates memory by converting the short-term form into a long-term form. These physiological consequences of repeated training have been best studied for sensitization. In Aplysia a single training session (or a single application of serotonin to the sensory neurons) gives rise to short-term sensitization, lasting only minutes, that does not require new protein synthesis. However, five training sessions produce long-term sensitization, lasting several days, that requires new protein synthesis. Further spaced training produces sensitization that persists for weeks. These behavioral studies of Aplysia (and similar ones in vertebrates) suggest that short-term and long-term memory are two independent but overlapping processes that blend into one another. Several findings point to this interpretation.

First, both short- and long-term memory for sensitization of the gill-withdrawal reflexes involve changes in the strength of connections at several synaptic sites, including the synaptic connections between sensory and motor neurons (Figure 63-5A). Second, in both the long-term and short-term processes the increase in the synaptic strength of the connections between the sensory and motor neurons is due to the enhanced release of transmitter. Third, the same transmitter (serotonin) released by stimulation of the tail produces short-term facilitation after a single exposure and long-term facilitation after five or more repeated exposures. Finally, cAMP and PKA, intracellular second-messenger pathways that are critically involved in short-term memory, are also recruited for long-term memory (Figure 63-5B).

Despite these similarities, short- and long-term memory are distinct processes that can be distinguished by several criteria. In humans, epileptic seizure or head trauma affects long-term memory but not short-term memory. A similar dissociation between short- and long-term memory can be demonstrated in experimental animals using inhibitors of protein or mRNA synthesis to block long-term memory selectively.

As we saw in the preceding chapter, the process by which transient short-term memory is converted into a stable long-term memory is called consolidation. Consolidation of long-term implicit memory for simple forms involves three processes: gene expression, new protein synthesis, and the growth (or pruning) of synaptic connections.
Figure 63-5 (Facing page) Persistent synaptic enhancement with long-term sensitization.

A. Long-term sensitization of the gill-withdrawal reflex of Aplysia involves facilitation of transmitter release at the connections between sensory and motor neurons. 1. The recordings show representative synaptic potentials in a siphon sensory neuron and a gill motor neuron in a control animal and in an animal that received long-term sensitization training by repeated stimulation of its tail. The record was obtained one day after the end of training. 2. The median amplitude of the post-synaptic potentials (PSP) in an identified gill motor neuron is greater in sensitized animals one day after training than in control animals. 3. The effect of sensitization on the neural circuit of the gill- and siphon-withdrawal reflex is measured here by the median duration of withdrawal of the siphon (see Figure 63-1). (Pre = score before training; post = score after training.) The experimental group was tested one day after the end of training. (Adapted from Frost et al. 1985.)

B. Long-term sensitization of the gill-withdrawal reflex of Aplysia leads to two major changes in the sensory neurons of the reflex: persistent activity of protein kinase A and structural changes in the form of the growth of new synaptic connections.

Both the short-term and long-term facilitation are initiated by a serotonergic interneuron. Short-term facilitation (lasting minutes to hours), resulting from a single tail shock or a single pulse of serotonin, leads to covalent modification of preexisting proteins (short-term pathway). As shown in Figure 63-3, serotonin acts on a postsynaptic receptor to activate the enzyme adenyl cyclase, which converts ATP to the second messenger cAMP. In turn, cAMP activates the cAMP-dependent protein kinase A, which phosphorylates and covalently modifies a number of target proteins, leading to enhanced transmitter availability and release. The duration of these modifications is a measure of the short-term memory.

Long-term facilitation (lasting one or more days) involves the synthesis of new proteins. The switch for this inductive mechanism is initiated by protein kinase A (PKA), which recruits the mitogen-activated kinase (MAPK) and together they translocate to the nucleus (long-term pathway), where PKA phosphorylates the cAMP-response element binding (CREB) protein. The transcriptional activators bind to cAMP response elements (CRE) located in the upstream region of two types of cAMP-inducible genes. To activate CREB-1, PKA needs also to remove the repressive action of CREB-2, which is capable of inhibiting the activation capability of CREB-1. PKA is thought to mediate the derepression of CREB-2 by means of another protein, MAPK. One gene activated by CREB encodes a ubiquitin hydrolase, a component of a specific ubiquitin protease that leads to the regulated proteolysis of the regulatory subunit of PKA. This cleavage of the (inhibitory) regulatory subunit results in persistent activity of PKA, leading to persistent phosphorylation of the substrate proteins of PKA, including both CREB-1 and the protein involved in the short-term process. The second gene activated by CREB encodes another transcription factor C/EBP. This binds to the DNA response element CAAT, which activates genes that encode proteins important for the growth of new synaptic connections.
How do genes and proteins operate in the consolidation of long-term functional changes? Studies of long-term sensitization of the gill-withdrawal reflex indicate that with repeated application of serotonin the catalytic subunit of PKA recruits another second messenger kinase, the mitogen-activated protein (MAP) kinase, a kinase commonly associated with cellular growth. Together the two kinases translocate to the nucleus of the sensory neurons, where they activate a genetic switch (see the discussion of transcriptional regulation in Chapter 13). Specifically, the catalytic subunit phosphorylates and thereby activates a transcription factor called CREB-1 (cAMP response element binding protein). This transcriptional activator, when phosphorylated, binds to a promoter element called CRE (the cAMP response element). By means of the MAP kinase the catalytic subunit of PKA also acts indirectly to relieve the inhibitory actions of CREB-2, a repressor of transcription.

The presence of both a repressor (CREB-2) and an activator (CREB-1) of transcription at the very first step in long-term facilitation suggests that the threshold for putting information into long-term memory is highly regulated. Indeed, we can see in everyday life that the ease with which short-term memory is transferred into long-term memory varies greatly depending on attention, mood, and social context. In fact, when the repressive action of CREB-2 is relieved (by injecting, for example, a specific antibody to CREB-2), a single pulse of serotonin, which normally produces only short-term facilitation lasting minutes, is able to produce long-term facilitation, the cellular homolog of long-term memory.

Under normal circumstances the physiological relief of the repressive action of CREB-2 and the activation of CREB-1 induce expression of downstream target genes, two of which are particularly important: (1) the enzyme ubiquitin carboxyterminal hydrolase, which activates proteasomes to make PKA persistently active, and (2) the transcription factor C/EBP, one of the components of a gene cascade necessary for the growth of new synaptic connections. The induction of the hydrolase is a key step in the recruitment of a regulated proteolytic complex: the ubiquitin-dependent proteasome. As in other cellular contexts, ubiquitin-mediated proteolysis also produces a cellular change of state, here by removing inhibitory constraints on memory. One of the substrates of this proteolytic process is the regulatory subunit of PKA.

PKA is made up of four subunits: two regulatory subunits inhibit two catalytic subunits (Chapter 13). Long-term training and the induction of the hydrolase degrades about 25% of the regulatory (inhibitory) subunits in the sensory neurons. As a result, the catalytic subunits continue phosphorylating proteins important for enhancing transmitter release and strengthening the synaptic connections, including CREB-1, long after the second messenger, cAMP, has returned to its basal level (Figure 63-58). This is the simplest mechanism for long-term memory: a second-messenger kinase critical for the short-term process is made persistently active for up to 24 hours by repeated training, without requiring a continuous signal of any sort. The kinase becomes autonomous and does not require either serotonin, cAMP, or PKA.

The second and more enduring consequence of the activation of CREB-1 is a cascade of gene activation that leads to the growth of new synaptic connections. It is this growth process that provides the stable, self-maintained state of long-term memory. In Aplysia the number of presynaptic terminals in the sensory neurons of the gill-withdrawal pathway increases and becomes twice as great in the long term in sensitized animals as in control animals (Figure 63-6) (Bailey and Chen 1983). Long-term habituation leads to a loss of synapses and long-term sensitization leads to an increase in synapses.