CHAPTER 10

Memory, Learning, and Synaptic Plasticity

学而时习之，不亦说乎？

Is it not a pleasure, to have learned something, and to practice it at regular intervals?

Confucius (~500 BC)

A hallmark of the nervous system is its ability to change depending on experiences. In the preceding chapters, we have learned how the nervous system processes sensory information and how it organizes motor output. However, the nervous system is much more than a giant sensorimotor circuit. In addition to acquiring sensory information from the environment and making appropriate responses, animals are constantly learning from their sensory experiences and from the consequences of their actions. These learning processes and events can cause lasting changes in the brain that make it possible to retain the learned information we call **memory**. Learning enables animals to adapt to their changing world much faster than by evolutionary mechanisms, and its importance to animals and humans cannot be overstated. Memory gives us much of our individuality, as we are profoundly shaped by what we can remember from our past experiences.

Memory and learning have fascinated human beings throughout our written history. The epigraph above, taken from the opening statement of the *Analects of Confucius*, reveals that the importance of practicing what has been learned was already recognized 2500 years ago. The French philosopher Rene Descartes described memory as an imprint made in the brain by external experience (**Figure 10–1**). Over a century ago, psychologists had already established important concepts, such as the distinct steps of the memory process including acquisition, storage, and retrieval. But our understanding of the neurobiological basis of memory and learning comes mostly from research conducted during the past few decades, fostered by our increasing knowledge about the workings of the brain at molecular, cellular, and systems levels.

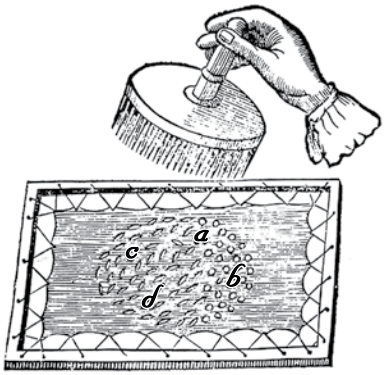

Figure 10–1 Memory as an imprint. According to Rene Descartes, memory could be considered as the imprints left on a linen cloth after needles had passed through it; some of the needle holes would stay open (as near points *a* and *b*), and for holes that close (as near points *c* and *d*), some traces would remain that make it easier to reopen them afterwards. (Adapted from Descartes R [1664] Treatise of Man.)

PRELUDE: WHAT IS MEMORY, AND HOW IS IT ACQUIRED BY LEARNING?

That different parts of the brain perform different functions seems an obvious concept today, but historically it took a long time for this concept to take root (see Section 1.10). Prior to the 1950s, the prevailing view was that memories for specific events and skills are distributed across large areas of the cerebral cortex. For example, in the 1920s, Karl Lashley carried out systematic lesions of the cerebral cortex of rats that had learned maze navigation to search for brain areas that, when removed, would affect the learned task. He did not identify a particular area that was necessary for memory; instead, task performance deteriorated progressively as increasingly larger areas were removed. From the 1950s onward, this concept of distributed memory changed, at least with regard to memory acquisition, as a result of studies in human patients, particularly the patient H.M.

Figure 10–2 Henry Molaison (H.M.), a famous amnesic patient. Bilateral removal of the medial temporal lobes to alleviate his epilepsy resulted in profound defects in H.M.'s ability to form new memories of facts and events. (Adapted from Corkin S [2013] Permanent Present Tense. Basic Books.]

10.1 Memory can be explicit or implicit, short-term, or long-term: Insights from amnesic patients

Henry Molaison (**Figure 10–2**), widely known as H.M. to protect his privacy until his death at the age of 82 in 2008, suffered from intractable seizures as a young

man. In 1953, he underwent a bilateral surgical removal of the medial temporal lobes for the treatment of his seizure. While his seizures improved significantly, he emerged from the surgery with irreparable damage: he appeared to have lost his ability to form new memories. He did not recognize doctors who saw him frequently. Within half an hour of eating lunch, he could not remember a single item he had eaten; in fact, he could not remember having eaten lunch at all.

Extensive studies were performed on H.M. His personality and general intelligence, including perception, abstract thinking and reasoning abilities, were not affected by the surgery. In fact, his IQ improved slightly, from 104 pre-surgery to 112 post-surgery, likely because he was less affected by seizures after the surgery. However, he could not retain memory during intensive tasks such as trying to remember a three-digit number with repeated rehearsals; as soon as his attention shifted to a new task, he did not recall the old task or having ever been exposed to it. However, H.M. still had vivid memories of childhood and had largely intact memories of events until about 3 years prior to his surgery. He remembered the address of his old house (but not the address of the new house he moved to after the surgery).

Interestingly, not all forms of memory were impaired in H.M. In a mirror drawing task, subjects are asked to trace a line between the two borders of a double-outlined star (**Figure 10–3**A) while looking at their hands only in a mirror. Healthy people improve at this task with practice, so that the number of errors they make—defined by the number of times the traced line crosses one of the borders—decreases in later trials. H.M. could learn this task with a decreasing error rate just as normal subjects do. He showed steady improvement in this task across three days (Figure 10–3B), although each day he could not recall ever having performed the task before.

Studies of amnesic patients like H.M. have provided important insights into human memory. First, memory can be divided into two broad categories: explicit and implicit (**Figure 10–4**). **Explicit memory** (also called **declarative memory**) refers to memory that requires conscious recall, such as memories of names, facts, and events. When we use the term 'memory' in daily life, we are usually referring to explicit memory. **Implicit memory** (also called **non-declarative memory** or **procedural memory**) refers to memory in which previous experience aids in the performance of a task without conscious recall. The skill that H.M. acquired in the mirror drawing task and the ability to ride a bicycle involve implicit memory; so do habituation and sensitization, memory types that will be introduced later in this chapter. H.M. was selectively deficient for forming new explicit memories after his surgery.

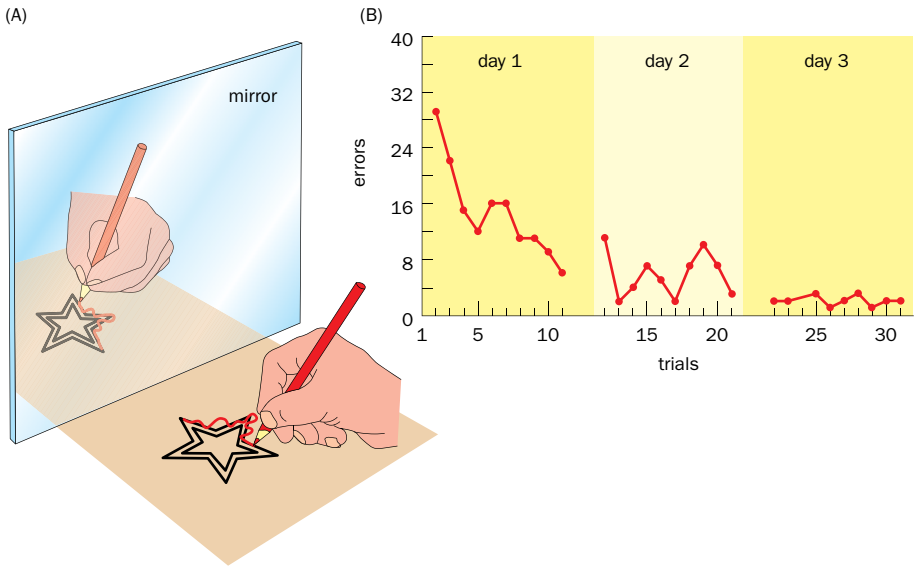

Figure 10–3 Memory of motor-skill learning displayed by H.M. (A) In this task, subjects are asked to view a double-outlined star in a mirror and draw a line in the space between its two borders. Subjects can only see their hands in the mirror. **(B)** With practice (number of trials, *x* axis), H.M. improved his performance in the mirror drawing task within and across days, as seen by the decreasing number of errors (occasions on which the traced line crosses a border, *y* axis). (B, adapted from Milner B, Squire LR & Kandel ER [1998] *Neuron* 20:445–468. With permission from Elsevier Inc.)

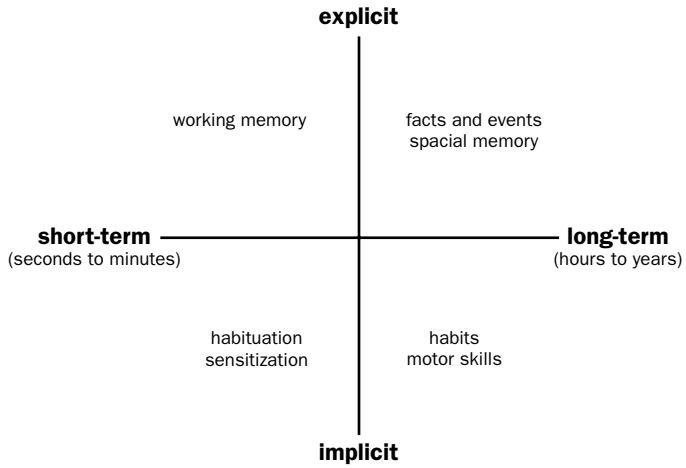

Figure 10–4 Different types of memory. One major division of memory is explicit (for example, facts and events that require conscious recall) versus implicit (for example, habits and motor skills that do not require conscious recall). Another distinction among different types of memory is their duration: short-term memory lasts for seconds to minutes, while long-term memory can remain intact throughout the lifespan of a human or other animal.

Second, memory has different temporal phases, which are usually divided into short-term and long-term memory (Figure 10–4). **Working memory**, where facts are temporarily held (such as doing multi-step mental arithmetic, or remembering a telephone number before dialing before the era of smart phones), is a form of **short-term memory**. H.M. had intact working memory, which enabled him to hold normal conversations with others, but he could not convert facts and events into **long-term memory**. Implicit memory also has short- and long-term components. The exact temporal window can vary for different types of memories and in different organisms, but typically the memories we define as short-term are retained for seconds to minutes, whereas long-term memories can last for hours to years (Figure 10–4). As we will learn later in the chapter, there are mechanistic differences between short-term and long-term memory.

Third, distinct steps of the memory process and different types of memory require the function of specific parts of the brain. As we alluded to in the introduction, nineteenth century psychologists had divided memory into distinct steps. **Acquisition** is the initial formation of a memory as a consequence of experience and learning. **Retrieval** is the recall of a memory. **Storage** is the step in between acquisition and retrieval, where memory is held somewhere in the nervous system. More recently, a distinct step called **consolidation** has been proposed between acquisition and storage, during which newly acquired memory is solidified. Systematic comparisons of the lesions of H.M. and other amnesic patients have revealed that the region of the medial temporal lobe essential for the acquisition of new explicit memories is the **hippocampus**, located underneath the cortical surface of the temporal lobes (see Figure 1–8).

Importantly, H.M. still had largely intact explicit memory after surgery for the facts and events he had encountered prior to surgery. This suggests that the hippocampus is required for the acquisition of new explicit memories, but not for the long-term storage or retrieval of remote explicit memories. This also implies that the memories formed by utilizing the hippocampus are then stored elsewhere in the brain, such that they can be recalled even when hippocampal function is disrupted (as with H.M.). The fact that H.M. appeared to have intact working memory (which enabled him to hold conversations) and implicit memory (which enabled him to perform the mirror drawing task) implies that working memory and implicit memory also do not require the presence of the hippocampus. It is generally accepted that the prefrontal cortex plays a central role in working memory, whereas the cerebellum and the basal ganglia are instrumental for many types of motor learning (see Sections 8.8 and 8.9).

10.2 Hypothesis I: Memory is stored as strengths of synaptic connections in neural circuits

A key question that connects memory to the neurobiology we have studied in the preceding chapters is: What is the cellular basis of memory storage? Finding

a satisfactory answer to this question would allow researchers to then study the mechanisms by which memory is acquired and retrieved. The leading hypothesis, which is strongly supported by the experimental evidence presented in this chapter, is that memory is stored as strengths of synaptic connections in neural circuits.

Let's first discuss this hypothesis from a theoretical perspective. Suppose that we have a synaptic connection matrix between five input neurons and five output neurons, which have the potential to form 25 synaptic connections. To simplify the discussion, we use a binary code for the connection matrix, where 1 designates a connection (purple dots in **Figure 10–5**, left) and 0 indicates the lack of a connection. Suppose further that the firing threshold of each output neuron obeys the following integration rule: if two or more of its connected presynaptic input neurons are firing simultaneously, it will fire its own action potential. The input–output function of this circuit, determined by the synaptic connection matrix, can in principle be used for event-triggered memory recall, where each input pattern can be considered an event and each output pattern can be considered a memory recall. Each input pattern is represented by a specific combination of firing patterns of the five input neurons at a given time. Three input patterns are shown as X_1, X_2, and X_3 (Figure 10–5, right), where 1 means that a presynaptic neuron is firing an action potential, and 0 means the presynaptic neuron is not firing an action potential. After passing through the connection matrix, each input pattern produces a corresponding output pattern, Y_1, Y_2, and Y_3, represented by the firing pattern of output neurons at a given time as determined by the integration rule. Through this synaptic connection matrix, each input pattern produces a defined output pattern; in other words, each event (X_1, X_2, X_3, and so forth), by interacting with this synaptic matrix, triggers the recall of specific memories (Y_1, Y_2, Y_3, and so forth) (**Movie 10–1**).

Instead of only five input and five output neurons like the above example, neural circuits in the mammalian brain usually comprise many more neurons. As

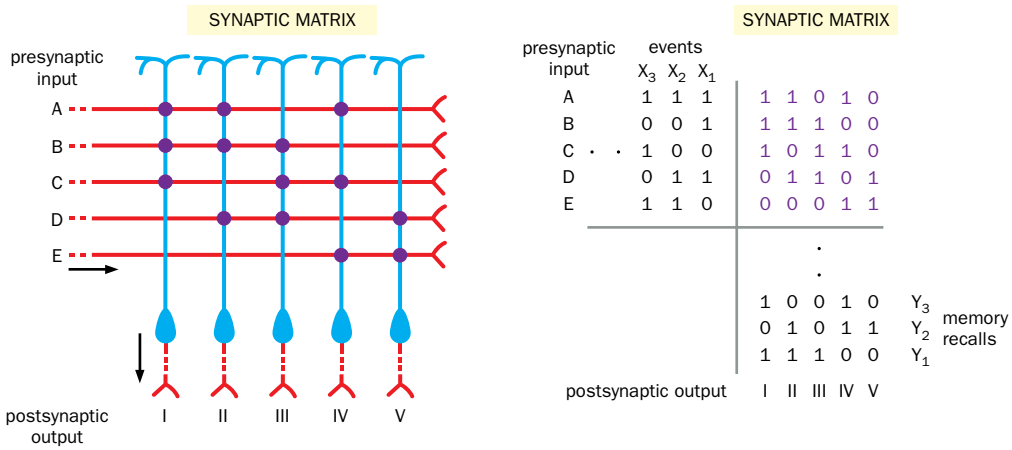

Figure 10–5 The synaptic weight matrix as a memory device. **Left**, a highly simplified model is used to illustrate how a synaptic matrix can store memory. In this synaptic matrix, axons of five presynaptic input neurons (A–E, red) form specific connections with dendrites of five postsynaptic output neurons (I–V, blue) that are represented by a binary code: each purple dot signifies a synaptic connection (value = 1); the absence of a purple dot indicates that no synaptic connection exists (value = 0). (In reality, rather than binary, synaptic connection strengths are continuous—from 0 or no connection to 1 or connection with maximal strength.) Blue cell bodies of the postsynaptic output neurons are shown below the matrix. Arrows indicate the direction of information flow. **Right**, this synaptic matrix can transform specific events, represented by the firing pattern of five input axons at any given time, to specific memory recalls represented by the firing pattern of output neurons. As examples, three specific input patterns, X_1, X_2, and X_3, are transformed to three corresponding output patterns, Y_1, Y_2, and Y_3. In these input and output patterns 1 and 0 represent an action potential or no action potential, respectively. The integration rule of each postsynaptic neuron is set such that it fires when two or more of its presynaptic partners are firing an action potential at a given time (in other words, when the matrix product is equal to or greater than 2). For example, for X_1, presynaptic neurons A, B, and D fire action potentials; neurons C and E do not. Neuron A synapses on output neurons I, II, and IV, neuron B synapses on neurons I, II, and III, and neuron D synapses on neurons II, III, and V. Thus, two presynaptic partner neurons fire on postsynaptic output neurons I, II, and III (matrix product ≥ 2), whereas only one presynaptic partner fires on neurons IV and V (matrix product <2). The resulting Y_1 is that neurons I, II, and III fire action potentials, while neurons IV and V do not. This 5×5 matrix has 2^{25} or ~30 million binary codes that can be used as a memory device to mediate event (X_N) triggered recall (Y_N).

the number of neurons increases, the number of possible synaptic connections goes up astronomically. Whereas the 5 × 5 matrix in Figure 10–5 has $2^{(5\times5)}$ or ~30 million possible binary codes, a 100 × 100 matrix has $2^{(100\times100)}$ or ~10^{3000} possible binary codes, more than there are atoms in the universe. At the same time, suppose that input patterns are represented by the simultaneous firing of 10 out of 100 input neurons; choosing 10 active input fibers out of 100 provides ~10^{13} different events. Even if the input fibers encode a different event each millisecond, the system can run for more than 300 years without repeating an event. Furthermore, we have simplified the synaptic connection matrix as consisting of 0–1 binary codes, but in reality the strength (or the weight) of synaptic connections can be any value between 0 (no connection) and 1 (maximal strength of connection). This greatly expands the coding capacity. In summary, these **synaptic weight matrices** can in principle store enormous amounts of information that can be used to transform specific input patterns (events) to specific output patterns (memory recalls). In Section 10.18, we will see a discrete example of how information in the synaptic weight matrix is read out by different downstream neurons to instruct distinct behavior.

As an example of synaptic weight matrices, let's examine the circuit organization of the mammalian hippocampus (**Figure 10–6**). The hippocampus receives input from the neocortex via the adjacent entorhinal cortex. Axons that project from neurons in the superficial layers of the entorhinal cortex, which constitute the **perforant path**, synapse onto the dendrites of **granule cells** in the **dentate gyrus**, the input part of the hippocampus. The axons of dentate gyrus granule cells, called **mossy fibers** because of their elaborate axon terminals, form synapses with the dendrites of CA3 pyramidal neurons, while the axons of CA3 pyramidal neurons form extensive recurrent connections via association fibers (that is, they synapse onto CA3 pyramidal neurons, including themselves). CA3 axons also form branches called **Schaffer collaterals**, which synapse onto the dendrites of CA1 pyramidal neurons. In addition to receiving trisynaptic input (perforant path → granule cells → CA3 → CA1), CA1 dendrites also receive direct input from the entorhinal cortex via the perforant path (Figure 10–6).

Thus, the hippocampus contains not just one but multiple synaptic matrices for information processing. These include the perforant path → granule cell synapses, the granule cell mossy fiber → CA3 synapses, the recurrent network among CA3 neurons, the CA3 Schaffer collateral → CA1 synapses, and the direct

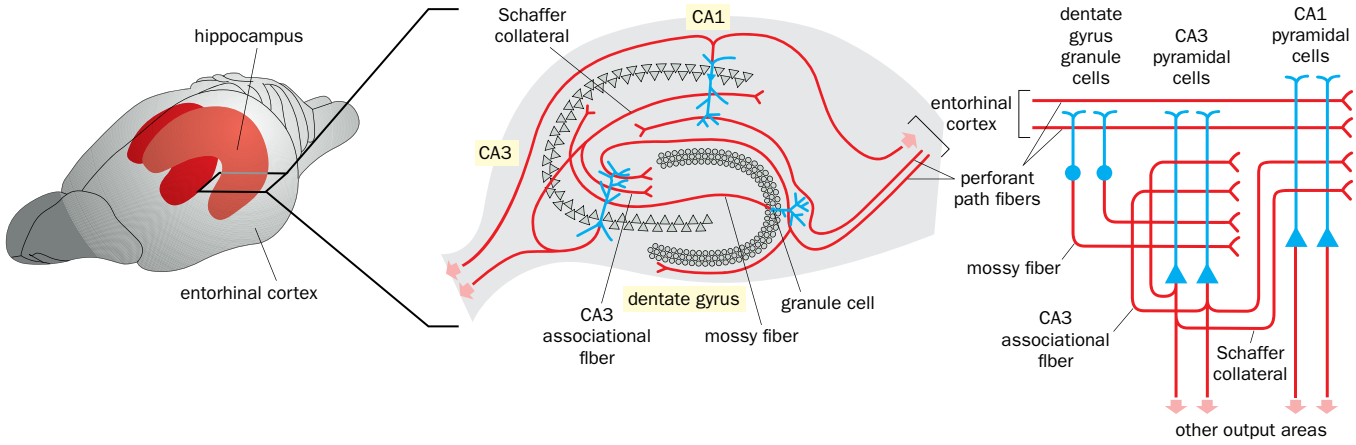

Figure 10–6 The hippocampal circuit. Left, location of the hippocampus and entorhinal cortex in the rat brain. A magnified section of the hippocampus (middle) and a circuit diagram (right) highlight the principal neurons (circles, granule cells; triangles, pyramidal neurons) and their major connections. Blue, dendrites and cell bodies; red, axons. Synapses can form where blue and red lines intersect. Perforant pathway axons from superficial layers of the entorhinal cortex can reach hippocampal CA1 pyramidal neurons directly via a monosynaptic connection, or indirectly via a trisynaptic connection in which the dentate gyrus granule cells and CA3 pyramidal neurons act as intermediates. CA3 pyramidal neurons also form extensive recurrent connections. Both CA3 and CA1 axons project to subcortical areas (middle panel, bottom left; right panel, bottom). In addition, CA1 axons project directly and via intermediate neurons (not shown) to deep layers of the entorhinal cortex (middle panel, top right).

perforant path → CA1 synapses. In the rat hippocampus, there are hundreds of thousands of CA1 and CA3 pyramidal neurons and over a million dentate gyrus granule cells. Each neuron is connected with thousands to tens of thousands of other neurons in these synaptic matrices, thus providing huge capacity for memory acquisition and storage.

10.3 Hypothesis II: Learning modifies the strengths of synaptic connections

If memory is stored as weights of synaptic matrices, then the essence of learning is to alter such weights based on experience. We have already studied one such mechanism—Hebb's rule—in Chapter 5. According to Hebb's rule, when the firing of a presynaptic neuron repeatedly participates in causing the postsynaptic neuron to fire, their synaptic connection becomes strengthened; conversely, when the firing of the presynaptic neuron repeatedly fails to elicit the firing of the postsynaptic neuron, their synaptic connection becomes weakened (see Figure 5–25). In principle, Hebb's rule can be used to modify the weights of synaptic connection matrices, including the formation of new synapses and the dismantling of existing ones. In a synaptic weight matrix (for example, see Figure 10–5), a change of synaptic weight at specific synapses means that the same input must produce different outputs before and after learning. The term **synaptic plasticity** is used to describe changes of the strengths of synaptic connections in response to experience and neuronal activity.

In summary, synaptic connections can be modified (that is, formed, dismantled, strengthened, or weakened), and neuroscientists hypothesize that these modifiable synaptic connections represent a major form of plasticity underlying memory and learning. We will devote the rest of this chapter to examining how well the experimental evidence supports this conceptual framework. In addition to synaptic plasticity, other plastic changes, such as the expression level and subcellular distribution of ion channels that underlie intrinsic properties of neurons (see Section 8.5), can also contribute to memory and learning. One specific example of an intrinsic property is the concentration of voltage-gated Na^+ channels at the axon initial segment, which determines the efficacy by which input (collective synaptic potentials) is transformed into output (action potentials) (see Sections 3.24–3.25).

Memory and learning have been studied on a variety of levels of organization, including genes and proteins, individual neurons and their synapses, the circuits comprising those neurons, and the animal behaviors effected by the activity of those circuits. Researchers can study memory and learning by taking two complementary approaches: a top-down approach that deconstructs complex phenomena to reveal the underlying mechanisms, or a bottom-up approach that starts with more basic, smaller-scale phenomena and explores how they relate to high-level events (**Figure 10–7**). A full understanding of the complexities of memory and learning requires investigations at all of these levels of organization. We begin at the level of neurons and synapses, focusing on the mechanisms that underlie synaptic plasticity.

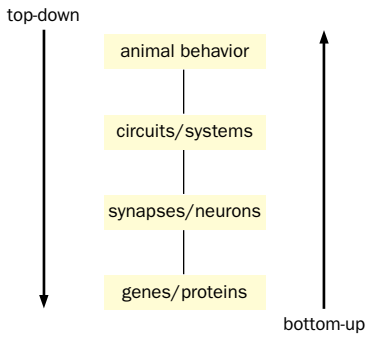

Figure 10–7 Memory and learning can be studied at multiple levels. When researchers start by observing a complex, high-level phenomenon and work to discover its underlying mechanisms, the approach is described as top-down or reductionist. By contrast, when researchers start by examining a low-level phenomenon and try to elucidate its relationship to more complex, high-level events, the approach is termed bottom-up or integrative.

HOW IS SYNAPTIC PLASTICITY ACHIEVED?

The ability of synapses to change their strengths according to experience is one of the most remarkable properties of the nervous system. Most mechanistic studies of synaptic plasticity in mammals have centered on the hippocampus; this focus has been prompted by human (see Section 10.1) and animal studies indicating that the hippocampus plays an essential role in memory acquisition, by the highly organized architecture of the synaptic input and output of hippocampal principal neurons (that is, excitatory projection neurons; see Figure 10–6), by the opportunity to investigate many synaptic connections *in vitro* using brain slices, and by the discovery of the plasticity phenomena to which we now turn.

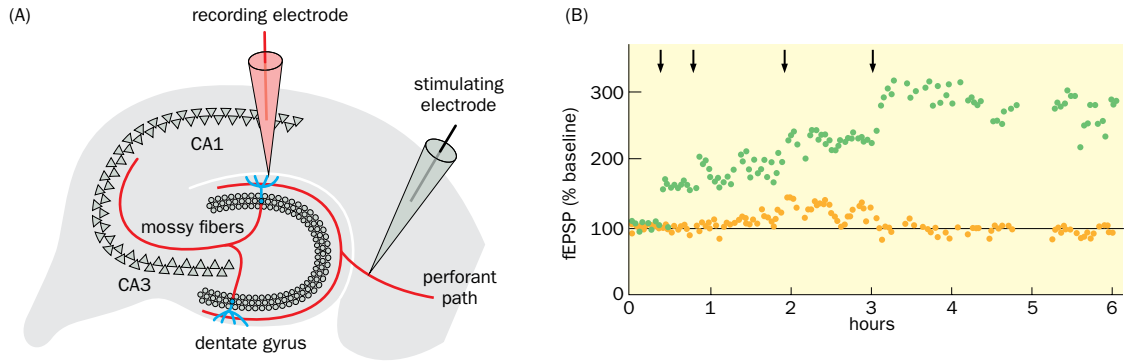

Figure 10–8 Long-term potentiation (LTP) induced by high-frequency stimulation. (A) Experimental setup. The stimulating electrode was placed at the perforant path, which consists of axons that innervate dentate gyrus granule cells. A second electrode was placed near the granule cell bodies to record the field excitatory postsynaptic potential (fEPSP), which represents the collective EPSPs from the population of granule cells near the recording electrode. Axons of dentate gyrus granule cells form the mossy fibers. **(B)** High-frequency stimulations (downward arrows, each representing 10 s of 15-Hz stimulation) caused an increase in the amplitude of fEPSPs produced afterward by single stimuli (green dots) compared to controls (yellow dots, no high-frequency stimulation). (Adapted from Bliss TVP & Lomo T [1973] *J Physiol* 232:331–356. With permission from the Physiological Society.)

10.4 Long-term potentiation (LTP) of synaptic efficacy can be induced by high-frequency stimulation

In the early 1970s, it was discovered that the connection strengths of hippocampal neurons could be altered in response to high-frequency stimulation (Figure 10–8). In these experiments, an extracellular recording electrode was implanted in the dentate gyrus of anesthetized rabbits to record the activity of granule cell populations near the electrode. A stimulating electrode was placed in the perforant path to provide synaptic input to the granule cells. A single stimulus applied to the stimulating electrode would depolarize the granule cell populations via the perforant path → granule cell synapses. This was recorded as a **field excitatory postsynaptic potential** (fEPSP; see Section 3.15 for EPSP and Section 13.20 for field potential), whose amplitude (or in later experiments, initial slope) is a measure of the strength of synaptic transmission between the stimulated axons of the perforant path and the granule cell population near the recording electrode. After brief trains of high-frequency stimulation were delivered through the stimulating electrode, each single stimulus thereafter produced an fEPSP with a two- to threefold greater magnitude than the baseline. This indicates that the strength of synaptic transmission (**synaptic efficacy** in short) between the perforant path axons and granule cells was enhanced as a result of the high-frequency stimulation. Importantly, this enhancement could last for many hours to several days (Figure 10–8). This phenomenon is thus called **long-term potentiation** (**LTP**).

LTP in response to high-frequency stimulation has since been observed at all excitatory synapses in the hippocampus, including the mossy fiber → CA3 synapse, the CA3 → CA3 recurrent synapse, the CA3 Schaffer collateral → CA1 synapse (which we will refer to as the CA3 → CA1 synapse), and the perforant path → CA1 synapse (see Figure 10–6). LTP has also been found in many regions of the nervous system including the neocortex, striatum, amygdala, thalamus, cerebellum, and spinal cord. Importantly, LTP can be reproduced *in vitro* in **brain slices**, which largely preserve the local three-dimensional architecture of brain tissues *in vivo* while allowing easier experimental access for mechanistic studies. These studies have revealed that LTP at different synapses can exhibit different properties through distinct mechanisms. Below, we focus on LTP at the CA3 → CA1 synapse, which is one of the most studied synapses in the mammalian brain.

10.5 LTP at the hippocampal CA3 → CA1 synapse exhibits input specificity, cooperativity, and associativity

The reproduction of LTP in hippocampal slices has enabled many studies to probe its properties. In one experiment, two separate electrodes were placed on the

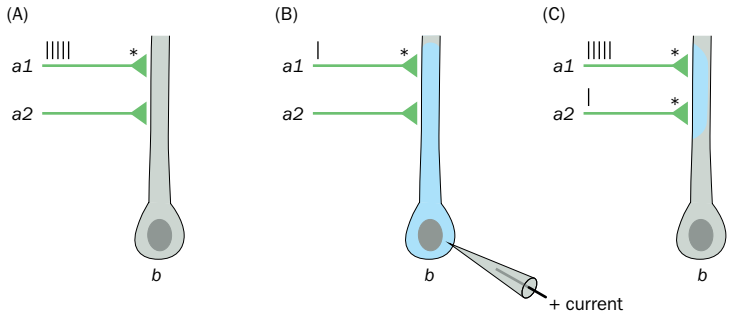

Figure 10–9 Input specificity, cooperativity, and associativity of long-term potentiation. In each experiment, two sets of presynaptic axons from CA3, *a1* and *a2*, form synapses with the same postsynaptic CA1 neuron *b*. **(A)** LTP exhibits input specificity. In the schematic shown here, only the *a1* → *b* synapses that have undergone high-frequency stimulation (represented by repeated vertical bars) exhibit LTP (* indicates potentiated synapses). **(B)** LTP exhibits cooperativity. Depolarization (blue) of postsynaptic cell *b* by current injection enables a weak stimulus (single shock) at axon *a1* to induce LTP. **(C)** LTP exhibits associativity. A weak stimulus at the *a2* → *b* synapses normally would not induce LTP at that synapse. However, when the timing of a weak *a2* stimulus coincides with high-frequency stimulation of *a1*, the *a2* → *b* synapse also becomes potentiated, because local depolarization at the *a1* → *b* synapses spreads to the *a2* → *b* synapses (blue represents the extent of depolarization spread). (See Bliss TVP & Collingridge GL [1993] *Nature* 361:31–39.)

Schaffer collaterals to stimulate two sets of presynaptic axons (from two groups of CA3 neurons), *a1* and *a2*, which synapsed onto the dendrites of cell *b*, a CA1 postsynaptic neuron that was being recorded. LTP was induced by high-frequency stimulation of *a1* (**Figure 10–9**A). When the synaptic efficacy was measured afterwards, only the strength of the *a1* → *b* connection was potentiated, whereas the strength of the *a2* → *b* connection remained unchanged. Thus, LTP exhibits **input specificity**: it occurs at the synapses that have experienced high-frequency stimulation but does not occur at inactive synapses of the same postsynaptic neuron.

A second property of LTP was derived from experiments attempting to induce LTP by directly manipulating the postsynaptic neurons. When a weak axonal stimulation that was insufficient to induce LTP (such as a single stimulus, also called a shock) was paired with coincident injection of depolarizing currents into the postsynaptic cell from the recording electrode, LTP could be induced (Figure 10–9B). Thus, LTP is induced at a synapse when two events coincide: (1) the presynaptic cell fires and releases neurotransmitters and (2) the postsynaptic cell is in a depolarized state. This property is called **cooperativity** of LTP.

The cooperativity of LTP explains why high-frequency stimulation can induce LTP. Early in the train, action potentials from *a1* depolarize cell *b* at the *a1* → *b* synapses, such that the arrival of action potentials late in the train coincides with a depolarized state of the postsynaptic cell, hence potentiating the *a1* → *b* synapses. (Indeed, cooperativity was originally used to describe the phenomenon that high-frequency stimulation of one or few axons is insufficient to induce LTP, and 'cooperation' of many active axons is needed to induce LTP. The underlying mechanism is the same as defined above—to produce sufficient depolarization in the postsynaptic cell coincident with presynaptic axon firing.) Cooperativity can also explain a third property of LTP illustrated in the following experiment. While high-frequency stimulation was applied to *a1* to induce LTP at the *a1* → *b* synapses, *a2* was also stimulated at a level (for example, a single shock) that by itself would not reach the threshold of inducing LTP. The coincident stimulation was found to potentiate the *a2* → *b* synapses as well (Figure 10–9C). This is because high-frequency stimulation of *a1* causes depolarization in a region of cell *b* that includes the site of the *a2* → *b* synapses. If *a2* receives a weak stimulus (such as a single shock) during the time *b* is depolarized at the *a2* → *b* synapses, the synapses become potentiated. This potentiation of synapses that experience a weak stimulus by a coincident strong stimulus is called **associativity** of LTP.

These properties of LTP make it a suitable mechanism for adjusting the synaptic weight matrix that is hypothesized to underlie memory. Using Figure 10–5 as an example, input specificity allows the strengths of different synapses of a postsynaptic neuron with different input neurons to be altered independently, while cooperativity allows a given input to alter the strengths of synapses with a specific subset of co-active postsynaptic neurons. Together, these properties allow experience to adjust synaptic weights in the matrix on a synapse-by-synapse basis. Associativity makes it possible for coincident inputs to influence each other's synaptic strengths and is particularly well suited for associative learning, which we will discuss later in the chapter.

10.6 The NMDA receptor is a coincidence detector for LTP induction

The cooperativity of LTP is consistent with Hebb's rule (see also Section 10.3 and Figure 5–25). Indeed, this property made the CA3 → CA1 synapse the first known example of what is now called a **Hebbian synapse**, that is, a synapse whose strength can be enhanced by co-activating pre- and postsynaptic partners. Recall that we have already studied a molecule capable of implementing Hebb's rule: the **NMDA receptor**. The opening of the NMDA receptor channel requires simultaneous glutamate release from the presynaptic terminal and depolarization of the postsynaptic neuron to remove the blockade by Mg^{2+} (see Figure 3–24). This property accounts for the cooperativity and associativity of LTP. Indeed, ample evidence supports a key role for the NMDA receptor in the establishment of LTP (termed LTP induction) at the CA3 → CA1 synapse.

First, the NMDA receptor is highly expressed in developing and adult hippocampal neurons (**Figure 10–10**A). Second, pharmacological inhibition of the NMDA receptor by a specific NMDA receptor antagonist, **2-amino-5-phosphonovaleric acid** (**AP5**), blocked LTP induction in hippocampal slices without affecting baseline synaptic transmission. Third, when the gene encoding the required GluN1 subunit of the NMDA receptor was selectively knocked out in hippocampal CA1 neurons of mice (Figure 10–10B), LTP at the CA3 → CA1 synapse was abolished (Figure 10–10C), but basal synaptic transmission was unaffected. Because GluN1 was knocked out only in the postsynaptic CA1 neurons and remained functional in the presynaptic CA3 neurons, this experiment also demonstrated a postsynaptic requirement for the NMDA receptor in the induction of LTP at the CA3 → CA1 synapse.

10.7 Recruitment of AMPA receptors to the postsynaptic surface is the predominant mechanism of LTP expression

It is widely accepted that at most CNS synapses, LTP induction occurs through postsynaptic activation of the NMDA receptor. (A notable exception is the mossy fiber → CA3 synapse, where LTP induction is independent of the NMDA receptor and instead involves a largely presynaptic mechanism in which cAMP and protein kinase A act to regulate neurotransmitter release probability.) The means by which NMDA receptor activation leads to long-lasting increases in the synaptic efficacy, called LTP expression, has been the subject of intense debate. Two major

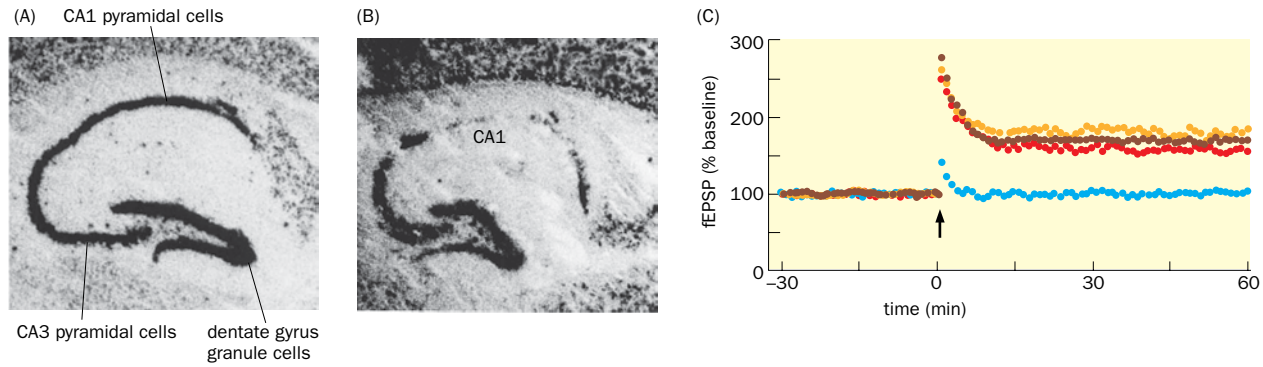

Figure 10–10 The NMDA receptor in the postsynaptic neuron is essential for LTP induction at the CA3 → CA1 synapse. (A) *In situ* hybridization shows that mRNA for the GluN1 subunit of the NMDA receptor is highly expressed in CA3 and CA1 pyramidal neurons as well as dentate gyrus granule cells in the hippocampus. GluN1 is also expressed in the cerebral cortex above CA1. **(B)** Conditional knockout of GluN1 using a transgene that expresses the Cre recombinase specifically in CA1 neurons (see Section 13.7) selectively disrupts GluN1 mRNA expression in the CA1 pyramidal neurons. **(C)** In *CA1-Cre*-mediated GluN1 conditional knockout mice CA3 → CA1 LTP is blocked (blue trace) compared to normal LTP exhibited control mice that are wild type (yellow trace), that have the GluN1 conditional allele but lack the *CA1-Cre* transgene (red trace), or that have *CA1-Cre* transgene alone (brown trace). The upward arrow indicates high-frequency stimulation to induce LTP at $t = 0$. (Adapted from Tsien JZ, Huerta PT & Tonegawa S [1996] *Cell* 87:1327–1338. With permission from Elsevier Inc.)

types of mechanisms have been proposed: a presynaptic mechanism involving an increase in the probability that action potential arrival triggers neurotransmitter release (see Section 3.10), and a postsynaptic mechanism involving an increase in the sensitivity of the postsynaptic cell to the release of the same amount of neurotransmitter. These two mechanisms are not mutually exclusive.

At the CA3 → CA1 synapse, accumulating evidence suggests that the predominant mechanism of LTP expression is an increase in the number of AMPA-type glutamate receptors at the postsynaptic surface. As discussed in Chapter 3 (see Figure 3–24), the AMPA receptor is essential for basal synaptic transmission under conditions in which postsynaptic cells are insufficiently depolarized to activate the NMDA receptor. Following activation of the NMDA receptor during LTP induction, more AMPA receptors are inserted on the postsynaptic membrane. Subsequent glutamate release can thus trigger the opening of more AMPA receptors and hence stronger depolarization.

In fact, some glutamate synapses in the CNS, including a large fraction of the CA3 → CA1 synapses, initially contain only NMDA receptors on the postsynaptic surface. These synapses cannot be activated by presynaptic glutamate release alone and are therefore called **silent synapses**. However, coincident postsynaptic depolarization (presumably through AMPA receptors at other synapses) and presynaptic glutamate release activate the NMDA receptors at silent synapses and thereby cause the insertion of AMPA receptors into the postsynaptic membrane, transforming silent synapses into synapses that can be activated by presynaptic activity alone (**Figure 10–11**A–C). LTP expression involves both the activation of

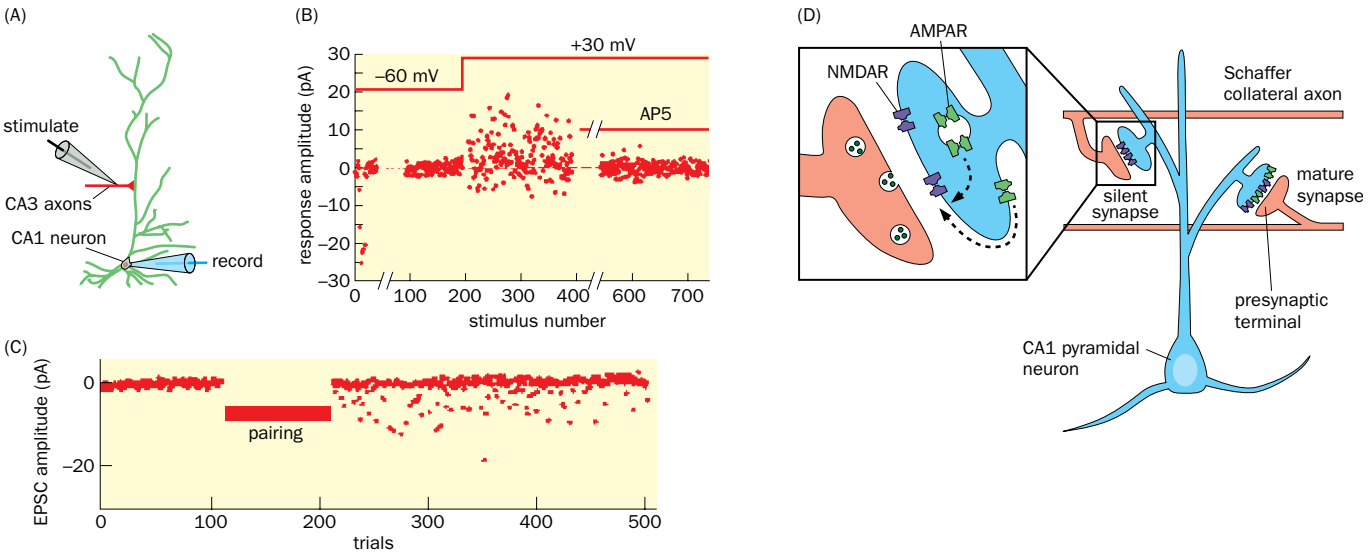

Figure 10–11 Silent synapses and their activation by LTP. **(A)** Schematic of the experiment. In a hippocampal slice, a CA1 neuron's responses to stimulation of a set of CA3 axons were measured by whole-cell patch recording (see Box 13–2). **(B)** Demonstration of silent synapses. At the beginning of this experiment, the CA1 cell was held at −60 mV, and after obtaining small excitatory postsynaptic currents (EPSCs) by stimulating CA3 axons, the stimulation strength was reduced (resulting in stimulating fewer axons) so the stimuli 100–200 did not produce any EPSCs. This means that no AMPA receptor was activated by the weak stimulus. However, when the cell was held at +30 mV, the same weak stimulus now evoked EPSCs that were blocked by AP5, indicating that the stimulated synapses contained NMDA receptors. In other words, the weak stimulus activated synapses that contained NMDA but not AMPA receptors. **(C)** Activating silent synapses. In this experiment, for the first 100 trials, CA1 neurons were held at −65 mV so that only AMPA currents could be induced by CA3 axon stimulation. Prior to pairing, EPSCs were not elicited, indicating that either the stimulated CA3 axons did not connect with the recorded CA1 neurons, or that they were connected via silent synapses. After repeated pairing of CA3 axon stimulation with depolarization of the postsynaptic CA1 neurons, a condition that induces LTP (see Figure 10–9B), a subset of CA3 stimulations elicited EPSCs, indicating that this subset was previously connected via silent synapses, which were activated (unsilenced) by the pairing of presynaptic stimulation and postsynaptic depolarization. Note that EPSCs were outward when the cell was clamped at +30 mV (B) and inward at −65 mV (C). This is because the reversal potentials for AMPA and NMDA receptors are near 0 mV (see Section 3.15). **(D)** Schematic summary. Left, silent synapses have only NMDA receptors (NMDAR) at their postsynaptic surface. LTP causes a net insertion of AMPA receptors (AMPAR) at the postsynaptic surface via exocytosis of AMPA-receptor-containing vesicles, recruitment of AMPA receptors from extra-synaptic areas, or both (dashed arrows). Right, mature synapses contain both AMPA and NMDA receptors. (B, adapted from Isaac JTR, Nicoll RA & Malenka RC [1995] *Neuron* 15:427–434. With permission from Elsevier Inc.; C, adapted from Liao D, Hessler NA & Mallnow R [1995] *Nature* 375:400–404. With permission from Macmillan Publishers Ltd; D, adapted from Kerchner GA & Nicoll RA [2008] *Nat Rev Neurosci* 9:813–825. With permission from Macmillan Publishers Ltd.)

silent synapses (Figure 10–11D) and an increased number of AMPA receptors in synapses that already have AMPA receptors.

In LTP and other forms of synaptic plasticity (discussed in following sections), AMPA receptor trafficking is subjected to many forms of regulation as a consequence of NMDA receptor activation. These include increasing the exocytosis of AMPA-receptor-containing vesicles leading to an increase in the number of cell-surface AMPA receptors, enhancing the binding of AMPA receptors to postsynaptic density scaffolding proteins to increase their residence time at the postsynaptic surface, facilitating lateral diffusion of AMPA receptors toward the synaptic surface, and altering the subunit compositions and phosphorylation status of AMPA receptors to increase their conductance. Exactly how these regulations are triggered by the activation of the NMDA receptor is the subject of intense research; we turn now to one mechanism that involves the activation of a specific protein kinase.

10.8 CaMKII auto-phosphorylation creates a molecular memory that links LTP induction and expression

As we learned in Chapter 3, a key property of the NMDA receptor, distinct from other glutamate receptors, is its high conductance for Ca^{2+} (see Figure 3–24). NMDA receptor activation causes an increase of $[Ca^{2+}]_i$ that activates a number of signaling pathways; for example, Ca^{2+}-activated adenylate cyclases increase the production of cAMP and activation of protein kinase A (see Figure 3–41). Another key signaling molecule is Ca^{2+}/calmodulin-dependent protein kinase II (**CaMKII**), which is activated by Ca^{2+}/calmodulin binding and is highly enriched in the postsynaptic density (see Figures 3–27 and 3–34). The holoenzyme of CaMKII consists of 12 subunits. Each subunit contains a catalytic domain plus an auto-inhibitory domain that binds to the catalytic domain and inhibits its function. Binding of Ca^{2+}/calmodulin to CaMKII transiently displaces the auto-inhibitory domain and thus activates the kinase. When $[Ca^{2+}]_i$ decreases, Ca^{2+}/calmodulin dissociates, deactivating CaMKII if no further modification occurs to CaMKII (**Figure 10–12**A, top).

The combination of the multi-subunit structure and auto-inhibitory domains that can be regulated by phosphorylation endows CaMKII with an interesting property. Active CaMKII can phosphorylate a threonine residue at amino acid 286 (T286) in the auto-inhibitory domain of a neighboring CaMKII subunit; T286 phosphorylation impairs the auto-inhibitory function, so that the activity of the phosphorylated subunits persists even after Ca^{2+}/calmodulin dissociates. Thus, if the initial Ca^{2+} signal is sufficiently strong to cause T286 phosphorylation at

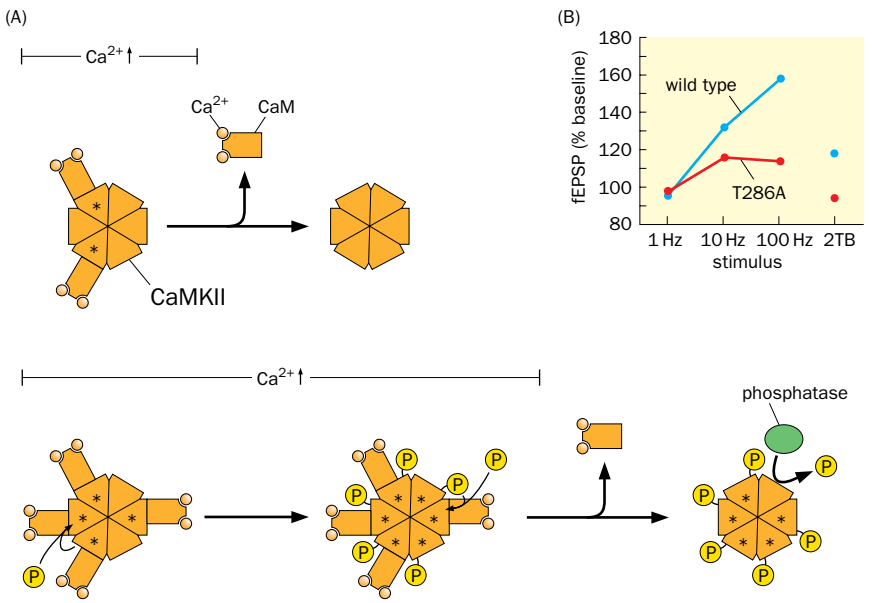

Figure 10–12 Auto-phosphorylation of CaMKII and its requirement in LTP. **(A)** The CaMKII holoenzyme has 12 subunits; only six are shown here for simplicity. Top, binding of Ca^{2+}/calmodulin to a particular subunit transiently activates that subunit (* denotes an active subunit). When Ca^{2+}/calmodulin dissociates after $[Ca^{2+}]_i$ drops, the subunit becomes inactive. Bottom, if a sufficient number of CaMKII subunits become activated in response to a prolonged $[Ca^{2+}]_i$ elevation, specific threonine residues (T286) in multiple subunits are phosphorylated by neighboring subunits in the same complex. This cross-subunit phosphorylation maintains CaMKII in an activated state after $[Ca^{2+}]_i$ drops and Ca^{2+}/CaM complexes dissociate, until phosphatase activity overrides the auto-activation. **(B)** LTP in the CA3 → CA1 synapse can be induced by 10-Hz or 100-Hz high-frequency stimulation, or by two theta bursts (2TB) each consisting of four stimuli at 100 Hz with 200 ms separating the onset of each burst, which mimic endogenous firing of hippocampal neurons. In mutant mice in which T286 of CaMKII was replaced with an alanine residue (T286A), all these forms of LTP were disrupted. (A, adapted from Lisman J, Schulman H & Cline H [2002] *Nat Rev Neurosci* 3:175–190. With permission from Macmillan Publishers Ltd; B, adapted from Giese KP, Federov NB, Filipkowski RK et al. [1998] *Science* 279:870–873. With permission from AAAS.)

multiple subunits, subsequent CaMKII cross-phosphorylation can lead to sustained activity that outlasts Ca^{2+}/calmodulin binding. This process creates a 'memory' in the CaMKII molecule—a historical record of Ca^{2+} signaling—until phosphatases erase the memory through T286 dephosphorylation (Figure 10–12A, bottom). This molecular memory contributes to sustained changes in synaptic efficacy after transient NMDA receptor activation. Supporting this proposal, mice in which auto-phosphorylation of CaMKII at T286 was prevented by mutating the T286 residue to an alanine exhibited profound defects in LTP (Figure 10–12B).

Activation of CaMKII also appears to be sufficient for LTP induction. When a truncated, constitutively active form of CaMKII that lacks the auto-inhibitory domain was injected directly into CA1 pyramidal neurons, CA3 → CA1 synaptic transmission was potentiated. Furthermore, synapses potentiated by constitutively active CaMKII could no longer be induced to exhibit LTP by high-frequency stimulation, while synapses at which LTP had been induced by high-frequency stimulation could no longer be potentiated by constitutively active CaMKII (**Figure 10–13**). Thus, the two mechanisms of synaptic potentiation—high-frequency stimulation and CaMKII activation—occlude each other. These occlusion experiments provide strong evidence that CaMKII activation is an integral component of LTP induction and maintenance.

CaMKII activity contributes to the regulation of synaptic transmission strength through multiple mechanisms. For example, CaMKII-catalyzed phosphorylation of AMPA receptors increases their ion conductance and influences their trafficking (see Section 10.9 below). CaMKII also phosphorylates postsynaptic scaffolding proteins (see Section 3.16), which creates locking sites for AMPA receptors in the postsynaptic membrane. Another key output mediated by CaMKII and other signaling molecules, which is essential for long-lasting changes in synaptic efficacy, involves transcription factor activation and gene expression (see Figure 3–41). One process that these genes likely regulate is the structural alteration of synapses (see Section 10.13).

10.9 Long-term depression weakens synaptic efficacy

So far we have focused on LTP and its mechanisms of induction and expression. However, if synaptic connections could only be made stronger, the entire synaptic weight matrix (see Figure 10–5) would eventually become saturated, and there would be no room to encode new memories. In fact, many additional plasticity mechanisms co-exist with LTP so that the synaptic weight can be adjusted bidirectionally, as is discussed below and in the next section.

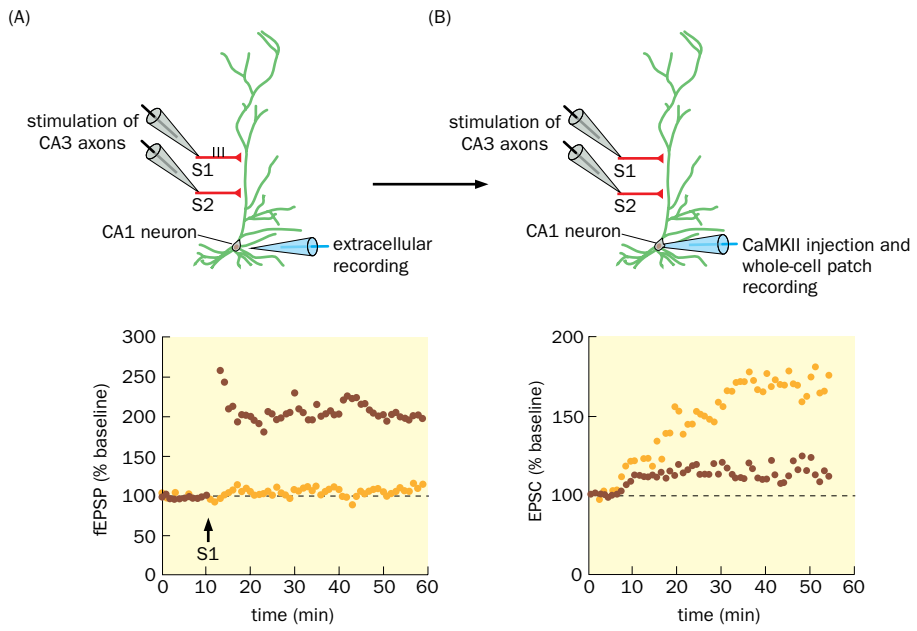

Figure 10–13 LTP induction occludes CaMKII-induced synaptic potentiation. Top, experimental design schematics; bottom, experimental data. The arrow that links the two schematics indicates that experiment B was a continuation of experiment A in the same preparation. **(A)** High-frequency stimulation was applied via the S1-stimulating electrode at the time indicated in the graph by the upward arrow. Only the S1 → CA1 neuron synapses were potentiated (brown trace) whereas the efficacy of the S2 → CA1 synapses remains unchanged (yellow trace), showing input specificity. An extracellular recording electrode was used to measure field excitatory postsynaptic potential (fEPSP) in response to S1 or S2 stimulation. **(B)** Subsequent to potentiation and extracellular recording in (A), a postsynaptic cell was patched for whole-cell recording, and constitutively active CaMKII enzyme was injected into the CA1 neuron through the patch electrode (at $t = 0$). Only the previously unpotentiated S2 synapses were potentiated, as indicated by gradually increased excitatory postsynaptic current (EPSC) in response to stimulation of S2 but not S1. Thus, CaMKII potentiation of the S1 synapses was occluded by prior LTP. (Adapted from Lledo P, Hjelmstad GO, Mukherji S et al. [1995] *Proc Natl Acad Sci USA* 92:11175–11179. With permission from the National Academy of Sciences.)

One counterbalancing mechanism is **long-term depression**, or **LTD**. Just like LTP, LTD has also been found in many CNS synapses (see Section 8.8 for an example of LTD at the parallel fiber → Purkinje cell synapse in the cerebellum). LTD can be induced at hippocampal CA3 → CA1 synapses by low-frequency stimulation of presynaptic axons; note that the same synapses exhibit LTP in response to high-frequency stimulation (**Figure 10–14**A). Like LTP induction, LTD induction is dependent on the NMDA receptor and Ca^{2+} influx. The increase of $[Ca^{2+}]_i$ resulting from low-frequency stimulation is lower than that resulting from high-frequency stimulation. This lower increase of $[Ca^{2+}]_i$ is thought to preferentially activate Ca^{2+}-dependent phosphatases, which do the opposite of what LTP-activated kinases do: the phosphatases reduce the number of AMPA receptors at the postsynaptic plasma membrane so that subsequent glutamate release from the presynaptic terminal induces a smaller depolarization.

LTD and LTP can affect the same synapse sequentially. Low-frequency stimulation can depress a synapse that has previously been potentiated by LTP; high-frequency stimulation can potentiate a synapse that has previously been depressed by LTD. Regulation of the phosphorylation status of the AMPA receptor GluA1 subunit at specific amino acid residues by CaMKII, protein kinase A (PKA), and protein kinase C (PKC) likely plays a role in LTP or LTD expression. One model proposes that in the context of LTP, GluA1 phosphorylation not only increases the channel conductance of AMPA receptors, but also stabilizes AMPA receptors newly added to the postsynaptic membrane, whereas GluA1 dephosphorylation triggers endocytosis of AMPA receptors from the postsynaptic membrane, leading to LTD (Figure 10–14B). Indeed, knock-in mice in which two such phosphorylation sites on GluA1 were replaced with alanines (so that neither could be phosphorylated) had significantly reduced LTP and LTD expression. These and other experiments support the notion that, at a given synapse, LTD and LTP represent a continuum of modifications of synaptic strength. The ability to control synaptic

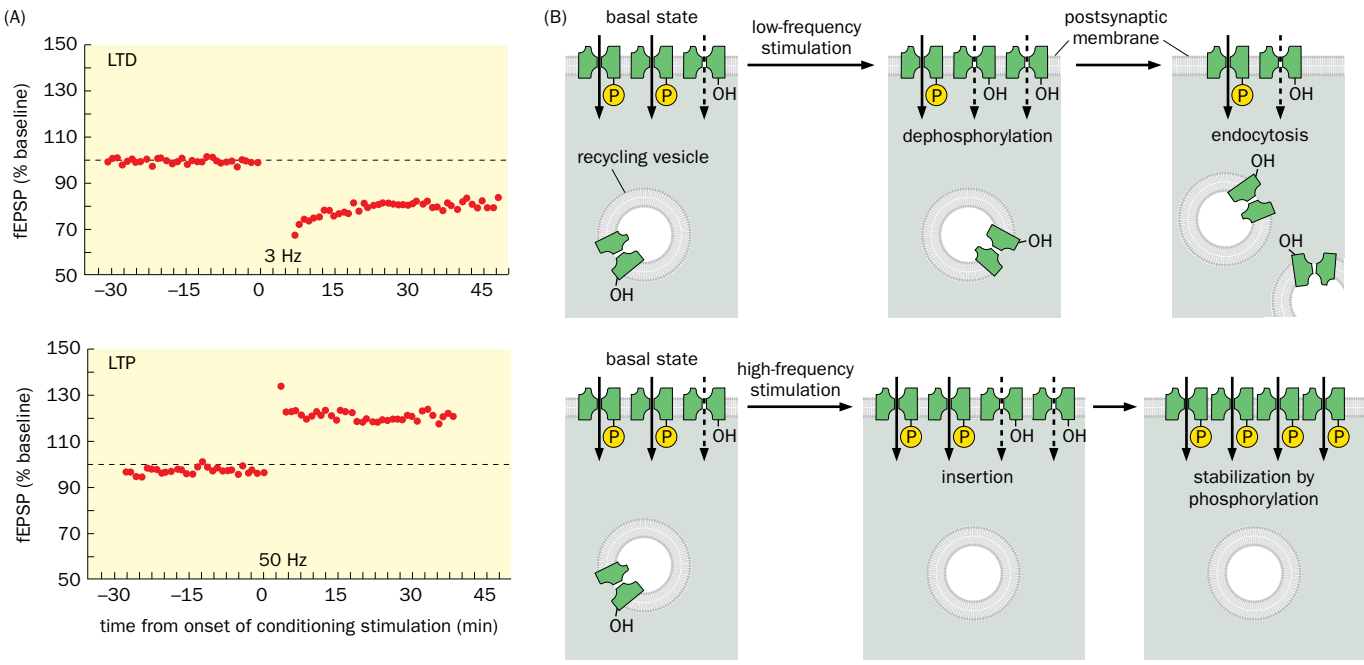

Figure 10–14 Long-term depression at the CA3 → CA1 synapse. **(A)** Whereas high-frequency (50-Hz) stimulation induces LTP (bottom panel), low-frequency (3-Hz) stimulation of CA3 axons innervating a CA1 neuron causes long-term depression (LTD) of the efficacy of synaptic transmission (top panel). **(B)** In this model, AMPA receptors are in a dynamic equilibrium between cell surface and intracellular recycling vesicles. Low-frequency stimulation induces dephosphorylation of GluA1, which promotes endocytosis of AMPA receptors (top panel). High-frequency stimulation causes phosphorylation of GluR1, which stabilizes AMPA receptors at the postsynaptic membrane (bottom panel). In addition, phosphorylated GluA1 has higher AMPA channel conductance (solid arrow for larger ion flow) compared to non-phosphorylated GluA1 (dashed arrow). Together, low-frequency stimulation promotes LTD whereas high-frequency stimulation promotes LTP. (A, adapted from Dudek SM & Bear MF [1992] *Proc Natl Acad Sci USA* 89:4363–4367. With permission from the National Academy of Sciences; B, adapted from Lee HK, Takamiya K, Han JS et al. [2003] *Cell* 112:631–643. With permission from Elsevier Inc.)

weights bidirectionally via LTP and LTD greatly increases the flexibility and storage capacity of synaptic memory matrices.

10.10 Spike-timing-dependent plasticity can adjust synaptic efficacy bidirectionally

Although high- and low-frequency stimulations are commonly used experimentally to induce synaptic plasticity, under physiological conditions, neurons are not usually activated at those precise frequencies. In reality, interconnected neurons can fire action potentials at many frequencies. Another plasticity mechanism that can influence synaptic strength is termed **spike-timing-dependent plasticity** (**STDP**). Originally discovered in the 1990s by researchers using patch clamp methods to study pairs of pyramidal neurons in rat cortical slices and in cultures of dissociated hippocampal neurons, STDP has since been found in many different preparations. In STDP, the precise timing of pre- and post-synaptic firing is critical in determining the sign of the synaptic strength change. For a typical synapse between two excitatory neurons, if the presynaptic neuron fires prior to the postsynaptic neuron within a narrow window (usually tens of milliseconds), and if these pairings are repeated, then subsequent synaptic efficacy increases. If repeated firing of the presynaptic neuron takes place within tens of milliseconds after the firing of the postsynaptic neuron, then the efficacy of subsequent synaptic transmission decreases (**Figure 10–15**). Thus, STDP incorporates features of both LTP and LTD. Indeed, it shares many similarities to LTP and LTD, such as dependence on NMDA receptor activation.

STDP is well suited for implementing Hebb's rule. If the presynaptic cell fires repeatedly before the postsynaptic cell, then it is likely that firing of the presynaptic cell contributes to the stimuli that cause the postsynaptic cell to fire; the synapses between the two cells should be strengthened. If the presynaptic cell fires repeatedly after the postsynaptic cell, then it is unlikely that the presynaptic cell contributes to causing the firing of the postsynaptic cell; synapses between the two cells should be weakened. In addition to serving a role in balancing potentiation and depression of synaptic strength in the synaptic weight matrix, the timing property of STDP can be used for other purposes, including activity-dependent wiring of the nervous system discussed in Chapters 5 and 7.

10.11 Dendritic integration in the postsynaptic neuron also contributes to synaptic plasticity

Not all forms of synaptic plasticity follow Hebb's rule as do LTP and STDP. In fact, synaptic plasticity can occur through dendritic integration without having to cause the firing of the postsynaptic neuron. We use a specific example involving hippocampal CA1 neurons to illustrate.

CA1 neurons receive direct perforant path input from the entorhinal cortex at their distal dendrites and Schaffer collateral input from CA3 neurons at more

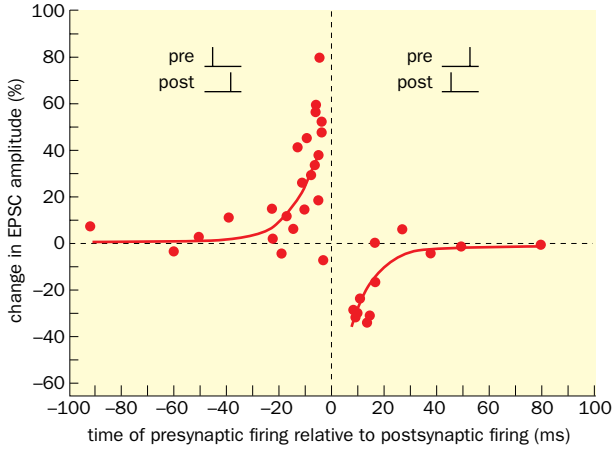

Figure 10–15 Spike timing-dependent plasticity (STDP). If the presynaptic neuron repeatedly fires before the postsynaptic neuron, the synapse is potentiated (left). If the presynaptic neuron repeatedly fires after the postsynaptic neuron, the synapse is depressed (right). Data here were taken from retinotectal synapses in developing *Xenopus in vivo,* where the presynaptic neuron was a retinal ganglion cell and the postsynaptic neuron was a tectal neuron. (Adapted from Zhang IL, Tao HW, Holt CE et al. [1998] *Nature* 395:37–44. With permission from Macmillan Publishers Ltd.)

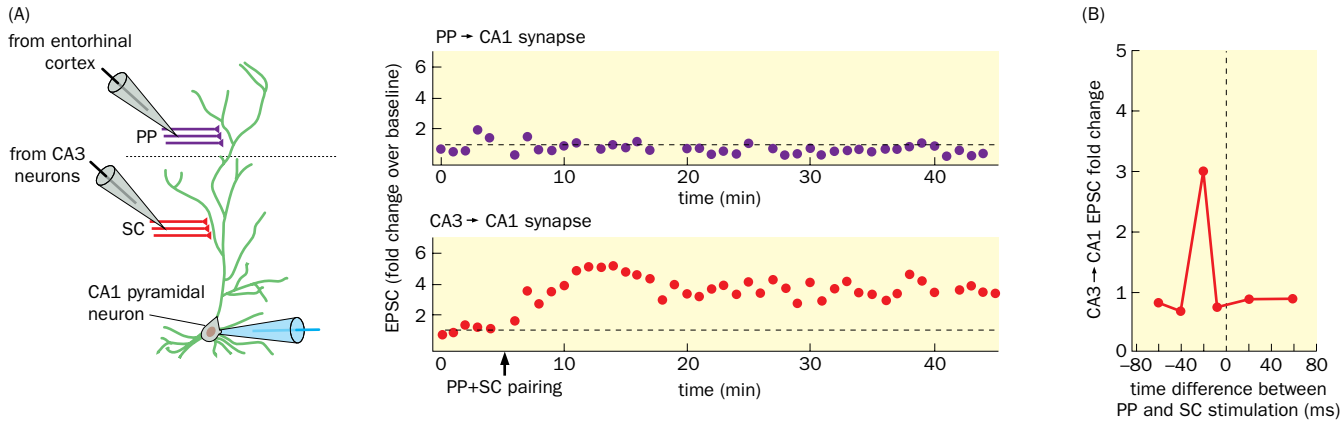

Figure 10–16 Input-timing-dependent plasticity (ITDP) in CA1 neurons. (A) Left, experimental setup. In a hippocampal slice, whole-cell patch clamp recording was performed on a CA1 neuron; stimulating electrodes were placed at the perforant path (PP) and the Schaffer collaterals (SC) that innervate the CA1 neuron's distal and proximal dendrites, respectively, which constitute different layers separated by the dotted line. Right, after paired sub-threshold stimulation of 1 Hz for 90 s, with PP stimulation preceding SC stimulation by 20 ms, average EPSC magnitude of the CA3 → CA1 synapse was enhanced whereas average EPSC magnitude of the PP → CA1 synapse remained unchanged. Thus, synaptic plasticity can be induced in the absence of postsynaptic cell firing. **(B)** Experiments with variable timing intervals: PP stimulation preceding SC stimulation by 20 ms was optimal for potentiating the CA3 → CA1 synapse. (Adapted from Dudman JT, Tsay D & Siegelbaum SA [2007] *Neuron* 56:866–879. With permission from Elsevier Inc.)

proximal dendrites (see Figure 10–6). An interesting means by which the perforant path → CA1 input contributes to CA1 neuronal activity is to influence the CA3 → CA1 synaptic efficacy. In a brain slice preparation in which whole-cell recording was performed on a CA1 pyramidal neuron, repeated pairing of perforant path and Schaffer collateral stimulations, with perforant path stimulation preceding the Schaffer collateral stimulation by ~20 ms, greatly potentiated the efficacy of CA3 → CA1 synapses (**Figure 10–16**A, bottom). The efficacy of the perforant path → CA1 synapses was unaffected (Figure 10–16A, top). Studies using varied time intervals indicated that the 20-ms difference was optimal for potentiating the CA3 → CA1 synapse (Figure 10–16B). This phenomenon has been termed input-timing-dependent plasticity (ITDP).

How do dendritic properties of CA1 neurons contribute to ITDP? Computational modeling suggests that 20 ms is the amount of time needed for perforant path → CA1 EPSCs from distal synapses to travel to the proximal dendrites, so that they can optimally summate with CA3 → CA1 EPSCs (see Section 3.24). This creates a prolonged depolarization at the proximal dendrite that is conducive to NMDA receptor activation and subsequent strengthening of the CA3 → CA1 synapse. What is the biological significance of the 20-ms difference in ITDP? As we saw in Figure 10–6, entorhinal cortical input can reach CA1 neurons through either the monosynaptic perforant path or the trisynaptic dentate gyrus → CA3 → CA1 loop. It takes about 20 ms longer for the entorhinal input to reach CA1 via the Schaffer collaterals than via the perforant path directly. Thus, the 20-ms difference coincides with a window during which individual CA1 neurons can assess the saliency of information processed by the trisynaptic loop by comparing it to direct input from entorhinal cortex. Thus, a combination of the properties of CA1 dendritic integration and the hippocampal circuit enables the perforant path input from the entorhinal cortex to selectively potentiate the efficacy of those CA3 → CA1 synapses that likely transmit the same entorhinal cortical input.

10.12 Postsynaptic cells can produce retrograde messengers to regulate the release of neurotransmitters by their presynaptic partners

Our discussions thus far have largely focused on postsynaptic mechanisms for modifying the efficacy of synaptic transmission, but synaptic plasticity can also engage presynaptic mechanisms. For example, synapses can be facilitated

or depressed as a consequence of an increase or a decrease of the probability of neurotransmitter release in response to a train of action potentials (see Section 3.10). Longer-term changes of synaptic efficacy, such as LTP of the hippocampal mossy fiber → CA3 synapse, can also be induced by a presynaptic mechanism resulting in enhancement of neurotransmitter release probability. In other cases, however, modulation of presynaptic release probability is triggered by an initial change in the postsynaptic neuron. This implies that the postsynaptic neuron must send a retrograde messenger back to its presynaptic partner against the direction of the chemical synapse.

Endocannabinoids (endogenous cannabinoids) are among the best-studied retrograde messengers produced by postsynaptic neurons to regulate presynaptic neurotransmitter release probability. These lipophilic molecules, which include anandamide and 2-arachidonylglycerol, are ligands for a G-protein-coupled receptor, **CB1**, which is abundantly expressed in the brain and which was first identified as the receptor for cannabinoids from the marijuana plant (genus *Cannabis*). Upon depolarization, hippocampal CA1 pyramidal neurons rapidly produce endocannabinoids. In the 1990s, while some researchers discovered endocannabinoids and investigated their properties, others identified an interesting plasticity phenomenon called depolarization-induced suppression of inhibition (DSI) in hippocampal CA1 pyramidal neurons. CA1 pyramidal neurons receive inhibitory input from GABAergic neurons in addition to receiving excitatory input from CA3 neurons and entorhinal cortex. During intracellular recording of CA1 neurons in hippocampal slices, it was found that depolarization elicited by intracellular current injection or high-frequency stimulation of incoming CA3 axons caused a transient suppression of inhibitory input to the CA1 neuron (**Figure 10–17**A).

Further experiments indicated that DSI required Ca^{2+} influx into the postsynaptic CA1 neuron yet did not affect the sensitivity of the CA1 neuron to exogenous GABA application. These data suggest that DSI is most likely mediated by

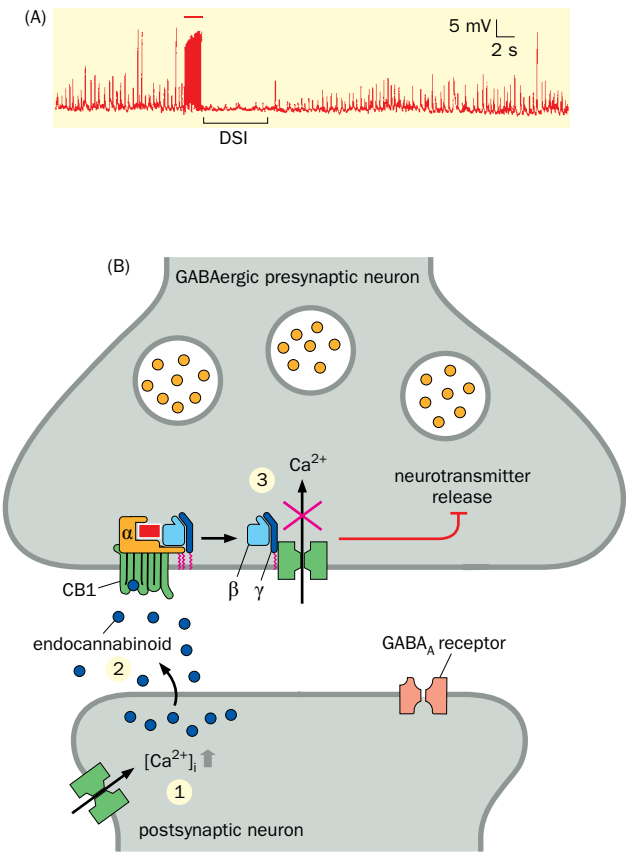

Figure 10–17 Depolarization-induced suppression of inhibition (DSI) and endocannabinoid signaling. (A) Following stimulation by a train of action potentials (indicated by the horizontal red bar), a hippocampal CA1 neuron exhibited DSI, as seen by a transient reduction of the frequency of spontaneous inhibitory postsynaptic potentials (IPSPs). Because the intracellular recording electrode was filled with KCl, diffusion of Cl^- from the electrode into the cell reversed the Cl^- gradient and caused IPSPs to be positive. **(B)** Schematic summary of endocannabinoid signaling in DSI. (1) CA1 neurons produce endocannabinoids in response to a rise of $[Ca^{2+}]_i$ through voltage-gated Ca^{2+} channels or NMDA receptors (not shown) as a consequence of postsynaptic depolarization. (2) Endocannabinoids diffuse across the postsynaptic membrane and synaptic cleft, where they bind to the G-protein-coupled CB1 receptor enriched in the presynaptic terminals of GABAergic neurons. (3) Activation of CB1 releases Gβγ, which binds to and causes closure of presynaptic voltage-gated Ca^{2+} channels, resulting in inhibition of GABA release. (A, adapted from Pitler TA & Alger BE [1992] *J Neurosci* 12:4122–4132. With permission from the Society for Neuroscience; B, adapted from Wilson RI & Nicoll RA [2002] *Science* 296:678–682. With permission from AAAS.)

a reduction of GABA release from its presynaptic partners. Indeed, in the early 2000s, it was found that cannabinoid agonists could induce DSI in the absence of postsynaptic depolarization, whereas cannabinoid antagonists blocked DSI. Moreover, cannabinoid agonists and high-frequency stimulation of CA3 input occluded each other in causing DSI, and DSI was abolished in CB1 receptor knockout mice. These and other lines of evidence led to the model illustrated in Figure 10–17B. Depolarization of postsynaptic cells causes Ca^{2+} influx through voltage-gated Ca^{2+} channels (1), which triggers the synthesis of endocannabinoids from their precursors. These lipid-soluble endocannabinoids diffuse across the postsynaptic membrane and the synaptic cleft (2) to activate the CB1 receptor on the presynaptic membrane. CB1 activation triggers the release of G protein βγ subunits (3), which bind to and cause the closure of voltage-gated Ca^{2+} channels in the presynaptic terminal, thereby inhibiting neurotransmitter release. In principle, DSI should facilitate LTP at excitatory synapses. For example, depolarization of CA1 neurons due to excitatory input from CA3 would induce DSI, which would reduce inhibitory input onto the CA1 neurons, in turn facilitating depolarization and thus LTP induction.

In addition to CA1 pyramidal neurons, cerebellar Purkinje cells also exhibit DSI, as well as an analogous phenomenon called DSE (depolarization-induced suppression of excitation), depending on whether inhibitory or excitatory inputs are examined. Endocannabinoid signaling was also found to be responsible for cerebellar DSI and DSE. Given the wide range of brain tissues in which the CB1 receptor is expressed, it is likely that many synapses use this retrograde system to adjust presynaptic input based on the activity of the postsynaptic neurons. Unlike LTP and LTD, whose expression lasts many minutes to hours and days, DSI and DSE are transient (seconds, see Figure 10–17A) and only regulate short-term synaptic plasticity.

10.13 Long-lasting changes of connection strengths involve formation of new synapses

In addition to changing the probability of presynaptic release of neurotransmitters and the postsynaptic sensitivity to neurotransmitter release, which are two major mechanisms that account for synaptic plasticity we have discussed so far, long-lasting changes of synaptic efficacy can also be accomplished through structural changes to synapses. These include altering the size of existing synapses, forming new synapses, and dismantling old ones. These long-lasting changes typically depend on new gene expression (see **Box 10–1**). Structural changes in response to stimuli have been extensively documented in dendritic spines, where most excitatory synapses in the mammalian CNS are located, because of the relative ease of using fluorescence microscopy to image these structures in slice preparations and *in vivo* (see Section 13.22). For example, LTP induction was found to be accompanied by the growth of existing dendritic spines and the formation of new spines on CA1 pyramidal neurons in cultured hippocampal slices (**Figure 10–18**); this effect depended on the function of the NMDA receptor, suggesting that the structural changes are also mediated by signaling events initiated by Ca^{2+} entry.

LTP-associated structural changes have also been studied by serial electron microscopic reconstructions (see Section 13.19). High-frequency stimulation

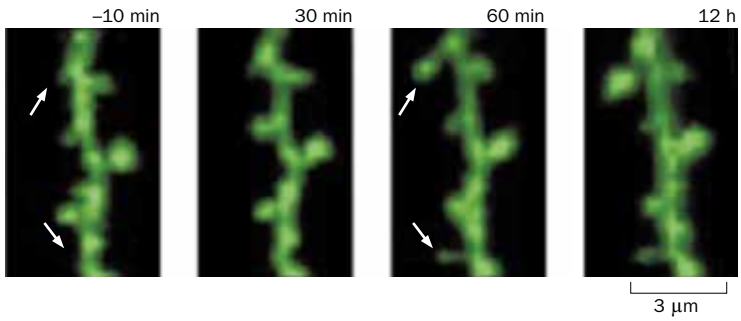

Figure 10–18 Growth of dendritic spines correlates with LTP. LTP is accompanied by the formation of two new spines (arrows) in CA1 pyramidal neurons from a cultured hippocampal slice that was imaged using two-photon microscopy. Time-lapse images were taken at −10, +30, +60 min, and +12 h relative to the onset of LTP induction (not shown). (From Engert F & Bonhoeffer T [1999] *Nature* 399:66–70. With permission from Macmillan Publishers Inc.)

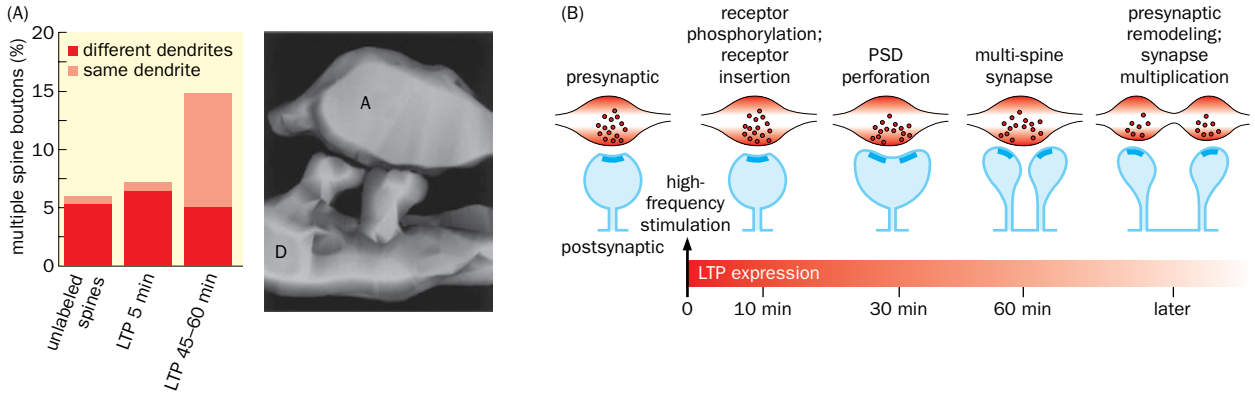

Figure 10–19 LTP correlates with formation of multiple-spine boutons. (A) Left, quantification of the fraction of axon terminals that contact more than one dendritic spine. Dendritic spines activated by LTP were labeled by a staining procedure that produces precipitates in EM micrographs of recently active spines to distinguish them from dendritic spines unrelated to LTP. A selective increase in the fraction of axon terminals that contact two dendritic spines from the same dendrite can be seen 45–60 min after LTP induction. Right, an example of serial EM reconstruction, showing two dendritic spines from the same dendrite, D, contacting the same presynaptic axon terminal, A. **(B)** A model of the temporal sequence of LTP expression. The initial enhancement of synaptic efficacy is caused by the phosphorylation of AMPA receptors and their insertion in the postsynaptic membrane. This is followed by a split of postsynaptic density (PSD), resulting in the formation of a multi-spine synapse. A further hypothetical split of the presynaptic terminal results in the duplication of synapses between the same two neurons. (A, adapted from Toni N, Buchs PA, Nikonenko I et al. [1999] *Nature* 402:421–425. With permission from Macmillan Publishers Ltd; B, adapted from Lüscher C, Nicoll RA, Malenka RC et al. [2000] *Nat Neurosci* 3:545–550. With permission from Macmillan Publishers Ltd.)

that induces LTP was found to cause a selective increase of axons that contact multiple dendritic spines from the same dendrites at a late (60-min) but not early (5-min) phase after the initial stimulation (**Figure 10–19**A). Thus, whereas early stages of LTP involve modulations of AMPA receptors at existing synapses, late-stage LTP can be manifested by structural modifications of synapses, namely the duplication of spines that are contacted by the same axons, possibly followed by a split of presynaptic axon terminals that results in the duplication of synapses (Figure 10–19B). Because these structural changes occur specifically between

Box 10–1: Synaptic tagging: maintaining input specificity in light of new gene expression

As discussed in Section 10.4, high-frequency stimulations (HFSs) can induce long-term potentiation (LTP) in hippocampus *in vivo* that lasts for many hours to days. Repeated HFSs of Schaffer collaterals can also induce LTP at the CA3 → CA1 synapses in hippocampal slices *in vitro* that lasts 8 hours or more. Further studies suggest that LTP in the *in vitro* model can be separated into two phases, an early-phase that decays within 3 hours and is protein synthesis-independent, followed by a late-phase (called **late LTP**) that requires new protein synthesis and new gene expression. This property echoes what we will learn in Section 10.16: short-term memory does not require new protein synthesis whereas long-term memory does.

A question arises as to how LTP maintains its input specificity (see Figure 10–9A) in light of new protein synthesis and new gene expression. For new protein synthesis, one solution could be the use of local protein synthesis from mRNA targeted to dendrites close to the postsynaptic compartments (see Section 2.2); indeed, activity-dependent local protein synthesis has been well documented. However, for new gene expression, activity-induced signals must go to the nucleus to trigger new transcription, and information regarding which synapses initiated the signal is blind to the newly synthesized macromolecules (mRNAs and their protein products). To overcome this difficulty, a **synaptic tagging** hypothesis was proposed, which states that in parallel with enhancing synaptic efficacy, repetitive HFSs also produce a local synaptic tag that can selectively capture newly synthesized macromolecules distributed cell-wide, thereby conferring input specificity. The following experiments (**Figure 10–20**) provided strong support for the synaptic tagging hypothesis.

Two stimulating electrodes were placed at different depths of the CA1 dendritic field in a hippocampal slice preparation, ensuring that they would stimulate different populations of CA3 → CA1 synapses (S1 and S2) onto the same group of CA1 neurons, whose activity was monitored by a recording electrode. In the first experiment (Figure 10–20A) only S1 received HFSs, and only S1 synapses were potentiated, confirming input specificity. In the second experiment (Figure 10–20B), 35 min after HFSs at S1, protein synthesis inhibitors were applied to the slice (prior experiments had shown that this time lag would not inhibit late LTP formation at S1). 25 min later, HFSs were applied at S2 in the

Box 10–1: Synaptic tagging: maintaining input specificity in light of new gene expression

presence of protein synthesis inhibitors, which would normally block late LTP. However, S2 exhibited normal late LTP under this circumstance, thanks to the prior HFSs at S1. The simplest explanation is that HFSs at S2 produced a synaptic tag even in the presence of protein synthesis inhibitor, and the tag captured newly synthesized macromolecules due to HFSs at S1. In the third experiment, researchers tested how long the synaptic tag could last by first applying HFSs at S1 in the presence of a protein synthesis inhibitor, thus preventing it from inducing new gene expression but not inhibiting its ability to produce a synaptic tag. Then the protein synthesis inhibitor was washed away, and HFSs were applied to S2. If the two HFSs were separated by 3 hours, then S1 no longer exhibited late LTP (Figure 10–20C), suggesting that the synaptic tag is transient and lasts no more than 3 hours.

Although the molecular nature of the synaptic tag and the newly synthesized macromolecules they interact with are still incompletely understood (they may involve multiple molecular pathways in parallel), the concept of synaptic tag has been widely accepted. Similar phenomena have also been observed in the *Aplysia* model for learning and memory that we will discuss in later sections. While the Hebbian mechanisms of synaptic plasticity relies on the precise timing between activation of pre- and postsynaptic neurons (within tens of milliseconds of each other), the synaptic tagging model suggests that plasticity at one synapse may affect the plasticity of other synapses in the same neuron over a wider temporal window (for an hour or two). This may provide a cellular mechanism to explain why inconsequential events are remembered longer if they occur within a short window of well-remembered events.

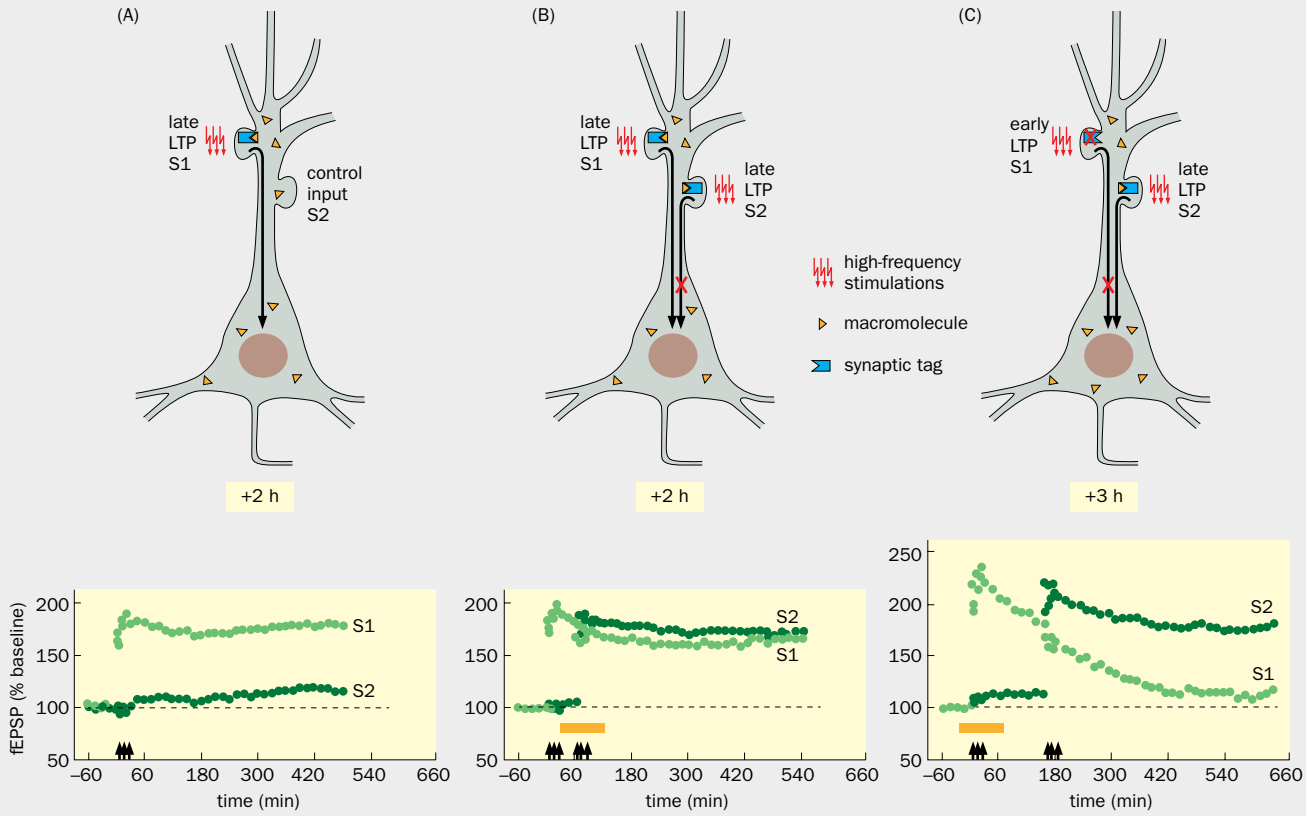

Figure 10–20 Experimental evidence for synaptic tagging hypothesis. The top schematics illustrate the experimental conditions and summarize the results at 2 or 3 hours after the first high-frequency stimulation (HFS) according to the synaptic tagging hypothesis. The bottom panels show the field EPSP changes over time. **(A)** HFSs were only applied to S1, and only S1 exhibited late LTP. This is because HFSs at S1, while inducing new gene expression (downward arrow to the nucleus), also produced a synaptic tag locally, which captured newly synthesized macromolecules necessary for late LTP. **(B)** 35 minutes after HFSs were applied to S1, protein synthesis inhibitors were added to the slice (duration represented by the horizontal bar in the bottom panel), during which time HFSs were applied to S2. Both S2 and S1 exhibited late LTP. This is because HFSs at S2, while incapable of inducing new gene expression (indicated by the cross on the downward arrow), nevertheless produced a synaptic tag, which captured newly synthesized macromolecules due to HFSs at S1. **(C)** HFSs were applied at S1 in the presence of protein synthesis inhibitors. Then HFSs were applied at S2 after protein synthesis inhibitors were washed away. When the two HFSs were 3 hours apart, late LTP at S1 was disrupted, presumably because the synaptic tag at S1 decayed (as indicated by the cross on the synaptic tag) by the time newly synthesized macromolecules due to HFSs at S2 arrived. (Adapted from Frey U & Morris RGM [1997] *Nature* 385:533–536. With permission from Macmillan Publishers Ltd.)

pre- and postsynaptic partners that have undergone LTP, this mechanism enhances the dynamic range of synaptic connections between a pair of neurons while at the same time maintaining the input specificity. This mechanism may be particularly important during development, when synapse formation and dendritic growth are influenced by experience, conveyed to the animal through patterned activity in sensory pathways (for example, see Box 5–3).

In summary, a wealth of mechanisms for synaptic plasticity, including changes in presynaptic neurotransmitter release probability and postsynaptic sensitivity to neurotransmitter release, as well as the structure and number of synapses, can be used to adjust the connection strengths between two neurons. These mechanisms allow experience and activity to adjust connection strengths both during development and in adulthood. Although we have focused largely on examples of mammalian hippocampal neurons and synapses, similar mechanisms likely occur throughout the nervous systems of both vertebrates and invertebrates. We next explore whether and how these plasticity mechanisms are linked to learning and memory.

WHAT IS THE RELATIONSHIP BETWEEN LEARNING AND SYNAPTIC PLASTICITY?

In this part of the chapter, we take a top-down approach to learning and memory, starting with animal behavior and seeking to link that behavior to the function of circuits, neurons, synapses, and molecules (see Figure 10–7). We first introduce different forms of learning and then study their underlying mechanisms in select model organisms. We end with a discussion of spatial learning and memory in mammals, noting how these processes relate to the hippocampal synaptic plasticity discussed in previous sections.

10.14 Animals exhibit many forms of learning

All animals must deal with changes in the environment. Those that adapt well have a greater chance of surviving and producing progeny. Consequently, many types of learning have evolved, each with specific properties. Psychologists and behavioral biologists have used these properties to categorize learning into different forms.

The simplest form of learning is **habituation**, which refers to a decrease in the magnitude of response to stimuli that are presented repeatedly. For instance, we may be startled when we hear a noise for the first time, but we respond less strongly to subsequent instances of the same noise—we 'get used' to it. Simple as it is, habituation reflects the ability of the nervous system to change its response to environmental stimuli. Another simple form of learning is **sensitization**, which refers to an increase of response magnitude to a stimulus after a different kind of stimulus, often noxious, has been applied. Sensitization is more complex than habituation, as the response reflects an interaction of two different kinds of stimuli. We will give specific examples of habituation and sensitization and study their mechanisms in the following two sections.

A more advanced form of learning is **classical conditioning** (also called Pavlovian conditioning), which refers to the ability of animals to produce a novel response to a previously neutral stimulus (the **conditioned stimulus**, or **CS**), after the CS has been repeatedly paired with a stimulus that always induces the response (the **unconditioned stimulus**, or **US**). A famous example is the experiment on salivation of dogs conducted by Ivan Pavlov, who discovered classical conditioning in the early twentieth century (**Figure 10–21**). Dogs always salivate in response to food in the mouth; this innate salivation constitutes the **unconditioned response**. After repeated pairing of food with a sound, which did not produce salivation before pairing, the sound alone induced salivation. In this example, food is the US, sound is the CS, and the process of pairing food and sound is called conditioning; the eventual salivation response to sound alone is called the **conditioned response**.

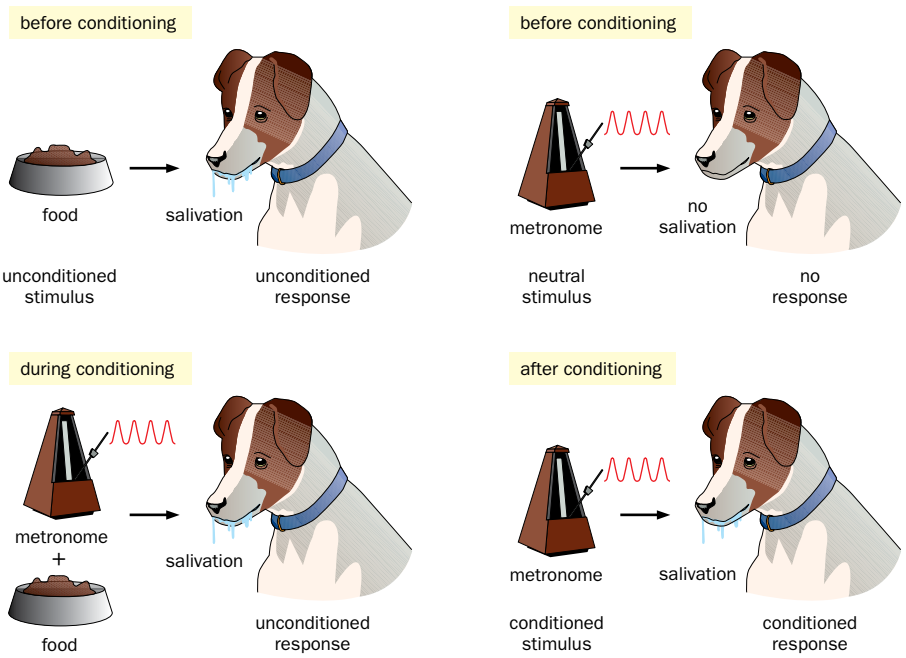

Figure 10–21 Pavlov's experiment that established the concept of classical conditioning. Before conditioning, the dog salivates in response to food in the mouth (top left), but does not salivate when hearing a sound from a metronome (top right). During conditioning, which consists of repeated pairing of the sound and food (bottom left), the dog learns to associate the sound with food, such that after conditioning the dog salivates in response to the sound alone (bottom right). (See Pavlov IP [1926] Conditioned Reflexes. Dover Publications Inc.)

Whereas sensitization merely changes the magnitude of the response to a stimulus due to the presentation of a second kind of stimulus, classical conditioning establishes a novel and qualitatively different stimulus–response (for example, sound–salivation) relationship. Classical conditioning requires that an association form between the CS and US. In order for conditioning to be effective, the proper timing of the CS and the US is critical; the CS usually precedes the US. Therefore classical conditioning is a form of **associative learning**. It is observed across the animal kingdom, including humans.

Another major form of associative learning, distinct from classical conditioning, is **operant conditioning** (also called **instrumental conditioning**). In operant conditioning, a reinforcer is given only when the animal performs an appropriate behavior. For instance, a hungry rat in a cage can be trained to press a lever to obtain a food pellet. Initially the rat may not know the association between the lever pressing and the food pellet; after the reinforcer (food pellet) is given each time the rat presses the lever, the rat gradually associates the lever pressing (its own action) with the food reward (**Figure 10–22**). After operant conditioning, the rat selects one action over many other possible actions in order to receive the food pellet. A 'law of effect' was proposed in the early twentieth century to explain the association process: responses (behavior) that are followed by a reward will be repeated, whereas responses that are followed by a punishment will diminish.

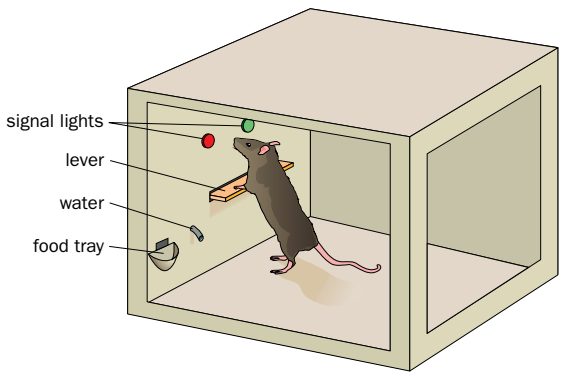

Figure 10–22 Basic design of an operant chamber. A hungry or thirsty rat placed in this chamber can learn through trial and error that pressing the lever results in the dispensing of either food or water (according to the particular experimental design); this reward reinforces the lever-pressing response. (See Skinner BF [1938] The Behavior of Organisms. B.F. Skinner Foundation.)

Timing is crucial in operant conditioning—as in classical conditioning—and the effect is greatest when the reinforcer is presented shortly after the behavior. Another property shared by classical and operant conditioning is **extinction**: in classical conditioning, when the CS is repeatedly *not* followed by the US, the conditioned response will diminish; in operant conditioning, when the behavior is repeatedly *not* followed by the reinforcer, the behavior will diminish. Operant conditioning is a prevalent learning mechanism in the animal kingdom and is widely used in the laboratory for training animals to perform tasks. Indeed, operant conditioning was used in many of the experiments discussed in this book, from motion perception to arm reaching (see Figures 4–52 and 8–27).

In our discussion so far, learning is viewed as the modification of behavior in response to experience, and the outcome of learning is measured by changes in behavior. There is a complementary view of learning. Psychologists use the term **cognitive learning** to refer to learning as an acquisition of new knowledge rather than simply modification of behavior. From this cognitive perspective, for instance, classical conditioning can be viewed as the animals having acquired the knowledge that the CS is followed by the US; the conditioned response is in fact a response to the predicted upcoming US rather than to the CS per se. While cognitive capabilities are usually thought to be specific to mammals with large cerebral cortices such as primates and particularly humans, the following example illustrates that even insects can master abstract concepts that qualify as cognitive learning.

Honeybees were trained to perform a task called delayed matching-to-sample, which is thought to utilize working memory (see Section 10.1). They first encountered a specific cue, such as a blue sign, after entering a Y-maze. After flying within the maze for a certain distance, they encountered the choice point, where the entrance into each arm of the Y-maze was marked by a blue sign or a yellow sign. If they chose to enter the arm marked by the same color as the color they encountered at the entrance of the maze, they would get a food reward (**Figure 10–23**A). After repeated training, bees not only can perform this task with a success rate well above chance (Figure 10–23B) but also can apply this skill to a completely new set of cues. For example, when the maze was outfitted with grid patterns that bees had not encountered previously, they could perform a pattern-matching task nearly as well as the original color-matching task (Figure 10–23C). Moreover, bees can apply the learned skill across different sensory modalities; for instance, training with a pair of odors improves the test results for matching a pair of colors. Lastly, bees can be trained to obtain a reward by entering the maze arm marked by a cue that differs from the one at the entrance—a task called delayed non-matching-to-sample—and can transfer the non-matching skill from colors to patterns. Thus, honeybees appear to be able to learn the abstract concepts of 'sameness' and 'difference' and use them to guide their behavior.

What are the neurobiological bases for these different forms of learning? Do they share common mechanisms? How are they related to the synaptic weight

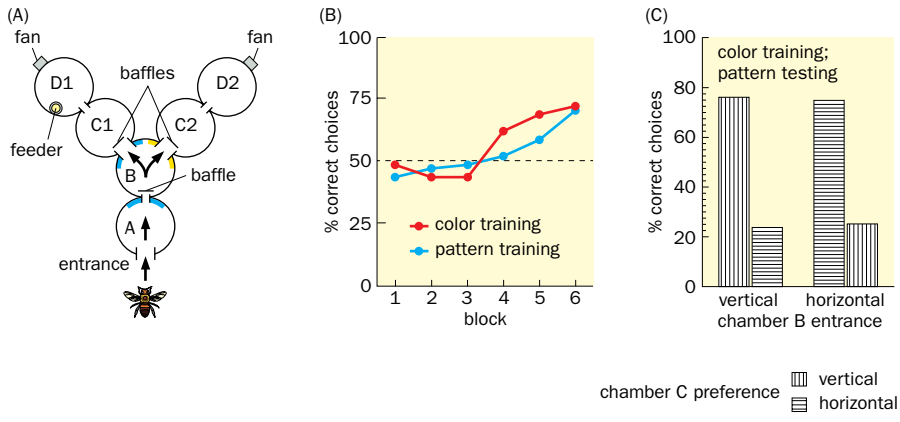

Figure 10–23 Cognitive learning in honeybees. (A) Experimental setup. At the entrance to chamber B, bees first encounter a stimulus (for example, a blue sign). They then face a choice of two different C chambers, with the entrances marked with two different stimuli (for example, a blue sign and a yellow sign). With the sugar solution in one of the D chambers as reward, bees can be trained after repeated trials to choose either the C chamber marked with a stimulus that matches the entrance to the B chamber (delayed matching-to-sample, as shown here), or the C chamber marked with a stimulus that differs from the B chamber (delayed non-matching-to-sample, which would apply if the feeder were placed at D2). **(B)** Learning curves for bees that performed color- or pattern-matching tasks. Each block consisted of 10 consecutive training sessions. After six blocks, the percentage of correct choices for either task exceeded 70%, significantly above random chance (50%, dashed line). **(C)** After being trained for delayed-matching-to-sample in color, bees were tested for the pattern-matching task. Whether the entrance to B was marked with a vertical (left) or horizontal (right) grid pattern, bees preferentially chose the C chamber whose entrance was marked with the same pattern as the pattern at entrance to B. (Adapted from Giurfa M, Zhang S, Jenett A et al. [2001] *Nature* 410:930–933. With permission from Macmillan Publishers Ltd.)

matrix hypothesis we introduced early in the chapter? We will now explore these questions, starting with simple forms of learning observed in a sea slug, *Aplysia*.

10.15 Habituation and sensitization in *Aplysia* are mediated by changes of synaptic strength

Aplysia has been used as a model for studying the cellular and molecular basis of learning and memory since the 1960s. *Aplysia* has only 20,000 neurons compared to about 10^8 neurons in the mouse. Many *Aplysia* neurons are large and individually identifiable such that electrophysiological recordings can easily be performed on multiple neurons in the same animal and with reproducible results across animals (as in the case of the crustacean stomatogastric ganglion discussed in Section 8.5). Importantly, *Aplysia* exhibits simple forms of learning and long-lasting memory that are similar to those found in more complex organisms.

The **gill-withdrawal reflex** has been used as a model behavior (**Figure 10–24**A). When a tactile stimulus is applied to the siphon, *Aplysia* reflexively withdraw their gill (and siphon) into the mantle shelf as a protective measure. This behavior shows habituation, as repeated siphon stimuli resulted in progressively smaller magnitudes of gill withdrawal (Figure 10–24B, left). However, if the habituated animal receives a noxious electric shock at the tail, the magnitude of gill withdrawal in response to the siphon stimulus applied shortly after the shock is drastically enhanced, indicating a sensitization of the gill-withdrawal reflex by the tail shock (Figure 10–24B, right).

The neural circuits underlying the gill-withdrawal reflex have been mapped (Figure 10–24C), thanks to the ease of electrophysiological recordings and manipulations. Siphon stimulation activates 24 sensory neurons; activating these neurons artificially was found to mimic siphon stimulation and induce the gill-withdrawal reflex. Six motor neurons control the muscle contraction that causes gill withdrawal. Activities of these motor neurons correlate with gill withdrawal, and direct electrical stimulation of these motor neurons is sufficient to cause gill withdrawal. These sensory and motor neurons form monosynaptic connections analogous to the sensorimotor circuit controlling our knee-jerk reflex (see Figure 1–19). A different group of sensory neurons transmits the tail-shock signal to a set of serotonin neurons, which in turn innervate the cell bodies of the

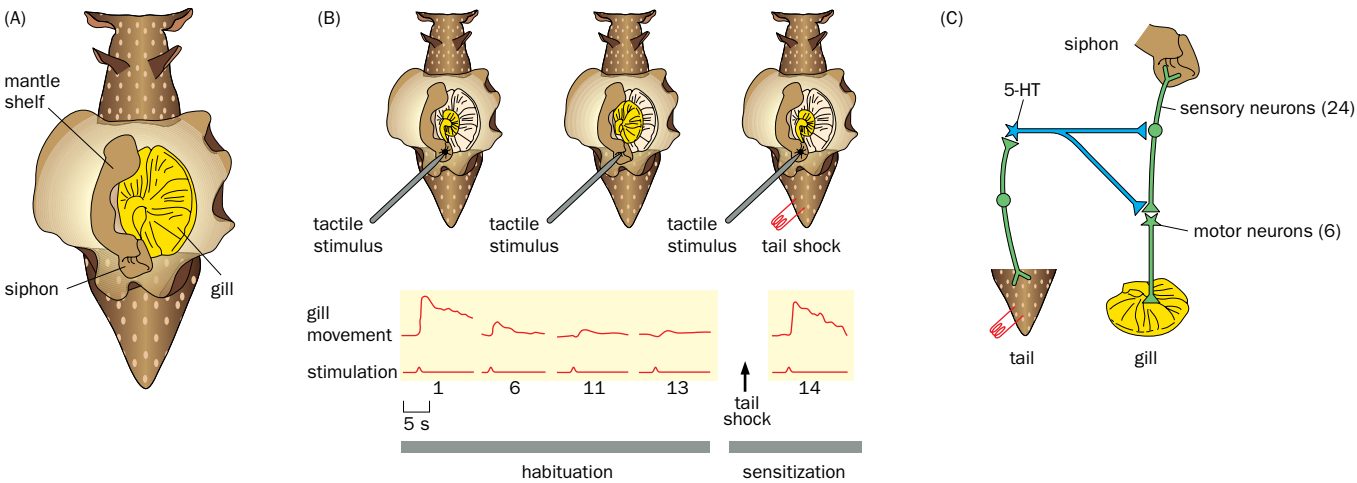

Figure 10–24 The gill-withdrawal reflex in *Aplysia* and the underlying neural circuits. (A) Schematic drawing of *Aplysia* highlighting the structures related to the gill-withdrawal reflex. **(B)** Top, schematic drawing of the gill-withdrawal reflex and its habituation (middle) and sensitization (right). Bottom, recording of the gill movement (top traces) shows progressive decrement in response to repetitive siphon stimulation (bottom traces). Numbers indicate repetitions. Shortly before the 14th stimulus, a tail shock was applied, which caused an increase of response to stimulus 14. **(C)** Circuit diagram of the gill-withdrawal reflex. The 24 sensory neurons that innervate the siphon connect directly with the six motor neurons that innervate the gill muscle. Sensory neurons activated by tail shock connect with serotonin (5-HT) neurons, which in turn innervate the siphon sensory neurons and their presynaptic terminals onto the gill motor neurons. (Adapted from Kandel ER [2001] *Science* 294:1030–1038. With permission from AAAS.)

24 sensory neurons that sense siphon stimulus and their presynaptic terminals on the motor neurons (Figure 10–24C). These connections would allow the tail shocks to modulate the activity of sensory neurons or neurotransmitter release from the sensory neurons to their motor neuron targets (see Figure 3–37).

Having mapped the neurons underlying the reflex circuit, researchers then asked the question: What is the nature of the circuit change responsible for behavioral habituation, that is, the reduction in the magnitude of gill withdrawal after repeated siphon stimulations? In principle, this could be caused by any of the following changes: (1) sensory neurons progressively reduce their response magnitude after repetitive stimuli, akin to sensory adaptation (see Section 4.7); (2) the efficacy of synaptic transmission between sensory and motor neurons is depressed; (3) the efficacy of synaptic transmission at the neuromuscular junctions is depressed; (4) the muscles become fatigued. A series of experiments using physiological recordings in conjunction with sensory stimulation and quantitative measure of behavioral responses were carried out to systematically examine these possibilities (**Figure 10–25**).

In Experiment 1, the gill-withdrawal responses to direct motor neuron stimulation were measured before and after behavioral habituation and were found to be the same. This ruled out the possibility that changes downstream of the motor neurons in the circuit, including a depression in synaptic efficacy at the neuromuscular junction or muscle fatigue, were responsible for habituation. To test for sensory adaptation at the peripheral sensory endings, a set of sensory stimuli were applied while motor neuron responses were recorded (Experiment 2). Responses became smaller as more stimuli were applied, correlating with behavioral habituation. As illustrated in Figure 10–25, during stimuli 10 through 18, a segment of the siphon nerve that connects the sensory nerve endings to the sensory neurons was bathed in a sodium-free solution to block action potential propagation. After the nerve block was relieved, the motor neuron response became larger, instead of becoming smaller as would be predicted if sensory adaptation at the periphery were responsible for habituation. This ruled out the possibility that habituation was due to an effect upstream of the sensory nerve. Collectively, these experiments suggested that changes at the sensorimotor synapses underlie behavioral habituation. Indeed, in studies carried out in an isolated ganglion, which facilitated stimulation and recording compared with intact *Aplysia*, motor neuron responses elicited by direct sensory neuron stimulation were found to undergo progressive depression after repeated trials

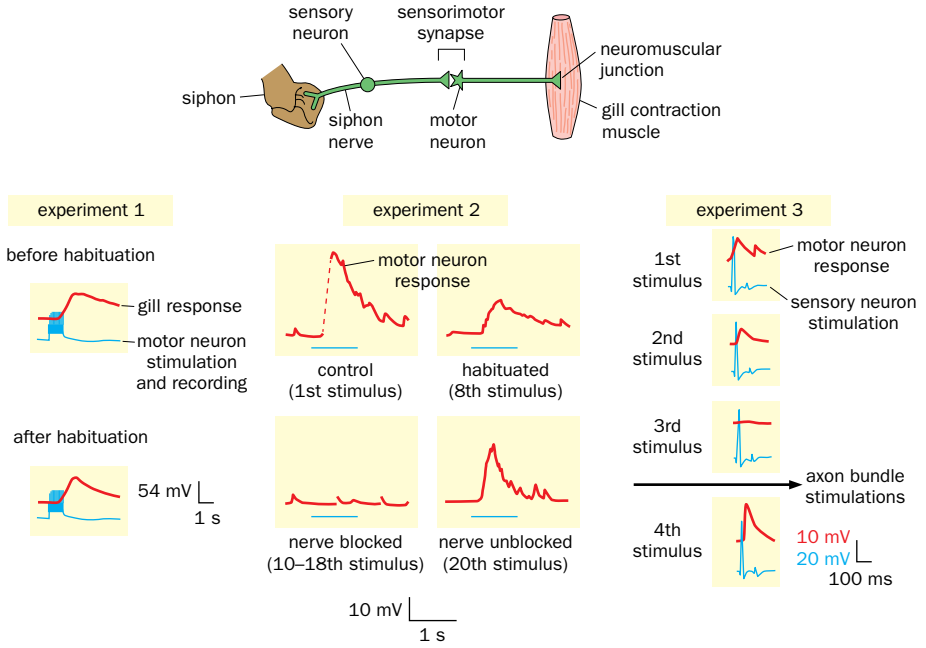

Figure 10–25 Neural mechanisms of habituation and sensitization of the *Aplysia* gill-withdrawal reflex. Top, diagram of information flow from siphon stimulation to gill withdrawal. Bottom, three experiments that investigate the neural mechanisms of behavioral habituation. **Experiment 1:** Gill responses (red traces) to direct motor neuron stimulation (spikes of motor neurons shown as blue traces) before and after habituation remained unchanged, arguing against the possibility that habituation affects processes downstream of the motor neuron. **Experiment 2:** Intracellular recording of a motor neuron (red traces) in response to a series of 20 siphon stimuli (blue line represents the duration of one stimulus). The first nine stimuli were applied under the normal condition, and the resulting motor neuron response was depressed (compare the top right with top left traces), correlating with habituated behavioral responses. During stimuli 10 through 18, action potentials from the siphon nerve were blocked so that the motor neuron did not respond (bottom left). After the siphon nerve was unblocked, the 20th stimulus gave a larger response than the 8th stimulus (compare the top right and bottom right traces), thus arguing against the possibility that habituation affects processes upstream of the siphon nerve. **Experiment 3:** In a reduced preparation consisting of an isolated ganglion that contains the sensory and motor neurons, motor neuron responses (red traces) were induced by intracellular stimulation of the sensory neuron that produced a single spike (blue traces). The top three pairs show three consecutive sensory neuron stimulations (mimicking behavioral habituation), which caused progressively reduced responses. In the bottom pair, the motor neuron response was facilitated due to stimulation of the axon bundle that includes axons of the serotonin neurons (mimicking behavioral sensitization) before the pairing. (Adapted from Kupfermann I, Castellucci V, Pinsker H et al. [1970] *Science* 167:1743–1745 and Castellucci V, Pinsker H, Kupfermann I et al. [1970] *Science* 167:1745–1748. With permission from AAAS.)

(Experiment 3), suggesting that depression of sensorimotor synaptic efficacy is the primary cause of behavioral habituation.

Analogous experiments were conducted to test the location of change during sensitization by tail shock. Remarkably, the same sensorimotor synapses that were depressed during habituation were potentiated during sensitization (Figure 10–25, Experiment 3). Together, these findings suggest that behavioral modifications, as measured by the magnitude of the gill-withdrawal reflex, are caused primarily by changes in the efficacy of synaptic transmission between sensory neurons and motor neurons—that is, habituation is caused by a depression and sensitization caused by a facilitation of the synaptic efficacy. These results provide compelling support for the hypothesis proposed in Section 10.3, namely that changes of synaptic strengths underlie learning.

10.16 Both short-term and long-term memory in *Aplysia* engage cAMP signaling

Studies of the *Aplysia* gill-withdrawal reflex have also provided important insights into the mechanisms of short-term and long-term memory. Behavioral studies in humans suggest that repeated training can strengthen memories, causing them to become long lasting (as noted in the epigraph of this chapter). Sensitization of the *Aplysia* gill-withdrawal reflex also exhibits these properties. Whereas one tail shock caused a transient increase in gill-withdrawal magnitude that returned to baseline within one hour, four shocks produced a memory (evidenced by a withdrawal response above baseline) that lasted at least a day. The memory produced by four trains of four shocks within a day was retained even after four days. Four trains of four shocks every day for four days produced a drastic increase in response magnitude that persisted for more than a week (**Figure 10–26**A).

In order to facilitate mechanistic studies, researchers established an *in vitro* co-culture system consisting of a siphon sensory neuron and a gill motor neuron (named L7, which can be identified in each animal based on its stereotyped size, shape, and location) that form synaptic connections in a dish. In this system, repeated stimulation of the sensory neuron caused progressive decreases of the magnitude of the postsynaptic potential (PSP) recorded from the motor neuron, mimicking behavioral habituation and consistent with the findings from studies in intact ganglion (for example, Figure 10–25, Experiment 3). Sensitization could also be recapitulated in the co-culture system by applying serotonin to the culture (Figure 10–26B). Whereas one pulse of serotonin application produced a short-term PSP facilitation that lasted for minutes, five repetitions of serotonin application separated by 15-minute intervals produced a long-term facilitation of the PSP that lasted for 24 hours (Figure 10–26C), comparable to the outcome of repeated tail shock (Figure 10–26A). These short-term and long-term facilitations of synaptic efficacy have been used as cellular models of short-term and long-term memory.

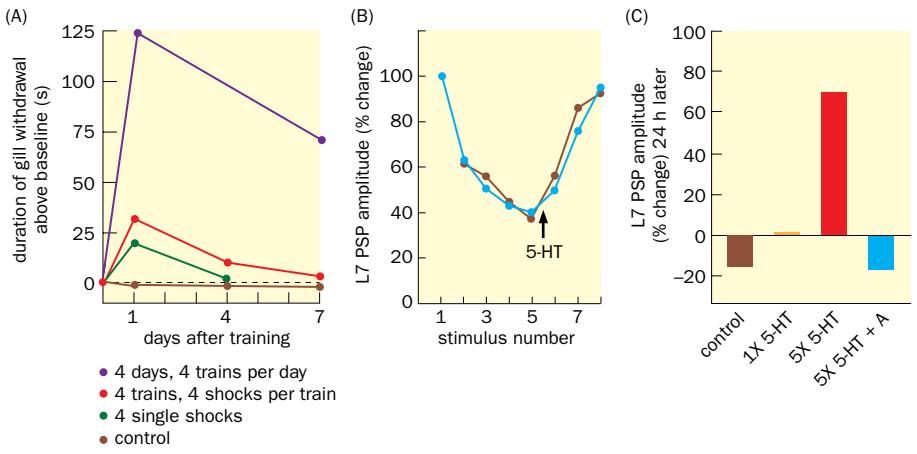

Figure 10–26 Long-term sensitization can be induced by repeated training or serotonin (5-HT) application, and is dependent on protein synthesis. **(A)** Duration of gill withdrawal above the baseline in response to three different tail-shock protocols as indicated. Increased training produced sensitization of the gill-withdrawal reflex that was longer lasting. **(B)** Behavioral habituation and sensitization can be recapitulated as changes of synaptic strength between a sensory and a motor neuron co-cultured *in vitro*. Here the relative magnitude of postsynaptic potential (PSP) of the L7 motor neuron in response to sensory neuron stimulation is plotted against the stimulus number. A progressive decline of the magnitude accompanied the application of successive stimuli. Application of 5-HT, which mimics tail shock, increased the PSP magnitude. Application of the protein synthesis inhibitor anisomycin (blue trace) had no effect on this short-term depression and facilitation compared to the control (brown trace). **(C)** A single 5-HT application (1 × 5-HT) did not produce long-term facilitation measured 24 hours later, whereas a sequence of five 5-HT applications did (5 × 5-HT). Application of the protein synthesis inhibitor anisomycin during the time of 5-HT application (5 × 5-HT + A) blocked the long-term facilitation. (A, adapted from Frost WN, Castelucci VF, Hawkins RD et al. [1985] *Proc Natl Acad Sci USA* 82:8266–8269. With permission from the National Academy of Sciences; B & C, adapted from Montarolo PG, Goelet P, Castellucci VF et al. [1986] *Science* 234:1249–1254. With permission from AAAS.)

Are there mechanistic differences between short-term and long-term memory? Studies in different animals from mice to goldfish indicated that long-term (but not short-term) memory formation is inhibited by applying drugs that inhibit protein synthesis at the time of training, suggesting that long-term-memory formation selectively requires new protein synthesis. Likewise, in the co-culture system in *Aplysia*, applying protein synthesis inhibitors at the time of serotonin application blocked long-term (Figure 10–26C) but not short-term (Figure 10–26B) facilitation of synaptic transmission caused by serotonin application. Furthermore, applying a protein synthesis inhibitor before or after serotonin application did not affect long-term facilitation. These studies support the notion that protein synthesis is required during the acquisition step of long-term memory.

We now have a good understanding of the molecular mechanisms that mediate short- and long-term facilitation in this system. During short-term facilitation, serotonin acts on a G-protein-coupled receptor in the presynaptic terminal of the sensory neuron to elevate the intracellular cAMP concentration through the activation of an adenylate cyclase (see Sections 3.19 and 3.21). Indeed, intracellular injection of cAMP into the sensory neuron was sufficient to cause an enhancement of synaptic transmission between sensory and motor neurons. As was discussed in Chapter 3, cAMP is a second messenger that activates protein kinase A (PKA). One effect of PKA activation at the presynaptic terminal of the sensory neuron is the phosphorylation of a specific type of K^+ channel that is active during resting state, resulting in its closure. This raises the resting membrane potential and makes it easier for action potentials arriving from the cell body to cause the opening of voltage-gated Ca^{2+} channels at the presynaptic terminal of the sensory neuron, thus facilitating neurotransmitter release (**Figure 10–27**, bottom). Serotonin also activates other intracellular signaling pathways, notably protein kinase C (see Figure 3–34), which can phosphorylate other substrates such as voltage-gated K^+ channels, leading to spike broadening and increased neurotransmitter release per action potential. Thus, short-term facilitation alters synaptic strength by post-translational modification of ion channels, consistent with action that takes place on a timescale of seconds to minutes and does not require new protein synthesis.

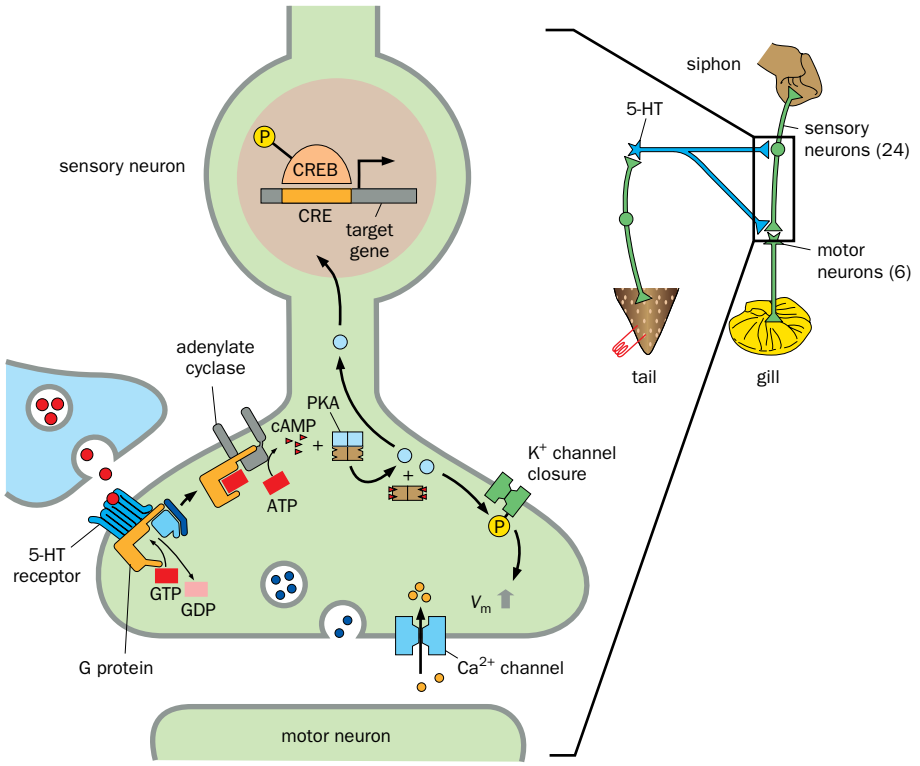

Figure 10–27 Short- and long-term facilitations in *Aplysia* both involve cAMP and PKA. During short-term facilitation, tail shock induces serotonin (5-HT) release at the presynaptic terminal of the sensory neuron, which activates a G-protein-coupled 5-HT receptor. One of the downstream mechanisms is the activation of adenylate cyclase, leading to cAMP production and PKA activation. PKA phosphorylates a specific type of presynaptic K^+ channel and causes its closure, which elevates the resting membrane potential and facilitates action potential-triggered neurotransmitter release. In conditions that produce long-term facilitation, the catalytic subunit of PKA enters the nucleus and phosphorylates nuclear substrates such as the transcription factor CREB and induces new gene expression. The circuit diagram of the gill-withdrawal reflex and sensitization is shown on the right; the box indicates where the scheme on the left is from. For simplicity, the 5-HT axon terminal at the cell body is skipped and the axon is shortened in the magnified diagram on the left. (Adapted from Kandel ER [2001] *Science* 294:1030–1038. With permission from AAAS.)

Remarkably, cAMP and PKA are also key components for long-term facilitation (Figure 10–27, top). Here, a widely used signaling pathway involving the transcription factor CREB is engaged (see Figures 3–41 and 7–36B): PKA phosphorylation activates CREB, which binds to the CRE (cAMP response element) sequences near the promoters of target genes to activate their transcription. How does activation of PKA affect events both locally at the synapse and remotely in the nucleus? Whereas transient serotonin application causes transient PKA activation locally at the synapse, imaging experiments indicated that repeated or prolonged serotonin application induces translocation of the catalytic subunit of PKA to the nucleus, where it can phosphorylate nuclear substrates including CREB. Just as long-term potentiation in the mammalian hippocampus is accompanied by structural changes (see Section 10.13), long-term facilitation in *Aplysia* is also accompanied by growth of synaptic contacts between the sensory and motor neurons. Therefore, some of the molecules whose expression is regulated by CREB are likely responsible for regulating synaptic growth.

10.17 Olfactory conditioning in *Drosophila* requires cAMP signaling

Whereas *Aplysia* offers large cells for physiological studies of learning and memory, the fruit fly *Drosophila* provides an unbiased way to identify genes required for learning and memory by using genetic screening (see Section 13.6). In this procedure, flies with mutations in random genes (produced by treating flies with a chemical mutagen, for example) can be screened using a behavioral assay that tests learning and memory. Mutant flies that perform poorly can be isolated, and the corresponding gene can be mapped using molecular-genetic procedures.

Flies can be trained to associate odors with electrical shocks. In a widely used classical conditioning paradigm, flies are exposed to odorant *A* while being shocked. They are also exposed to odorant *B* without shock. In this case, odorant *A* is designated as the CS+ as it is a conditioned stimulus that is associated with the unconditioned stimulus (US), electric shocks, whereas odorant *B* is designated as the CS−. To test their odorant preference, flies are placed in a T-maze (**Figure 10–28**A; see also Movie 6–1), where they choose to enter one arm (exposed to odorant *A*) or the other (exposed to odorant *B*). Prior to the odorant-shock pairing, flies are as likely to choose odorant *A* as they are *B*. However, after the odorant-shock pairing, 95% of wild-type flies avoid the odorant associated with

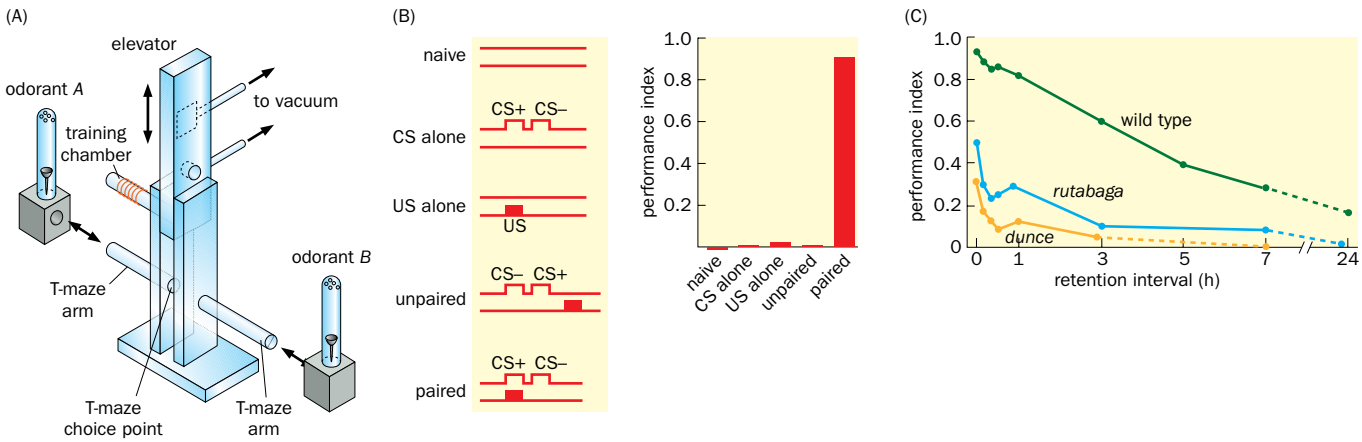

Figure 10–28 Olfactory conditioning in *Drosophila* and its disruption by mutations affecting cAMP metabolism. (A) Schematic of the olfactory conditioning procedure. About 100 flies in the training chamber were exposed to odorant A (CS+) paired with electric shock (US), and to odorant B without electric shock (CS−). These flies were then transferred via the sliding elevator to the bottom T-maze arms, where flies can freely choose a path to odorant A or odorant B. Performance index = [(number of flies in tube B − number of flies in tube A) / total number of flies] × 100. **(B)** Performance indices of flies under different training conditions. Flies learn the association only when US is paired with CS+. **(C)** Performance indices of wild-type and mutant flies. Performance indices represent learning when measured immediately after training (t = 0) and memory retention when measured as specific times thereafter. *rutabaga* and *dunce* mutant flies are defective in both learning and memory. (Adapted from Tully T & Quinn WG [1985] *J Comp Physiol* 157:263–277. With permission from Springer. See also Dudai Y, Jan Y, Byers D et al. [1976] *Proc Natl Acad Sci USA* 73:1684–1688 for the identification of the first learning mutant, *dunce*.)

shock (Figure 10–28B). Timing of the CS–US paring is crucial (Figure 10–28B), as would be predicted from a classical conditioning paradigm. In addition to learning, which is measured as the behavioral performance immediately after training, flies can also be tested for memory at specific times after training. One odorant–shock pairing (for 1 minute) produces a memory that lasts for several hours (Figure 10–28C). Repeated pairings with proper intervals (spaced training) can produce long-term memory that lasts for a week, similar to the *Aplysia* gill-withdrawal reflex following sensitization by tail shock (see Figure 10–26A).

Two of the first mutations identified through genetic screening, named *dunce* and *rutabaga*, affected both learning and memory. Performance of flies carrying either of these two mutations was drastically reduced compared with normal flies immediately after training, indicating a learning defect. In addition, they forgot quickly whatever they learned (Figure 10–28C). Separate tests showed that the abilities of these mutants to detect odorants and shocks were normal, indicating a specific defect in forming the odor–shock association. Molecular-genetic studies revealed that the *rutabaga* gene encodes an adenylate cyclase, an enzyme that catalyzes cAMP synthesis (see Figure 10–27), whereas the *dunce* gene encodes a phosphodiesterase, an enzyme that hydrolyzes cAMP (see Figure 6–4). Thus, proper regulation of cAMP metabolism is essential for learning and memory in a classical conditioning paradigm in *Drosophila*. Subsequent experiments found that perturbation of CREB, the transcription factor regulated by cAMP, affected long-term but not short-term memory of olfactory conditioning, again similar to sensitization of the *Aplysia* gill-withdrawal reflex (see Figure 10–27).

10.18 *Drosophila* mushroom body neurons are the site of CS–US convergence for olfactory conditioning

The identification of molecules required for *Drosophila* olfactory learning and memory also provided an entry point for cellular and circuit studies (see Figure 10–7). For example, it was found that both *dunce* and *rutabaga* genes have expression patterns that are highly enriched in mushroom body neurons, which are targets of olfactory projection neurons (see Figure 6–27). Indeed, expression of a wild-type *rutabaga* transgene in adult mushroom body neurons was sufficient to rescue the memory defects of *rutabaga* mutant flies, demonstrating that cAMP regulation in mushroom body neurons plays a crucial role in olfactory learning and memory.

A circuit model of olfactory learning has been proposed that is based on these studies and on the position of mushroom body neurons in the olfactory processing pathways (see Section 6.16). According to this model, odorants (the CS) are represented by ensembles of mushroom body neurons, whose connections with mushroom body output neurons are modified when the CS is paired with an unconditioned stimulus (the US) that is aversive (such as electric shocks) or appetitive (such as food). This plasticity is a cAMP-dependent process. Recent comprehensive mapping identified 21 types of mushroom body output neurons, most of which connect with one of 15 axonal compartments of mushroom body neurons. Information about US is likely carried by one or more of the twenty types of dopamine neurons, most of which also projects axons to one compartment. Behavioral studies suggest that specific types of mushroom body output neurons encode specific valence, such as aversive or appetitive, to guide behavior (**Figure 10–29**A).

As a specific example, we discuss below an experiment that tested the function of dopamine neurons in olfactory learning in an operant conditioning paradigm. In this paradigm, a single fly was allowed to walk freely in a chamber with two compartments, each of which contained a different odorant. During the training period, the fly received electric shocks whenever it entered the compartment containing one of the two odorants. Through its own actions, the fly learned to avoid the odorant associated with shock. To test the role of dopamine neurons in this learning paradigm, an ion channel that can be activated by light was selectively expressed in a subset of dopamine neurons. Researchers found that photoactivation of dopamine neurons could be used instead of electric shocks to train flies

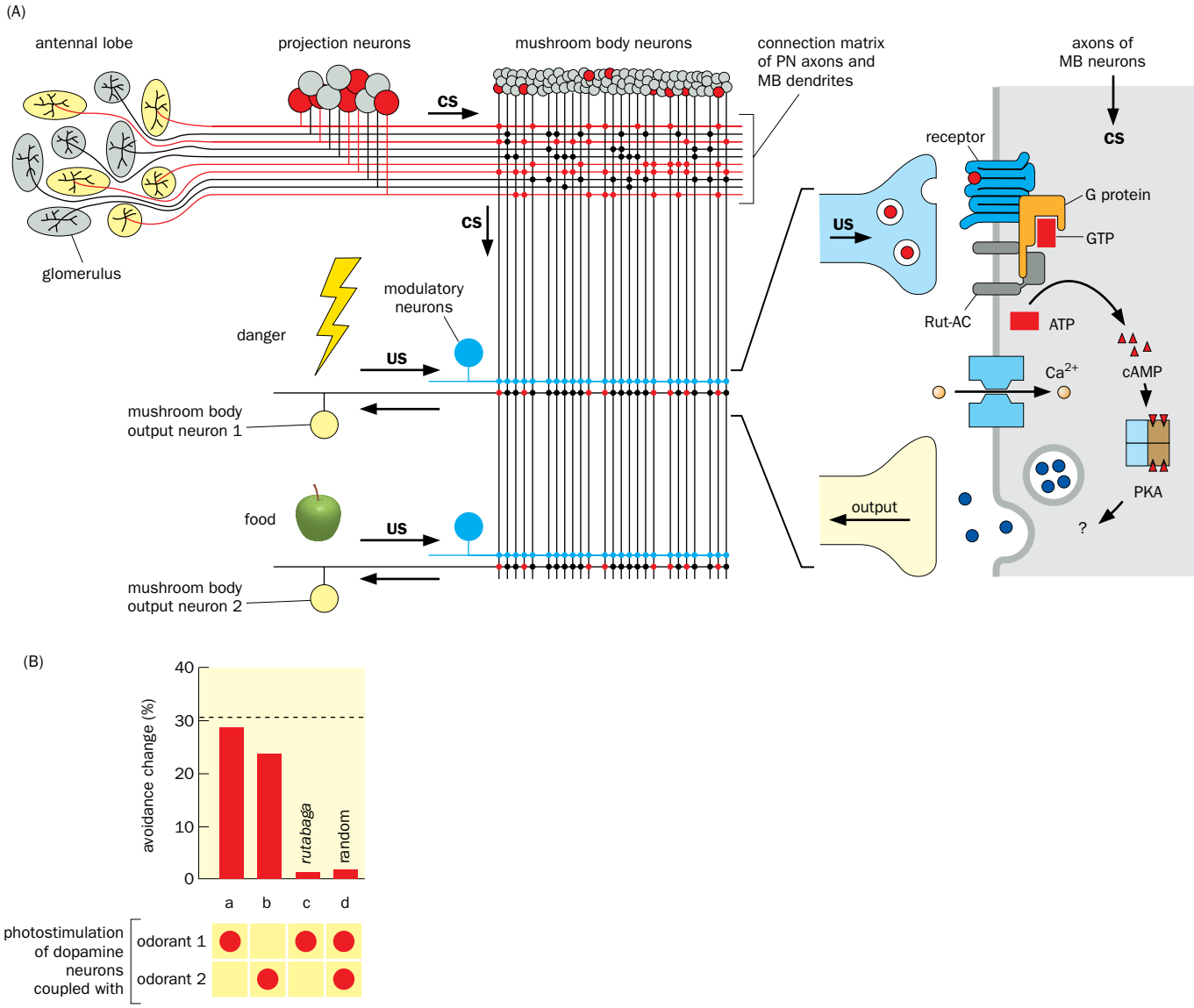

Figure 10–29 Neural circuit and mechanisms of *Drosophila* olfactory conditioning. (A) Left, a circuit model for olfactory conditioning. Odorants activate specific ensembles of glomeruli (ovals) in the antennal lobe, with active glomeruli shown in yellow (see Figure 6–27 for a schematic of the *Drosophila* olfactory system). Projection neurons (PNs) that innervate the active glomeruli become activated (red), which in turn activate a specific subset of mushroom body (MB) neurons. In this representative connection matrix between PN axons and MB neuron dendrites, each MB neuron is connected with three PN axons (dots); only when all three connected PNs are active would the MB neuron become active (red cell on top, with three red dots in the matrix). CS (odorant) information is represented by ensembles of active PNs and subsequently by ensembles of active MB neurons. Synapses between MB axons and dendrites of MB output neurons are modified by nearby input from modulatory neurons, such as dopamine neurons, that signal the presence of an aversive or appetitive US (bottom). Each dot represents a connection and each red or blue dot represents an active synapse. The co-activation of neurons representing the US and the CS modifies the synaptic efficacy between the MB neuron and the MB output neuron. Right, an enlarged diagram of the output synapses of MB neurons. Axons carrying the US information release modulatory neurotransmitters that activate G-protein-coupled receptors, resulting in the activation of the Rutabaga adenylate cyclase (Rut-AC). This causes an increase in cAMP production and thereby activates PKA, leading to changes in synaptic efficacy through mechanisms yet to be explored. **(B)** Photostimulation of dopamine neurons expressing a light-activated ion channel (see Figure 13–44 for details) is used to train flies to avoid a specific odorant in an operant conditioning paradigm. Individual flies were trained to avoid one of the two odorants by giving an electric shock whenever the fly enters a compartment associated with the odorant. The horizontal line indicates the level of avoidance change after electric shock-based training. Photostimulation of specific dopamine neurons can substitute for shock and achieve similar effect. When photostimulation was repeatedly coupled with the fly entering the compartment containing odorant 1 (a) or odorant 2 (b), flies increased avoidance of the stimulus-paired odorant. This effect was abolished in the *rutabaga* mutant (c), or when photostimulation was random and not uniquely associated with one odorant compartment or the other (d). (A, adapted from Heisenberg M [2003] *Nat Rev Neurosci* 4:266–275. With permission from Macmillan Publishers Ltd. See also Aso Y, Hattori D, Yu Y et al. [2014] *Elife* 3:e04577 and Aso Y, Sitaraman D, Ichinose T et al. [2014] *Elife* 3:e04580; B, adapted from Claridge-Chang A, Roorda RD, Vrontou E et al. [2009] *Cell* 139:405–415. With permission from Elsevier Inc.)

to avoid a specific compartment (Figure 10–29B), consistent with the notion that dopamine neurons provide information about electric shocks. Both shock- and photostimulation-mediated training became ineffective in the *rutabaga* mutant, indicating that this operant conditioning paradigm also requires cAMP.

In summary, studies of olfactory conditioning in flies have produced a circuit and molecular model (Figure 10–29A) with remarkable similarities to sensitization of the gill-withdrawal reflex in *Aplysia* (see Figure 10–27). At the circuit level, information about olfactory conditioned stimuli enters the mushroom body neuron dendrites through excitatory input from olfactory projection neurons. Input from dopamine neurons, representing the US, likely modifies synapses that link mushroom body neurons to their downstream mushroom body output neurons. Indeed, the connections between the mushroom body neurons and the output neurons represent a specific example of the synaptic matrix discussed in Figure 10–5 (Movie 10–1). Here, input patterns represent specific odorants, and through the synaptic matrix produce at least two distinct output patterns, the activation of aversive or appetitive output neurons, leading to activation of two distinct behaviors. Before training, neutral odorants do not activate either of the output neurons. During learning, coincident activation of modulatory neurons modifies the connection strengths between mushroom body neurons and output neurons, such that after training, activation of specific mushroom body neuron ensembles alone (representing odorants) would activate either the aversive or appetitive output neurons depending on the training condition (see Movie 10–1).

At the molecular level, the US causes the activation of the G-protein-coupled dopamine receptor, which in turn activates the adenylate cyclase, leading to cAMP production and PKA activation in mushroom body neurons. Together, the *Aplysia* and *Drosophila* studies demonstrate an evolutionarily conserved role of cAMP in different forms of learning and memory. Indeed, cAMP and PKA also play important roles in synaptic plasticity (see Sections 10.7–10.9) as well as learning and memory in mammals (see Section 10.20), including the hippocampus-dependent learning that we now turn to. Many hippocampus-dependent learning paradigms and memory tasks take advantage of an important function of the hippocampus: spatial representation (**Box 10–2**).

Box 10–2: Place cells, grid cells, and representations of space

Navigation is essential for animals to find food and return home safely. Animals from ants and honeybees to mammals use two types of navigation strategies: a **landmark-based strategy**, where animals use external cues to determine their location, and a **path-integration strategy**, where animals use information based on the speed, duration, and direction of their own movement to calculate their current positions with respect to their starting position. Both strategies require that animals have an internal representation of space.

In mammals, the hippocampus and entorhinal cortex are central to spatial representation. A seminal discovery was made in the 1970s when researchers performed single-unit recordings of hippocampal neurons in freely moving rats navigating an arena or a maze. Individual cells were found to fire robustly when the rat was at a particular location in the maze, regardless of what behavior the animal was performing (for example, passing through from various directions, exploring, or just resting); different cells fired at different locations (**Figure 10–30**A). These cells are called **place cells**, and the physical location that elicits place-cell firing is known as the cell's **place field**.

We now know that virtually all hippocampal CA1 and CA3 pyramidal neurons are place cells. Their place fields are influenced by external landmarks. For example, after the place field is established in a circular arena, if external landmarks are rotated, the place fields also rotate, preserving their relative positions to the external landmarks. However, once place fields form, place cells fire at the same locations in the dark, and place fields in the same environment can be stable for over a month (see Movie 13–3). Since different place cells fire when the rat occupies different locations in the same arena, it is possible to reconstruct the path of a moving rat from simultaneous recordings of dozens of place cells using a multi-electrode array (Figure 10–30B); in other words, a few dozen place cells contain sufficient information to reconstruct the rat's path. At the same time, a single place cell can be active in different environments, with differing place fields in each. Thus, each environment is represented by a unique population of active place cells (a **cell assembly**), and each cell participates in multiple cell assemblies that represent multiple environments. These remarkable properties led to the proposal that hippocampal place cells collectively form cognitive maps that

Box 10–2: Place cells, grid cells, and representations of space

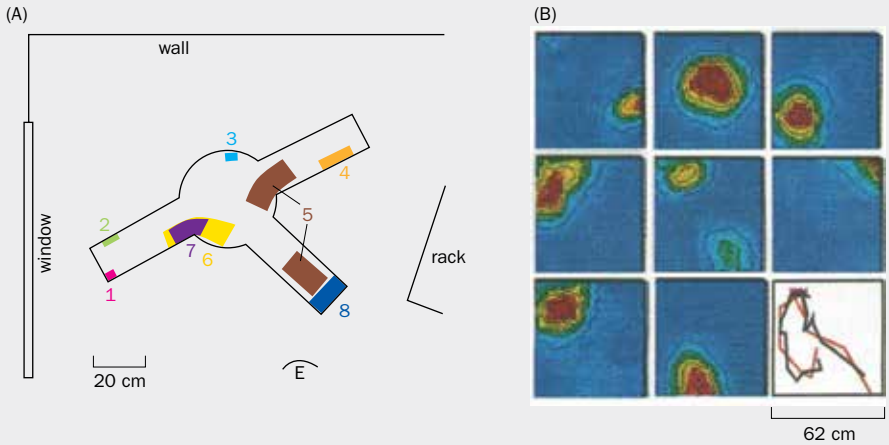

Figure 10–30 Hippocampal place cells. (A) Map of a maze showing the place fields (numbered and illustrated in different colors) of eight place cells in the hippocampus of a freely moving rat. Each place field represents the regions within the maze in which a given place cell exhibited increased firing rate. E, location of the experimenter. **(B)** The activity of place cells can be used to construct a map of a rat's travels. A multi-electrode array was used to simultaneously record 80 hippocampal cells. The place fields of eight selected place cells are represented here as heat maps in eight squares: for each place field, the colors indicate the firing rate of the cell when the rat occupied a corresponding position in a 62 cm × 62 cm square arena (red, maximal firing rate; dark blue, no firing). Note that the place fields of different cells vary in size and are situated at different locations in the arena. Bottom right, the vector of firing rates of a neuronal population during a 30-second period was used to reconstruct the spatial trajectory of the rat. The calculated trajectory (red) closely matches the actual trajectory (black). (A, adapted from O'Keefe J [1976] *Exp Neurol* 51:78–109. With permission from Academic Press; B, from Wilson MA & McNaughton BL [1993] *Science* 261:1055–1058. With permission from AAAS.)

animals can use to determine where they are in their environment and to aid their navigation using landmark-based and path-integration strategies. Unlike the topographic map discussed in the visual system (see Chapters 4 and 5), however, there is no obvious relationship between the positions of place cells in the hippocampus and the physical locations of their place fields.

How do hippocampal place cells acquire their firing properties? A partial answer to this question came from another remarkable discovery made in the mid-2000s. A large fraction of layer 2/3 neurons in the medial entorhinal cortex, which provide major input to the hippocampus (see Figure 10–6, right), were found to also have space-modulated firing patterns. The locations where these cells fire most are distributed across the environment in a periodic manner, forming grids that tile the entire space; each cell's peak firing rate occurs at the apices of the hexagonal grid unit (**Figure 10–31**A; see **Movie 10–2**). These cells are aptly named **grid cells**. Each grid cell has a characteristic grid size that remains constant in arenas of differing sizes and shapes (Figure 10–31A). Neighboring grid cells share similar grid sizes, but differ in the exact locations of the grid centers.

Grid cells and place cells share many similarities. The activities of simultaneously recorded grid cell populations, like those of place cells, can be used to reconstruct trajectories of movement (see Movie 10–2). As with place fields, grid patterns are influenced by external landmarks; when external landmarks are rotated in a circular arena, grid patterns rotate correspondingly. Grid patterns, like place fields, do not merely mirror sensory cues, since they are maintained when the animal moves in the dark. However, the properties of grid cells and place cells also differ in important ways. Grid cells tile space more efficiently: a few grid cells can cover a space that requires dozens or more place cells. After animals are introduced into a novel environment, grid cells retain their grid size but place fields may remap completely. Populations of grid cells maintain the positions of their grid centers relative to each other across different arenas, whereas place cells remap more randomly. These observations suggest that the grid cells provide a more fundamental metric of space for anchoring the place fields of hippocampal cells.

In addition to grid cells, the entorhinal cortex also contains **border cells**, which fire when the animal is at a specific edge of the arena (Figure 10–31B). Border cells provide information about the perimeters of the local environment, which can anchor grid patterns and place fields to geometric confines. **Head direction cells**, another intriguing cell type, fire when the animal's head is facing a specific direction independent of the animal's location in the arena (Figure 10–31C). Whereas grid cells and border cells have been found mostly in the entorhinal cortex, head direction cells are also present in brain areas that send input to the entorhinal cortex. Indeed, the entorhinal cortex receives diverse inputs representing visual, olfactory, and vestibular signals. In turn, intermingled populations of grid cells, border cells, and head direction cells in the entorhinal cortex all send direct projections via the perforant path (see Figure 10–6) to the hippocampus, which integrates these diverse streams of information to form place fields and send feedback signals

(*Continued*)

Box 10–2: Place cells, grid cells, and representations of space

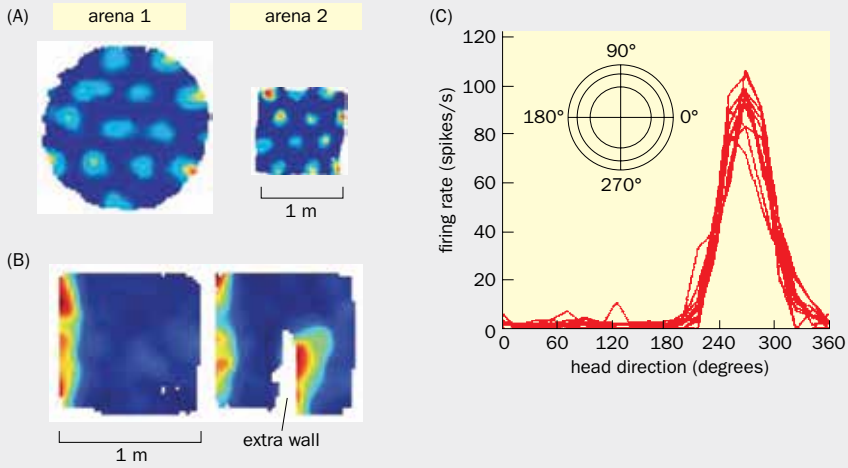

Figure 10–31 Grid cells, border cells, and head direction cells. **(A)** Firing patterns of two entorhinal cortex grid cells, one in a circular and another in a square arena. The color of each position within each arena reflects the firing rate of the grid cell when the rat occupied that position (red, maximal firing rate; dark blue, no firing). Periodic peaks in firing rate of each cell form hexagonal grids that tile each arena. **(B)** This entorhinal border cell fired selectively when the rat was located at the left border of a square arena (left). When an extra border was added, a new firing field along the new left border was created (right). **(C)** A head direction cell fired when the rat's head was facing a specific direction (peaking at ~270°, or when the rat's head was facing south) regardless of where the rat was located in the arena. Each of the 12 traces represents the firing rate of the cell when the rat was at one of the 12 divisions of the circular arena (inset at the top left). (A, from Hafting T, Fyhn M, Molden S et al. [2005] *Nature* 436:801–806. With permission from Macmillan Publishers Ltd; B, from Solstad T, Boccara CN, Kropff E et al. [2008] *Science* 322:1865–1868. With permission from AAAS; C, adapted from Taube JS, Muller RU & Ranck JB [1990] *J Neurosci* 10:420–435. With permission from the Society for Neuroscience.)

to the entorhinal cortex. Exactly how information is integrated and how the place code is read in order to guide navigation are still open questions.

The remarkable properties of place cells and grid cells in the hippocampal–entorhinal network, far removed from the sensory world, have provided a glimpse of how abstract information such as space is represented in the brain. What is the relationship between spatial representation and memory, another important function of the hippocampus? One hypothesis is that the hippocampal–entorhinal network is used in parallel for navigation and memory. Explicit memory often involves the binding of disparate details into a coherent event; this is conceptually similar to the process by which hippocampal place cells extract spatial information from the activities of grid cells, border cells, and head direction cells. An alternative hypothesis is that the location of an experience is so essential to its explicit memory that the formation of a memory is intimately tied with the representation of space. Indeed, the use of 'memory palaces,' that is, the organization of events into imaginary spaces, is an ancient and effective mnemonic technique, and space-based tasks have been among the most effective ways to assay memory in mammals. As researchers learn more about the functions of the hippocampal–entorhinal network in memory and in spatial representation, the connections between these two systems will become clearer.

10.19 In rodents, spatial learning and memory depend on the hippocampus

Does synaptic plasticity underlie learning and memory in mammals as it does in *Aplysia*? Put in another way, do activity-dependent changes induced at given synapses during the formation of a specific memory serve as a basis for the information storage that underlies that specific memory? In the following sections we will explore these questions using the mammalian hippocampus as a model, because rich synaptic plasticity mechanisms have been discovered in the mammalian hippocampus (see Sections 10.4–10.13), and the human hippocampus is essential for forming explicit memory (see Section 10.1).

An essential step for linking memory and hippocampal synaptic plasticity is to establish hippocampus-dependent behavioral tasks that test memory in rodents, the animal model in which synaptic plasticity has been most intensely investigated. Given that the mammalian hippocampus contains spatial maps of the external world (see Box 10–2), a number of hippocampus-dependent behavioral

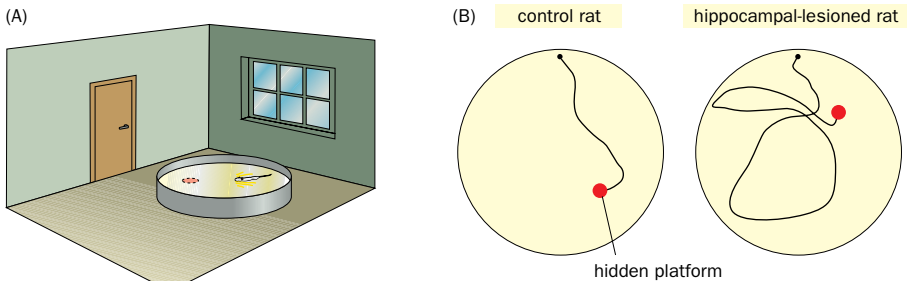

Figure 10–32 Spatial memory tested in the Morris water maze depends on the hippocampus. (A) Schematic of the Morris water maze. After training, a rat or a mouse can use distant spatial cues to help find the location of a hidden platform (dashed circle) in a large pool of milky water. **(B)** After training, a control rat swam directly to the hidden platform, whereas an experimental rat with hippocampal lesion found the platform after taking a circuitous route. (Adapted from Morris RGM, Garrud P, Rawlins JNP et al. [1982] *Nature* 297:681–683. With permission from Macmillan Publishers Ltd.)

assays have been established that require spatial recognition. One of the most widely used is the **Morris water maze** (Figure 10–32A), a navigation task in which rats (and mice) learn to locate a hidden platform in a pool of milky water to avoid having to swim. (Despite being able to swim, rats and mice prefer not to.) The rats cannot see, hear, smell, or touch the platform until they find it. Nevertheless, they can use distant cues in the room to learn the platform's spatial location, such that after training they can be placed at any position in the pool and will swim straight to the hidden platform (Figure 10–32B, left). Performance of this task is dependent on the hippocampus, as rats with hippocampal lesions no longer remember the location of the hidden platform even after extensive training (Figure 10–32B, right). When the platform was visible, both rats found it with equal ease. Of the forms of memory known in rodents, this spatial memory most closely resembles the explicit memory of humans.

10.20 Many manipulations that alter hippocampal LTP also alter spatial memory

The establishment of spatial memory tasks such as the Morris water maze enabled researchers to determine whether manipulations that affect synaptic plasticity in the hippocampus also affect spatial memory. One of the first such manipulations was to block the function of the NMDA receptor with a specific antagonist, AP5 (see Section 10.6). Infusion of AP5 into the hippocampus during the training session, at a concentration that blocked LTP *in vivo*, disrupted the subsequent recall of the platform position in the Morris water maze. When the hidden platform was removed after training, control rats focused their search preferentially in the quadrant where the hidden platform had been, whereas AP5-treated rats swam randomly (Figure 10–33A). Conditionally knocking out the essential NMDA

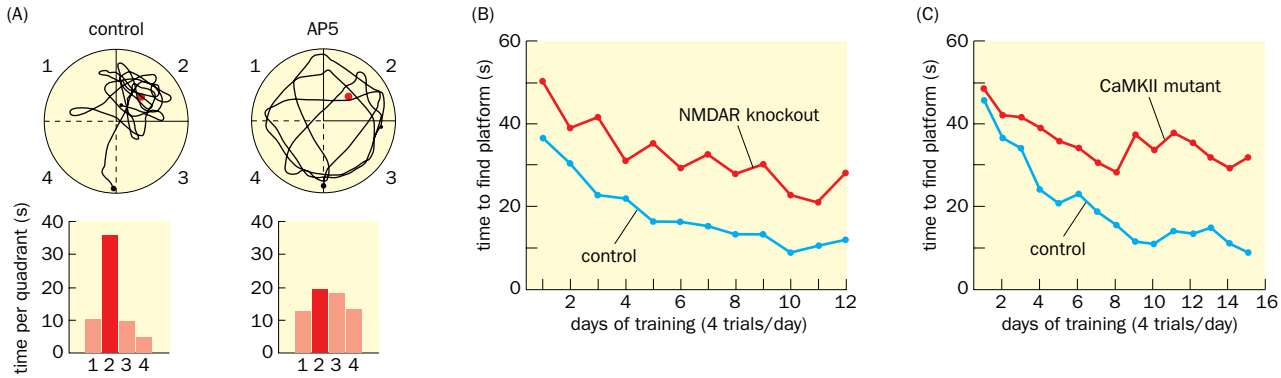

Figure 10–33 Manipulations that disrupt hippocampal LTP also interfere with performance in the Morris water maze. (A) Infusion of AP5, an antagonist of the NMDA receptor, disrupts the spatial memory of rats. Rats were trained with the hidden platform (circle) in quadrant 2. During the test, the platform was removed and the trajectory was recorded. Top, while the control rat focused its search near the phantom platform, the rat infused with AP5 during training swam randomly. Bottom, quantification of time spent in four quadrants. **(B)** Throughout the training regime, mice with CA1-specific knockout of the GluN1 subunit of the NMDA receptor (red trace) were slower to find the hidden platform than *CA1-Cre* only control mice (blue trace). **(C)** Mice in which the CaMKII auto-phosphorylation site is mutated (red trace) also took longer to find the hidden platform compared with controls (blue trace). See Figures 10–10 and 10–12 for LTP defects of the same mice as in panels B and C. (A, adapted from Morris RGM, Anderson E, Lynch GS et al. [1986] *Nature* 319:774–776. With permission from Macmillan Publishers Ltd; B, adapted from Tsien JZ, Huerta PT & Tonegawa S [1996] *Cell* 87:1327–1338. With permission from Elsevier Inc.; C, adapted from Giese KP, Federov NB, Filipkowski RK et al. [1998] *Science* 279:870–873. With permission from AAAS.)

receptor subunit GluN1 in CA1 pyramidal neurons, which blocked LTP at the CA3 → CA1 synapse (see Figure 10–10), also interfered with performance in the water maze assay (Figure 10–33B). These experiments demonstrated an essential function for the NMDA receptor in the hippocampus, and specifically in CA1 pyramidal neurons, in spatial learning and memory.

Many genetic manipulations in mice that disrupt hippocampal synaptic plasticity, notably LTP at the CA3 → CA1 synapse, also interfere with hippocampus-dependent spatial memory tasks. For example, mice that lack CaMKII or that have a point mutation in the CaMKII auto-phosphorylation site have impaired LTP at the CA3 → CA1 synapse (see Figure 10–12B) and perform poorly in the Morris water maze (Figure 10–33C). In concert with findings from *Aplysia* and *Drosophila*, the cAMP/PKA pathway is also essential for both hippocampal LTP and hippocampus-dependent memory. For example, in mice that carry double mutations for two Ca^{2+}-activated adenylate cyclase, which links Ca^{2+} entry to cAMP production (see Figure 3–41), CA3 → CA1 LTP was impaired (**Figure 10–34**A), as was a hippocampus-dependent memory task called passive avoidance. In this task, mice are placed in a chamber with two compartments, one of which is illuminated. Mice naturally prefer the dark, safer compartment. During training, entry into the dark chamber is paired with an electric shock. After training, mice are placed back in the illuminated compartment and the time it took for the mice to enter the dark compartment is a measure of their memory to avoid the shock-associated compartment. Adenylate cyclase double knockout mice performed poorly 30 minutes after training compared with control mice (Figure 10–34B).

Since the late 1990s, a number of genetic manipulations in mice have been reported to enhance memory performance compared to controls in a variety of memory tasks such as Morris water maze, passive avoidance, or fear conditioning that we will discuss in more detail later. For example, transgenic mice overexpressing the GluN2B subunit, which is normally preferentially expressed in developing neurons (see Box 5–3) and which has higher Ca^{2+} conductance than other GluN2 isoforms, exhibited superior performance in Morris water maze and several other memory tasks. Interestingly, GluN2B overexpressing mice and other genetically engineered mice with enhanced memory performance also exhibited enhanced hippocampal LTP. Together, these experiments have established a strong correlation between memory and hippocampal synaptic plasticity.

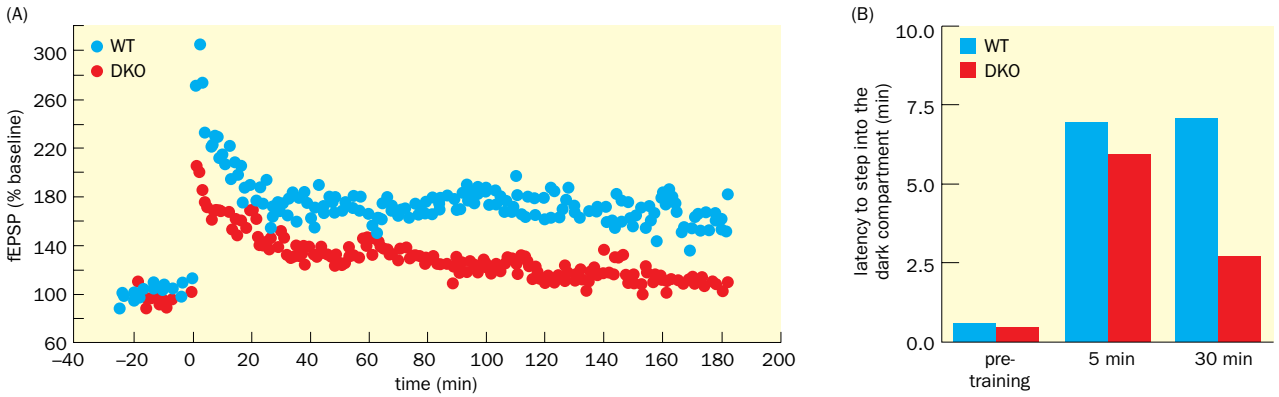

Figure 10–34 Interfering with the cAMP/PKA pathway affects hippocampal LTP and learning. (A) Compared with wild-type (WT) controls, mice in which two Ca^{2+}-dependent adenylate cyclase were doubly knocked out (DKO) exhibit reduced magnitude of LTP at the CA3 → CA1 synapse. **(B)** DKO mice also exhibit reduced memory in a passive avoidance task. In this assay, mice are placed in the illuminated compartment of a two-compartment chamber. Before training, mice quickly move into the dark compartment as a natural tendency to avoid predators. After training (pairing electric shocks with entrance to the dark compartment), DKO mice avoid the dark compartment similarly to controls 5 min after the training, but enter the dark compartment more quickly 30 min after the training, suggesting an impaired memory. (Adapted from Wong ST, Athos J, Figueroa XA et al. [1999] *Neuron* 23:787–798. With permission from Elsevier Inc.)

10.21 From correlation to causation: the synaptic weight matrix hypothesis revisited

While demonstrating strong correlations between hippocampal LTP and spatial memory in rodents, none of the genetic and pharmacological manipulations discussed in the previous section has proven that synaptic plasticity and memory are causally linked, that is, the synaptic changes cause the formation of memory. These manipulations could all affect synaptic plasticity and memory in parallel. To establish a causal link between modification of synaptic strength and learning, one would ideally perform experiments to specifically alter one and test the effect on the other.

One approach is to examine directly whether learning can induce hippocampal LTP. The key to this type of experiment is to identify which synapses in the hippocampus are related to a specific learning event. This difficult task was achieved in an experiment that combined passive avoidance training with use of a multi-electrode recording array in rats. These rats were implanted with a multi-electrode recording array at the CA1 dendritic fields and a stimulating electrode at the Schaffer collaterals (**Figure 10–35**A) such that synaptic transmission from Schaffer collaterals onto different populations of CA1 pyramidal neurons could be recorded before and after the training. Whereas none of the electrodes from control rats without training detected any potentiation, a small fraction of electrodes from trained rats detected potentiation after behavioral training (Figure 10–35B). Moreover, synapses that were potentiated by behavioral training became less likely to be potentiated further by subsequent high-frequency stimulation of the Schaffer collateral, a process known to induce LTP. Thus, learning can produce synaptic potentiation that partially occludes subsequent LTP at the same synapses.

Another approach to investigate the relationship between LTP and learning is to test whether saturation of LTP prevents further learning. Rats with unilateral hippocampal lesions (such that spatial memory must depend on the hippocampal tissues that remain) were implanted with a multi-electrode stimulating array at the perforant path in the unlesioned hippocampus. Repeated stimulation through this array could maximally induce and potentially saturate LTP at recording sites. Rats with nearly saturated LTP, measured *post hoc* by physiological recordings, were more impaired in the Morris water maze assay than were rats that still exhibited residual LTP. These experiments collectively provide stronger links between learning and changes in the strength of specific hippocampal synapses.

Let's revisit the hypotheses raised in Sections 10.2 and 10.3: memory is stored in the form of synaptic weight matrices in neural circuits, and learning is equivalent to altering the synaptic weight matrix as a result of experience. The strongest case for these hypotheses can be made in the context of simple forms of learning, such as the *Aplysia* gill-withdrawal reflex, where modifications of the strength of the sensory neuron–motor neuron synapse underlie behavioral habituation and sensitization (see Section 10.15). In the complex mammalian brain, the strongest evidence has come from studies of the hippocampus discussed in this and preceding sections. One way to strengthen the causal relationship between learning and alteration of synaptic weights would be to achieve the following: (1) identify the neurons and synapses in a circuit whose plasticity correlates with a learning experience; (2) determine the specific states of the synaptic weight matrix (for example, Figure 10–5) before the learning experience (state *A*) and after it (state *B*); (3) artificially change the synaptic weight matrix from state *A* to state *B* without learning; and (4) test whether the animal behaves as if the learning experience had occurred (that is, a mimicry experiment). This is a challenging task; the *in vivo* mimicry experiment has not been performed even in the *Aplysia* gill-withdrawal paradigm. Even though the complexity of the mammalian brain and the large number of neurons and synapses make this task even more challenging, researchers have employed modern circuit analysis tools to search for the potential physical substrates for memory (termed **memory traces** or **engrams**). **Box 10–3** provides an example of how such a search can be conducted.

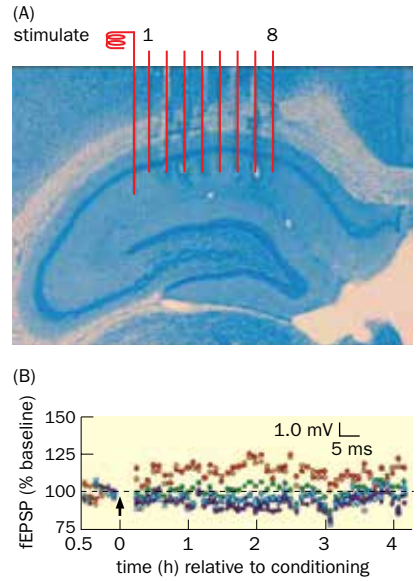

Figure 10–35 Learning can induce LTP. **(A)** A multi-electrode array (electrodes 1–8) was placed in the CA1 area of a rat hippocampus to record the responses of CA1 neurons to stimulation of Schaffer collaterals. **(B)** After passive avoidance training, recordings from the two electrodes indicated by red and orange dots showed an enhancement of field excitatory postsynaptic potentials (fEPSPs); recordings from the remaining six electrodes (represented by dots of other colors) did not demonstrate fEPSP enhancement. Additional experiments (not shown) revealed that synapses potentiated by passive avoidance training were less responsive to subsequent potentiation by high-frequency stimulation using the stimulating electrode. (Adapted from Whitlock JR, Heynen AJ, Shuler MG et al. [2006] *Science* 313:1093–1097. With permission from AAAS.)

Box 10–3: How to find an engram

Searching for engrams has had a long history. As mentioned in the introduction to the Prelude of this chapter, Lashley's lesion study led him to conclude that the engram for maze running is widely distributed in a rat's cerebral cortex. Studies of human patients such as H.M. have led to the identification of hippocampus as the site essential for forming new explicit memory. Tools in modern neuroscience have the potential to reveal engrams at the level of neurons and synapses. Neural substrates that represent an engram for a memory task should become active during training, and their reactivation should mimic the recall of the memory. We use a specific example to illustrate how researchers have utilized a combination of transgenic mice, viral transduction, and optogenetic manipulation to search for an engram (**Figure 10–36**).

To identify the active population of neurons, the *Fos-tTA* transgenic mouse was used, in which the expression of a **tetracycline-repressible transcriptional activator (tTA)** is controlled by the promoter of the immediate early gene *Fos*, such that tTA can be induced by neuronal activity (see Section 3.23 for this property of immediate early genes). tTA is a transcription factor that binds to DNA sequences called **tetracycline response elements (TRE)** to regulate gene expression; the activity of tTA is inhibited in the presence of a tetracycline analog, doxycycline (see Section 13.10 for more details of the tTA/TRE expression system). An adeno-associated virus (AAV) that enables the expression of channelrhodopsin (ChR2) under the control of TRE was used to transduce dentate gyrus granule cells, which provide input to CA3 pyramidal neurons (see Figure 10–6). These mice were tested for a hippocampus-dependent memory established by **contextual fear conditioning**. [In this paradigm, mice experience electric shocks during training in a specific environment (context *A*). Mice subsequently placed in the same environment exhibit a freezing response: they remain immobile, an adaptive response of rodents to avoid being

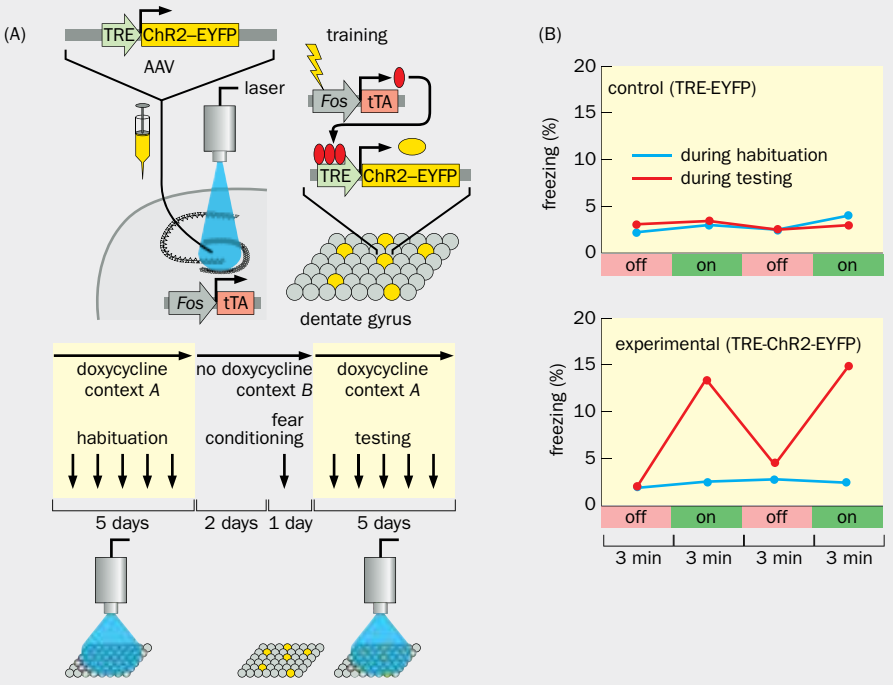

Figure 10–36 Optogenetic stimulation of a specific population of dentate gyrus granule cells activates fear memory. **(A)** Experimental design. Adeno-associated virus (AAV) enables the expression of channelrhodopsin (ChR2) fused with an enhanced yellow fluorescent protein (EYFP) under the control of a tetracycline response element (TRE). AAV was injected into the dentate gyrus (indicated by the needle) of a transgenic mouse that express the tetracycline-repressible transcription activator (tTA, red ovals) from the *Fos* promoter so that its expression is induced by neuronal activity. The matrix of circles represents dentate gyrus granule cells. Mice were first exposed to and became habituated to context *A* and photostimulation in the presence of doxycycline; the dentate gyrus granule cells activated in context *A* did not express ChR2, because tTA activity is inhibited by doxycycline. Fear conditioning was induced in context *B* in the absence of doxycycline such that dentate gyrus granule cells activated by this experience expressed ChR2 (yellow circles); ChR2 expression persisted for several days even after the mice were treated with doxycycline again to prevent further tTA-induced gene expression. Mice were then reintroduced to context *A* to test whether optogenetic stimulation of ChR2-expressing cells could induce fear memory recall. **(B)** In control mice, in which tTA induced expression of EYFP, optogenetic activation (light-on period in green) did not induce fear memory, as assayed by the percentage of time spent freezing (top). In experimental mice, optogenetic stimulation induced freezing in a light-dependent manner (bottom) during the testing period but not during the earlier habituation period. (Adapted from Liu X, Ramirez S, Pang PT et al. [2012] *Nature* 484:381–385. With permission from Macmillan Publishers Ltd.)

Box 10–3: How to find an engram

seen by predators when facing danger in the wild. Mice placed in a different environment (context *B*, which differs from *A* in ceiling shape, flooring, and lighting) do not exhibit a freezing response.] The mice were first exposed to and became habituated in context *A* in the presence of doxycycline to prevent tTA/TRE-induced expression of ChR2. After doxycycline removal, mice were exposed to context *B*, during which they received electric shocks to induce contextual fear conditioning. This resulted in tTA and ChR2 expression in the population of dentate gyrus granule cells that were activated during fear conditioning in context *B*.

To test the effect of reactivation of neurons that were active during fear conditioning in context *B*, mice were given food containing doxycycline to prevent new tTA/TRE-induced ChR2 expression, and were introduced to context *A* with or without optogenetic stimulation (Figure 10–36A). Control mice did not freeze in context *A*. However, ChR2-expressing mice froze in context *A* in response to optogenetic stimulation, as if they were in context *B* (Figure 10–36B). Thus, activation of a population of cells that were active during contextual fear conditioning was sufficient to induce fear recall in a different context, suggesting that this population of dentate gyrus granule cells contributes to the memory of context *B*.

This experiment did not show which synapses were modified and what additional properties in the circuits were changed to make mice fearful of context *B*. In principle, plasticity could occur anywhere in the neural pathway downstream of the granule cell population that leads to the motor behavior of freezing. In light of the hippocampal plasticity findings discussed in this chapter, it is likely that plasticity occurs in the downstream circuits within the hippocampus, such as at the dentate gyrus → CA3 synapse, the CA3 → CA3 recurrent synapse, the CA3 → CA1 synapse, or all of the above. Plasticity can also occur in the amygdala, whose function in fear conditioning will be discussed in Section 10.23.

WHERE DOES LEARNING OCCUR, AND WHERE IS MEMORY STORED IN THE BRAIN?

So far in this chapter, with the exception of the invertebrate systems, we have focused on the hippocampus as a model for studying mechanisms of synaptic plasticity and spatial (explicit) memory. However, synaptic plasticity occurs throughout the nervous system. For example, in Section 8.8 we discussed that the cerebellum plays an important role in motor skill learning, and long-term depression of parallel fiber–Purkinje cell synapses caused by co-stimulating parallel fibers and climbing fibers contributes to cerebellum-based motor learning. In the last four sections of this chapter, we will broaden our study of memory systems beyond the hippocampus with a few select examples.

10.22 The neocortex contributes to long-term storage of explicit memory

Although the medial temporal lobe including the hippocampus is essential for the initial formation of explicit memory, it does not appear to be required for long-term memory storage and retrieval, as suggested by the ability of H.M. to recall memories of his childhood (see Section 10.1). Where is long-term explicit memory stored?

A widely accepted view is that the neocortex is involved in long-term explicit memory storage, and that specific types of memory engage specific cortical regions. This idea, first proposed in the late nineteenth century, states that remembering involves reactivating the sensory and motor components of the original event that led to the formation of the memory. Two types of human studies are consistent with this view. First, lesions of specific parts of the neocortex lead to loss of specific types of memory. For example, patients with damage to the color- or face-processing areas of the visual cortex not only lose their ability to perceive colors or recognize faces, but also exhibit retrograde memory deficits in specific domains. Patients with adult-onset prosopagnosia (inability to distinguish faces), for instance, not only exhibit defects in face perception, but also cannot remember faces that were familiar before the onset of the disorder.

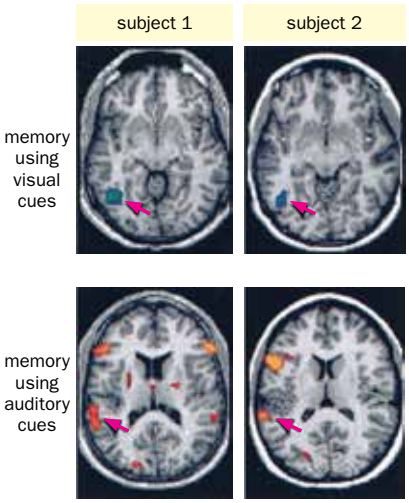

Figure 10–37 Reactivation of specific sensory cortices during long-term memory recall. fMRI images of two subjects, each performing the task of vividly remembering an object when presented with a word that had been extensively paired with either a picture or a sound during prior training. For image-based recall, the high-order visual cortex is activated (arrows in the top panels). For sound-based recall, the high-order auditory cortex is activated (arrows in the bottom panels). These fMRI images in the top and bottom rows were taken at two different horizontal planes. Both subjects have a bias for using the left cortex. (From Wheeler ME, Petersen SE & Buckner RL [2000] *Proc Natl Acad Sci USA* 97:11125–11129. With permission from the National Academy of Sciences.)

Second, functional imaging studies of healthy human subjects engaged in memory tasks revealed the reactivation of cortical areas relevant to specific memory tasks. In one study, for example, subjects were first extensively trained to associate words (for example, DOG) with either pictures (an image of a dog) or sounds (the bark of a dog). During subsequent testing, they were asked to vividly recall the items when given only the word as a cue, while their brains were being scanned via functional magnetic resonance imaging (fMRI). After the recall task/fMRI scan, subjects then indicated whether they vividly remembered an image or a sound; their answers to this question usually matched with their training. After pairing the word with an image during training, high-order visual cortical areas were selectively activated during the recall (**Figure 10–37**, top), whereas after sound-based training, the recall elicited the selective activation of high-order auditory cortical areas (Figure 10–37, bottom). These data suggest that the act of remembering indeed reactivates sensory-specific cortices.

How do the hippocampus and neocortex collaborate to form and store long-term memory? At present, we can only speculate. According to a prevalent hypothesis (**Figure 10–38**), signals that lead to the original hippocampus-dependent formation of explicit memory also activate primary and associative cortical areas. The hippocampus integrates distributed signals from multiple cortical areas during the initial memory formation. Over the course of long-term memory consolidation, the hippocampus 'trains' the establishment of new connections among cortical neurons, such that memories over the long term are no longer hippocampus-dependent. Exactly how this is achieved is not known.

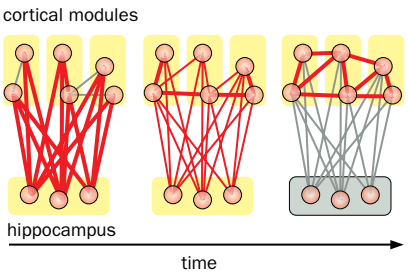

Figure 10–38 A model illustrating the interactions between the hippocampus and neocortex during long-term memory consolidation. During the initial memory formation (left), signals that pass through different cortical areas to the hippocampus establish links between the hippocampus and those cortical areas. Ongoing interactions between the hippocampus and the cortical areas after the initial memory formation gradually establish links among the different cortical areas (middle), until these intracortical links are sufficient to represent the remote memory, and the memory can be recalled independent of the hippocampus (right). Heavy and light lines represent strong and weak links, respectively. Links shown in gray at a given stage are not required for memory recall at that time. (Adapted from Frankland PW & Bontempi B [2005] *Nat Rev Neurosci* 6:119–130. With permission from Macmillan Publishers Inc.)

Animal studies have strengthened the notion that neocortex plays a role in remote memory, and have shed light on the interactions between the hippocampus and neocortex. Let's consider the three sets of experiments on contextual fear memory (see Box 10-3), a hippocampus-dependent form of memory that resembles human explicit memory. In the first experiment, rats received electric shocks when placed in a specific environment; subsequently, their hippocampi were bilaterally lesioned 1, 7, 14, or 28 days after the training. Seven days after the surgery, rats were returned to the training environment to measure their fear memory. Whereas control rats exhibited fear memory under all conditions, rats with hippocampal lesions lacked the contextual fear response if lesioning was performed 1 day after training, but had less severe deficits in memory recall as the duration between training and lesioning lengthened (**Figure 10–39**A). This experiment suggests that fear memory becomes increasingly less dependent on the hippocampus as time passes after the initial training.

Where is long-term fear memory stored? In the second experiment, researchers used immediate early gene expression to identify brain regions that were activated during retrieval of remote fear memory. Several frontal cortical areas were shown to have elevated expression of the immediate early genes *Fos* and *Egr1* (see Section 3.23). Inactivation of these specific cortical areas by focal injection of **lidocaine** (an anesthetic that blocks action potential propagation by inhibiting voltage-gated Na^+ channels) identified the **anterior cingulate cortex** (**ACC**), which is located near the midline of the frontal lobe, as a neocortical site

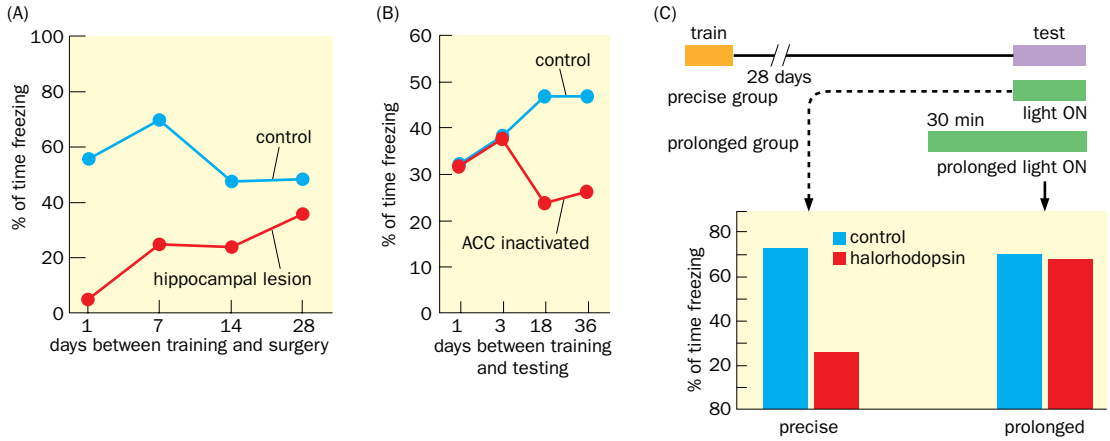

Figure 10–39 Interplay between the hippocampus and neocortex in contextual fear conditioning. (A) The *x* axis shows days elapsed between training and the surgery that bilaterally lesioned the hippocampi in experimental rats. All rats were tested for contextual fear conditioning (*y* axis) 7 days after the surgery. Compared with unlesioned controls that went through the same surgical procedure (blue trace), lesioned rats (red trace) did not exhibit fear memory (quantified by the percentage of time spent freezing) when lesioning was performed 1 day after training. The effect of lesioning became less pronounced as the period between training and lesioning lengthened. **(B)** Injection of lidocaine, an anesthetic that blocks action potentials, into the anterior cingulate cortex (ACC; red trace), reduced contextual fear memory compared with controls (blue trace) when drug administration and testing were performed 18 or 36 days, but not 1 or 3 days, after training. This finding suggests that the ACC is required for recall of remote memory but not recent memory. **(C)** Compared with controls that expressed a fluorescent protein, optogenetic silencing of mouse hippocampal neurons expressing a modified halorhodopsin significantly reduced contextual fear memory if silencing (light ON) was precisely timed with testing (precise group). If silencing occurred 30 minutes prior to as well as during the testing (prolonged group), the reduction in fear memory disappeared. This suggests that a compensatory mechanism was used for fear memory if hippocampal neurons were silenced under the prolonged condition. (A, adapted from Kim JJ & Fanselow MS [1992] *Science* 256:675–677. With permission from AAAS; B, adapted from Frankland PW, Bontempi B, Talton LE et al. [2004] *Science* 304:881–883. With permission from AAAS; C, adapted from Goshen I, Brodsky M, Prakash R et al. [2011] *Cell* 147:678–689. With permission from Elsevier Inc.)

involved in remote memory. Inactivation of ACC during testing caused significant loss of fear memory when testing occurred 18 or 36 days, but not 1 or 3 days, after initial training (Figure 10–39B). Interestingly, human fMRI studies consistently find activation of the frontal cortex, including the ACC, during different kinds of memory recall.

In the third experiment, researchers expressed halorhodopsin in hippocampal CA1 neurons and then used optogenetic manipulation to reversibly silence these cells (see Section 13.25) during remote contextual fear memory recall. Surprisingly, as shown on the left in Figure 10–39C, acute silencing of hippocampal CA1 neurons during testing 28 days after training severely reduced fear memory, suggesting that the hippocampus was indeed required for remote memory recall. (This acute silencing did not cause detectable nonspecific effects on global brain activity and did not interfere with a hippocampus-independent memory.) This result seemingly contradicted prior studies (see Figure 10–39A); however, when the hippocampal neurons were silenced during the 30 minutes prior to testing as well as during the testing, remote memory was intact (Figure 10–39C, right), consistent with previous pharmacological or lesion experiments in which the hippocampus had been inactivated for a longer period or permanently. In related experiments, optogenetic silencing of the ACC confirmed its requirement in remote fear memory. These findings suggest that (1) retrieval of remote memory may normally involve dynamic interplay between the hippocampus and neocortex, and (2) remote memory can be retrieved by more than one mechanism, such that if the hippocampus is inactivated, readjustment can be made within 30 minutes to allow the cortical network to perform the task. Such redundancy and flexibility likely increase the robustness of memory systems.

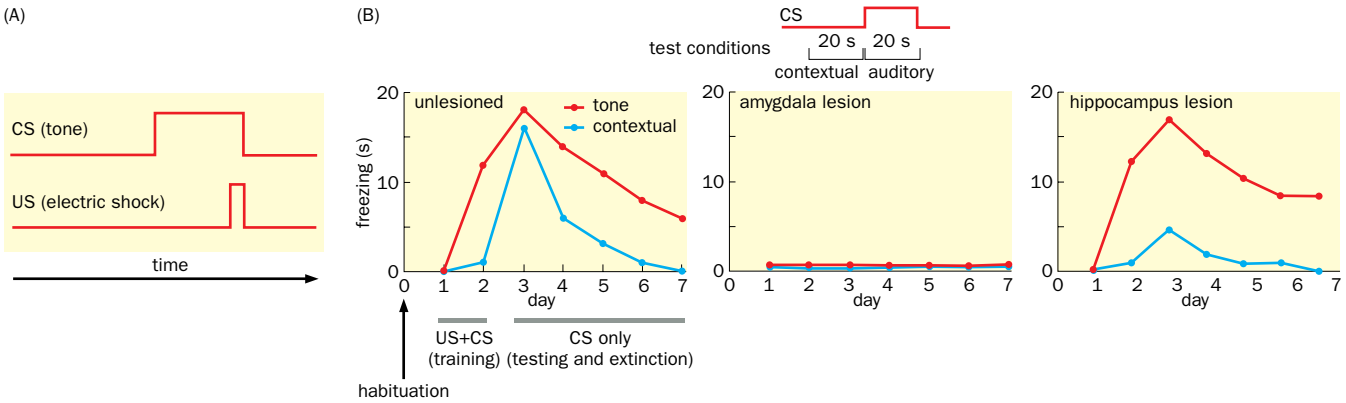

Figure 10–40 Both auditory and contextual fear conditioning require the amygdala. (A) Schematic of auditory fear conditioning, a form of classical conditioning in which the tone (~20 s) serves as a conditioned stimulus (CS) and the electric shock (~0.5–1 s) serves as an unconditioned stimulus (US) that co-terminates with the CS. **(B)** Left, learning curve as measured by average time spent freezing within a 20-s period (*y* axis) for control rats. On day 0, rats were introduced into the conditioning chamber without shock for habituation. On days 1 and 2, a 20-s tone was paired with a 0.5-s electric shock at the end of the tone, with two pairings per day. On days 3–7, only the tone was presented in the same conditioning chamber. As shown in the schematic above, contextual conditioning was measured as the freezing time during the 20 s immediately prior to the CS (tone) onset, whereas auditory conditioning was measured during the 20-s tone period. Middle, lesioning of the amygdala prior to training disrupted both contextual and auditory conditioning. Right, lesioning of the hippocampus disrupted contextual conditioning without affecting auditory conditioning. (B, adapted from Phillips RG & LeDoux JE [1992] *Behav Neurosci* 106:274–285. With permission from the American Psychological Association Inc.)

10.23 The amygdala plays a central role in fear conditioning

In the previous sections, we studied contextual fear conditioning as a hippocampus-dependent form of fear conditioning. There is also a different form of fear conditioning, called **cued fear conditioning**, in which an electric shock is applied at the end of a cue presentation during training. The most commonly used cue is a sound, in which case cued fear conditioning is called **auditory fear conditioning** (Figure 10–40A). Auditory fear conditioning is a form of classical conditioning (see Section 10.14); the shock and the tone serve as the US and CS, respectively. Lesion studies indicate that while contextual fear conditioning is dependent on the hippocampus, auditory fear conditioning is not. However, both forms of conditioning depend on the amygdala (Figure 10–40B). When compared side-by-side in control animals (Figure 10–40B, left panel), auditory conditioning was more rapidly acquired during training than contextual conditioning and was more resistant to extinction during the testing phase. After training, animals conditioned in one context to associate a tone with an electric shock also exhibit a robust fear response to the CS (tone) in a different context. These studies suggest that the amygdala is the location where the association of auditory CS and US occurs, whereas the hippocampus contributes specifically to the contextual aspect of the fear conditioning.

Extensive anatomical, physiological, and perturbation studies have delineated circuit diagrams that underlie fear conditioning (Figure 10–41). The amygdala complex consists of several major divisions: the lateral amygdala, the basal amygdala (collectively constituting the **basolateral amygdala**), and the **central amygdala**. (Note that this complex is adjacent to but distinct from the olfactory amygdala, which includes the cortical amygdala and medial amygdala and which receives direct input from mitral cells of the main and accessory olfactory systems; see Figure 9–32.) The central amygdala is the output site of the amygdala complex for fear and defensive responses. It sends descending projections to distinct sites in the hypothalamus and brainstem to regulate behavioral output (for example, freezing), autonomic nervous system response (for example, increased blood pressure), and neuroendocrine response (for example, stress hormone production). In auditory fear conditioning, information about the tone (CS) reaches the lateral amygdala via a direct pathway from the auditory thalamic nuclei, as well as

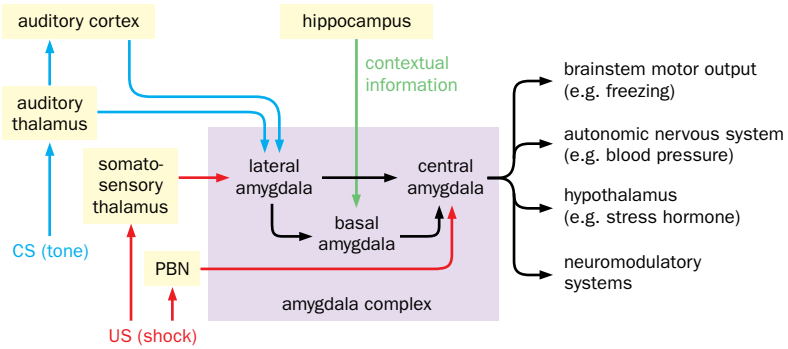

Figure 10–41 Circuit diagrams for fear conditioning. The tone signal (CS, blue) can reach the lateral amygdala directly from the auditory thalamus or indirectly from the auditory cortex. The shock signal (US, red) can reach the lateral amygdala via the somatosensory thalamus, or can reach the central amygdala directly through the pain pathway via the parabrachial nucleus (PBN). Contextual information from the hippocampus (green) enters through the basal amygdala. Within the amygdala complex, information flows from the lateral nucleus to the central nucleus either directly or via the basal amygdala. The central amygdala provides the output to brainstem and hypothalamus targets to regulate behavioral, autonomic, endocrine, and neuromodulatory systems. (See LeDoux JE [2000] *Annu Rev Neurosci* 23:155–184 and Pape HC & Pare D [2010] *Physiol Rev* 90:419–463.)

via an indirect pathway from high-order auditory cortex. Information can be sent by lateral amygdala neurons to the central amygdala either directly or indirectly via the basal amygdala. The foot shock (US) also reaches the amygdala by multiple pathways, including projections from the somatosensory thalamic nuclei to the lateral amygdala and from the pain pathway through the parabrachial nucleus (PBN) to the central amygdala (see also Figure 6–70B). In contextual fear conditioning, contextual input from the hippocampus enters the amygdala complex via the basal amygdala (Figure 10–41).

What is the neural basis of behavioral conditioning? The general framework is that the simultaneous presence of the CS and the US during training strengthens, through a Hebbian mechanism, the connection between neurons that represent the CS and neurons that produce fear response, such that the CS alone can elicit a fear response after the conditioning. The best evidence supporting this model has thus far come from studies of the synapses that connect auditory thalamic input neurons and excitatory projection neurons of the lateral amygdala, where strong correlations have been established between auditory fear conditioning and LTP of these synapses. For example, it has been shown that (1) auditory conditioning can enhance the response of lateral amygdala neurons to the shock-associated tone; (2) LTP can be induced by pairing presynaptic stimulation of thalamic axons with postsynaptic depolarization of lateral amygdala neurons (this postsynaptic depolarization can be achieved by the US during fear conditioning); and (3) LTP and fear conditioning share a common set of molecular mechanisms, including dependence on the postsynaptic NMDA receptor, CaMKII auto-phosphorylation, and AMPA receptor trafficking. This is very much analogous to the relationship between spatial learning and hippocampal LTP discussed earlier in this chapter. As in the hippocampus, plasticity can occur at multiple sites in the amygdala (**Box 10–4**).

Research on fear conditioning in rodent models has revealed the amygdala to be a center for emotional memory and for processing emotion-related signals, which has also been substantiated by human studies. For instance, fMRI studies have shown that the amygdala can be activated by stimuli that are emotionally negative (such as a fearful face) or emotionally positive (such as a pleasant picture). Similar to the rodent fear-conditioning model, the amygdala of human subjects is also activated by presentation of an image (for example, a blue square) that has previously been associated with mild electric shock to the wrist (**Figure 10–42**) but not by presentation of a comparable image (for example, a yellow circle) that has not been paired with a shock. In this fear-conditioning paradigm, patients with amygdala lesions do not exhibit physiological responses that normal subjects do, such as sweating, which can be measured by changes in skin conductance and which is due to activation of the sympathetic system as part of the fear response. Interestingly, the amygdala-lesioned patients remain aware of the explicit association of the CS (the blue square) and the US (mild electric shock), suggesting that amygdala-dependent fear conditioning utilizes a form of implicit memory distinct from the explicit memory that remains intact in these patients.

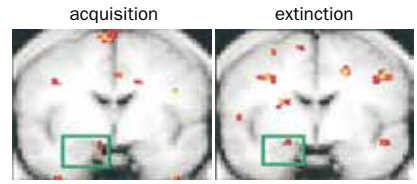

Figure 10–42 Acquisition and extinction of fear condition activate the amygdala in humans. Average fMRI images of 10 healthy human subjects during the acquisition (left) and extinction (right) phases of fear conditioning. The green box in each image highlights the right amygdala, showing activation (yellow to red colors) of the amygdala. During the acquisition phase, subjects were exposed to a blue square image paired with a mild electrical shock. During the extinction phase, previously trained subjects were exposed to a blue square without the shock. (From LaBar KS, Gatenby JC, Gore JC et al. [1998] *Neuron* 20:937–945. With permission from Elsevier Inc.)

Box 10–4: Microcircuits of the central amygdala

As is evident from the circuit diagram of fear conditioning (see Figure 10–41), CS and US can converge at multiple potential sites, where synaptic plasticity can contribute to fear conditioning. Indeed, each amygdala nucleus contains a heterogeneous population of neuronal types that have different properties and connections. For instance, the central amygdala can be divided into a lateral compartment (CEl), which receives input from the lateral and basal amygdala, and a medial compartment (CEm), which sends output to the brainstem to activate the freezing behavior. The lateral compartment further contains two separate populations of GABAergic neurons, CEl_{ON} and CEl_{OFF} cells, which mutually inhibit each other (**Figure 10–43**).

CEl_{ON} cells mostly restrict their projections within the CEl, and most projections from the CEl to the CEm are from CEl_{OFF} cells. After fear conditioning, presynaptic potentiation of excitatory input from the lateral and basal amygdala results in potentiation of the response of CEl_{ON} cells to tone stimuli. The responses of CEl_{OFF} cells to sound stimuli are depressed after fear conditioning due to presynaptic depression of excitatory input. Thus, the fear conditioning circuit in the amygdala contains a series of plastic synapses. Fear conditioning (1) enhances the excitatory input from the lateral and basal amygdala to the central amygdala, (2) enhances excitatory transmission to CEl_{ON} cells so that they are more activated by the tone, and (3) reduces excitatory transmission to CEl_{OFF} cells while increasing inhibition of CEl_{OFF} cells via activation of CEl_{ON} cells, so that the CEl_{OFF} cells become less activated by the tone. In combination, these changes result in a net disinhibition of the CEm output neurons (Figure 10–43), thus causing a fear response to the tone.

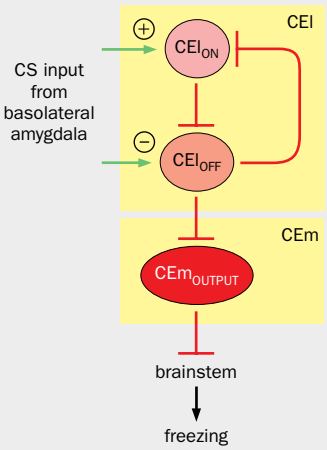

Figure 10–43 Microcircuits of the central amygdala. The central amygdala consists of a lateral compartment (CEl) and a medial compartment (CEm). Within the CEl, two separate GABAergic neuronal populations have been identified. CEl_{ON} neurons acquire a potentiated conditioned stimulus (CS) response after fear conditioning because of presynaptic potentiation (+) and less inhibition from CEl_{OFF} neurons; CEl_{OFF} neurons acquire a depressed CS response after fear conditioning because of presynaptic depression (−) and more inhibition from CEl_{ON} neurons. Thus, after fear conditioning, the CS signal from the basolateral amygdala preferentially activates CEl_{ON} neurons, which inhibit CEl_{OFF} neurons that normally inhibit CEm output neurons. This disinhibition ultimately activates CEm output neurons and the freezing response to the tone. Green, excitatory pathway; red, inhibitory pathway. (Adapted from Haubensak W, Kunwar PS, Cai H et al. [2010] *Nature* 468:270–276. With permission from Macmillan Publishers Inc.; See also Ciocchi S, Herry C, Grenier F et al. [2010] *Nature* 468:277–281 and Li H, Penzo MA, Taniguchi H et al. [2013] *Nat Neurosci* 16:332–339.)

10.24 Dopamine plays a key role in reward-based learning

In Section 10.14 we discussed the 'law of effect' in the context of operant conditioning: behaviors that are followed by a reward will be repeated, whereas behaviors that are followed by a punishment will be diminished. What is the neural basis for this effect? An interesting set of experiments to identify brain areas responsible for reward utilized electrical self-stimulation in an operant conditioning paradigm (**Figure 10–44**A). An electrode was implanted in a specific area of a rat's brain. Whenever the rat pressed a lever in an operant chamber, the electric circuit became connected and current from the electrode excited nearby neurons or axonal projections. When the electrode was placed in certain areas of the brain presumed to signal reward, the rat would keep pressing the lever in order to receive more electrical stimulations. When the stimulation was sufficiently strong, rats would keep pressing the lever at the expense of eating, drinking, or having sex; they also withstood substantial foot shock (an aversive stimulus) in order to receive more electrical stimulations (Figure 10–44B). Where are these presumed reward centers, the stimulation of which can override an animal's basic drives?

Systematic mapping revealed that the most effective self-stimulation sites coincide with midbrain dopamine neurons in the **ventral tegmental area** (**VTA**)

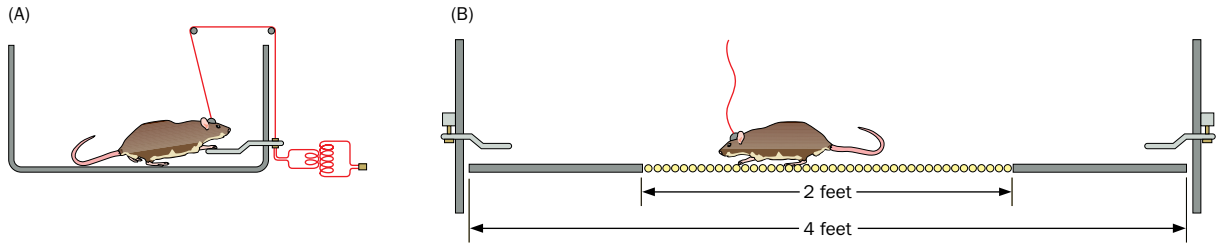

Figure 10–44 Electrical self-stimulation. (A) Design of the experiment. When the rat presses the lever, the electrode implanted in its brain connects to a source of current so that the neurons or axon bundles near the electrode tip become excited. If the excited neurons or axons signal reward, the rat will keep pressing the lever. **(B)** In this experiment, after the rat receives rewarding electric stimulation on one side of the arena, it must run across the center grid to the other side to receive more stimulation. After that rat had learned the task, electrical shock was applied in the central portion of the arena. If the electrode was implanted in the reward centers, rats withstood more foot shock when crossing the grid to receive electrical brain stimulation than they did when crossing the grid for food after 24 hours of food deprivation. (Adapted from Olds J [1958] *Science* 127:315–324. With permission from AAAS.)

and **substantia nigra pars compacta** (**SNc**) and their projections to the striatum (see Figure 8–22), in particular to the ventral striatum, also called the **nucleus accumbens**. The involvement of dopamine neurons was further supported by additional experiments. For example, lesions of dopamine neurons or their forebrain projections abolished the self-stimulation behavior, as did application of drugs that block dopamine synthesis. The addition of dopamine agonists in the nucleus accumbens, which bypassed the blockade of dopamine synthesis, restored reward behavior. Indeed, as we will learn in Chapter 11, most drugs of abuse act by enhancing the activity of midbrain dopamine neurons.

How does dopamine regulate reward and modify behavior? An important insight came from *in vivo* recording of dopamine neurons in alert monkeys performing behavioral tasks. Dopamine neurons normally fire in two different modes: in the **tonic** mode, dopamine neurons maintain a low and relatively constant basal firing rate; in the **phasic** mode, they fire in bursts in response to specific stimuli. In a specific example, a monkey was trained to touch a lever after a cue light was turned on. Following the cued lever touch, the monkey would receive a juice reward. Prior to and during the initial phase of training, dopamine neurons exhibited phasic firing in response to the juice reward. However, after extensive training, phasic firing was triggered by the cue (light on) that predicted the reward, but not by the actual reward delivery itself. After training, in trials in which the reward was omitted, the tonic firing rate was depressed at the time when reward was expected (**Figure 10–45**A). These data suggest that rather than signaling the reward per se, phasic firing of dopamine neurons signals a **reward prediction error**, that is, the difference between the actual reward and the predicted reward. Before training, reward came unexpectedly, resulting in a positive reward prediction error that triggered phasic firing. After the training, reward was predicted by the sensory cue, such that the sensory cue became the unexpected reward signal; when reward was actually delivered, it was fully predicted, and hence there was no reward prediction error and no phasic firing of dopamine neurons; when reward was omitted, a negative prediction error resulted in depression of the tonic firing.

Various learning theories have been proposed to account for these remarkable experimental findings. Let's first discuss an abstract model for reward-based learning (Figure 10–45B), and then place it in the context of a realistic dopamine circuit. In this abstract model, the connection between a signal neuron and a response neuron has an adjustable strength (ω). Through a negative feedback loop, the response magnitude (ωS) is compared to a reward signal (R). This difference, or reward prediction error carried by the dopamine neuron (blue in Figure 10–45B), is used to modify ω. Before training, ω is small such that the reward prediction error ($R - \omega S$) is large. Dopamine neurons fire and send a large signal to increase ω. As learning proceeds, ω increases until $R - \omega S = 0$; at that point, dopamine-neuron-mediated learning is accomplished and the dopamine

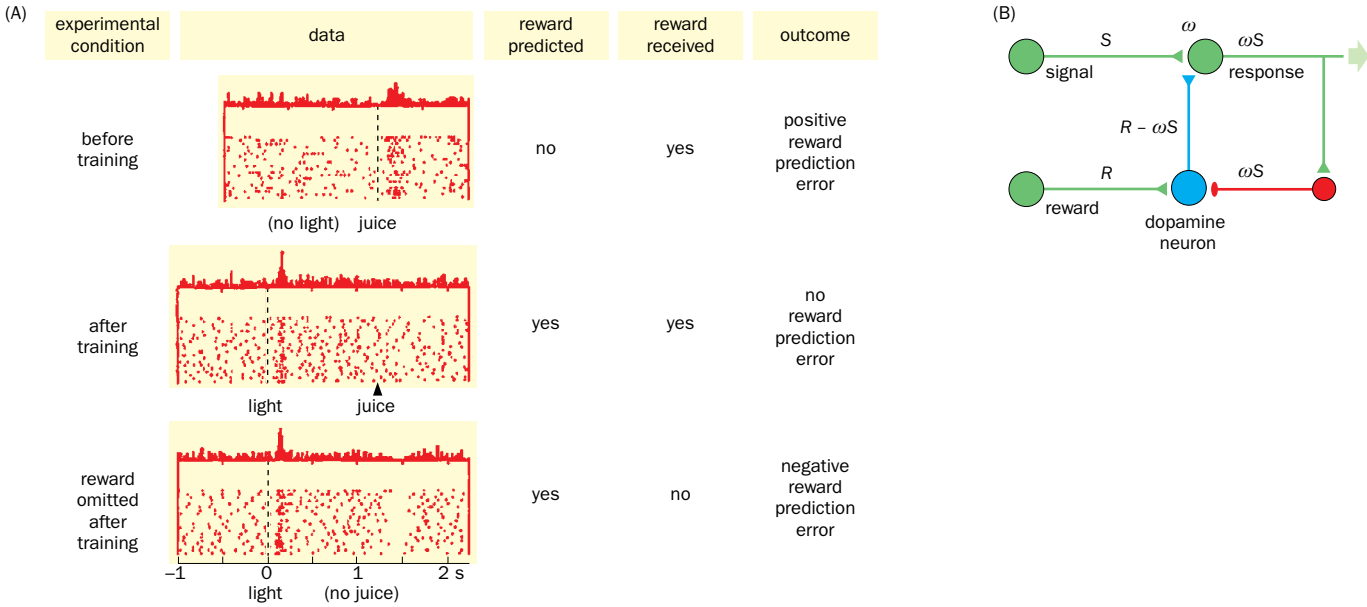

Figure 10–45 Dopamine neurons, reward prediction error, and reinforcement-based learning. (A) *In vivo* single-unit recordings of a midbrain dopamine neuron of a monkey trained to associate light onset with a reward (a drop of juice). Within each of the three blocks, each row is a separate trial and each vertical bar is an action potential. Above the individual trials is a histogram that combines the firing rates from all trials. Top, prior to training, phasic firing was triggered by reward (juice) delivery (vertical dashed line). Middle, after training, phasic firing was triggered by light onset (vertical dashed line) but not by reward delivery (arrowhead). Bottom, in trials when reward was omitted after training, tonic firing was depressed around the time reward was expected. Individual trials are aligned with reward delivery (top) or light onset (middle and bottom). Phasic firing of this dopamine neuron can be interpreted to signal the difference between the actual and expected reward, as summarized on the right. **(B)** An abstract circuit model for reward-based learning. A signal neuron produces a signal with the magnitude *S*, and connects with a response neuron through a synapse whose strength ω can be adjusted, resulting in a response whose magnitude is the product of ω and *S*. In addition to sending information to downstream circuits (arrow), the response is also transmitted to a feedback inhibitory neuron (red), which in turn sends output to a dopamine neuron (blue). The dopamine neuron also receives an excitatory input that delivers a reward signal with a magnitude of *R*. Thus, the dopamine neuron positively adjusts ω with an output magnitude of $R - \omega S$ (for simplicity we assume that synaptic transmission is faithful and integration is linear). Before training, ω is small and $R - \omega S$ is large, resulting in a large magnitude of dopamine release to increase ω. As training proceeds, ω increases and $R - \omega S$ decreases; when $R - \omega S$ becomes zero, training is accomplished. Note that although an excitatory neuron (green) is used as the response neuron, in the midbrain dopamine circuit, the response neuron is GABAergic spiny projection neurons (SPNs). SPNs can either signal directly to dopamine neurons to deliver ωS, or through an intermediate GABAergic neuron to signal to the feedback inhibitory neuron. (A, adapted from Schultz W, Dayan P & Montague R [1997] *Science* 275:1593–1599. With permission from AAAS; B, adapted from Schultz W & Dickinson A [2000] *Annu Rev Neurosci* 23:473–500. With permission from Annual Reviews.)

neurons no longer exhibit phasic firing in response to the reward (as in the middle panel of Figure 10–45A).

As discussed in Section 8.9, a major projection region for midbrain dopamine neurons is the striatum, with VTA dopamine neurons projecting preferentially to the nucleus accumbens, and SNc dopamine neurons projecting to the rest of the striatum (see Figure 8–22). There, dopamine release regulates the strength of connections between the cortical and thalamic excitatory input and the spiny projection neurons (SPNs). Thus, the signal neuron in Figure 10–45B is equivalent to the cortical and thalamic projection neurons into the striatum. The response neuron is equivalent to the GABAergic SPNs. Some SPNs connect directly to dopamine neurons, thus could serve both as response neurons and feedback neurons (red in Figure 10–45B). Other candidates to carry feedback signals are the midbrain GABAergic neurons, which receive striatal input and inhibit dopamine neurons. (Because SPNs are GABAergic themselves, another GABAergic neuron is required to deliver the ωS signal with a positive sign to these midbrain GABAergic neurons that in turn synapse onto dopamine neurons.) The dopamine neurons additionally receive input from sensory systems signaling the reward, such as juice in the experiment discussed in Figure 10–45A, or pleasure derived from sex in the example of pair bonding discussed in Section 9.24. As supporting evidence for this model, dopamine has been shown to regulate various forms of plasticity at the

cortical/thalamic → SPN synapse in *in vitro* slices. Whereas dopamine projection to the ventral striatum is associated with reward- and motivation-based learning, dopamine-guided synaptic plasticity in the dorsal striatum facilitates procedural learning and habit formation, likely through a similar circuit mechanism. Much remains to be learned about how striatal circuits are organized into subcircuits that carry out these distinct functions and whether striatal synaptic plasticity is causally linked with various forms of reinforcement-based learning.

Although midbrain dopamine neurons have been demonstrated to represent (that is, fire in response to) reward prediction errors in primates and rodents, recent studies have also identified heterogeneity among dopamine neurons, with some signaling aversive stimuli and others signaling salience of motivational stimuli; dopamine neurons in this latter group are activated by both strong appetitive and strong aversive signals and respond poorly to weak appetitive and weak aversive signals. This heterogeneity of dopamine neuron function may be accounted for by the heterogeneity of input to and output from different dopamine neurons. For example, according to a recent study, VTA dopamine neurons that project to the nucleus accumbens tend to signal the presence of appetitive stimuli, whereas those that project to the prefrontal cortex tend to signal the presence of aversive stimuli. Just as reward-based learning can increase the frequency of actions that lead to reward, aversion-based learning can reduce the frequency of actions that lead to punishment. Indeed, conceptually similar circuit designs can be applied to reinforcement-based learning that does not involve dopamine at all, such as the cerebellum-based motor learning (see Figure 8–21B).

10.25 Early experience can leave behind long-lasting memory traces to facilitate adult learning

We have seen that learning can occur and memory can be stored in neural circuits in many parts of the brain, including the hippocampus, cerebral cortex, amygdala, striatum, and cerebellum. Remarkably, memory can even be formed by artificially activating random populations of cortical neurons (**Box 10–5**). In the final section of this chapter, we further broaden the scope of learning and memory to developmental and structural plasticity by returning to the story of the barn owl introduced at the beginning of Chapter 1, integrating what we have learned about the organization and wiring of the brain in the intervening chapters.

Box 10–5: Memory can be formed by the activation of random populations of cortical neurons

Recent advances in genetically targeting specific neuronal populations *in vivo* for precise control of their activity has contributed much to our understanding of the neural basis of brain function and behavior. In particular, we have seen examples of the application of optogenetic approaches for dissecting memory circuits in model species ranging from flies (see Figure 10–29B) to mice (see Figure 10–36 and Figure 10–39C). Photostimulation of channelrhodopsin (ChR2)-expressing neurons has also been used to probe whether a random population of neurons can be associated with reward or punishment such that reactivation of those neurons changes the behavior of the animal. We discuss two examples of this approach below.

In the first example, a random population of piriform cortical neurons in mice was transduced with an adeno-associated virus to express ChR2, such that they fire action potentials in response to photostimulation. During training, the mouse was allowed to freely move in an arena, but whenever it moved to one side of the arena, foot shock was applied along with photostimulation. This elicited a robust flight response—mice ran quickly to the other side of the arena where no foot shock was applied. After training, photostimulation alone could elicit the flight response (**Figure 10–46**A). Thus, activation of a random population of piriform cortical neurons (~500) could serve as an effective CS with which the animal can be trained to associate a US (the shock) and subsequently to elicit a robust conditioned response. In separate experiments, photostimulation of ChR2-expressing piriform neurons was shown to also effectively serve as a CS for reward; indeed, activation of the same random population of ChR2-expressing neurons can be sequentially used as a CS for reward and subsequently as a CS for electrical shock.

(*Continued*)

Box 10–5: Memory can be formed by the activation of random populations of cortical neurons

Because the spatial representation of odor in the piriform cortex has no discernible order, these experiments have been interpreted as a support for the hypothesis that this brain area is a random network whose connectivity is sculpted by individual experience (see Sections 6.10 and 6.16). However, a second example shows that the ability of researchers to influence behavior by activating a random population of neurons is not restricted to the piriform cortex. In this experiment, a random set of layer 2/3 neurons in the mouse barrel cortex (see Box 5–3) was electroporated *in utero* to introduce a functional ChR2 gene, and thirsty mice were trained to associate photostimulation of ChR2-expressing neurons with a water reward at one of two choice ports (Figure 10–46B, left). In this task, the snout of the mouse must enter the center port in order for a drop of water to be delivered to the left or the right port. While the mouse's snout was at the center port, the mouse either received photostimulation, after which water would be delivered to the left port, or did not receive photostimulation, after which water would be delivered to the right port. After training, mice could report photostimulation reliably by choosing the correct port for the water reward. The effectiveness of photostimulation depended on the number of ChR2-expressing neurons and the strength and the duration of photostimulation (Figure 10–46B, right). Single action potentials (elicited by 1-ms photostimulation) in about 300 ChR2-expressing layer 2/3 neurons of the barrel cortex served as a sufficient cue to bias the mouse to the reward port.

These examples highlight the nervous system's remarkable plasticity for learning: given sufficient strength of stimulation and sufficient training, association can be established between reward or punishment and the activity of random populations of neurons in different brain areas. It is likely that these photostimulations mimic the perception of a smell or a touch, so that the animals use the normal neural pathways that process olfactory or somatosensory information to associate the photostimulation with punishment or reward. These experiments also offer valuable estimates as to the number of cortical neurons that must be activated and the number of action potentials that must be fired in order for animals to associate neuronal activity with reward or punishment and consequently alter their behavior.

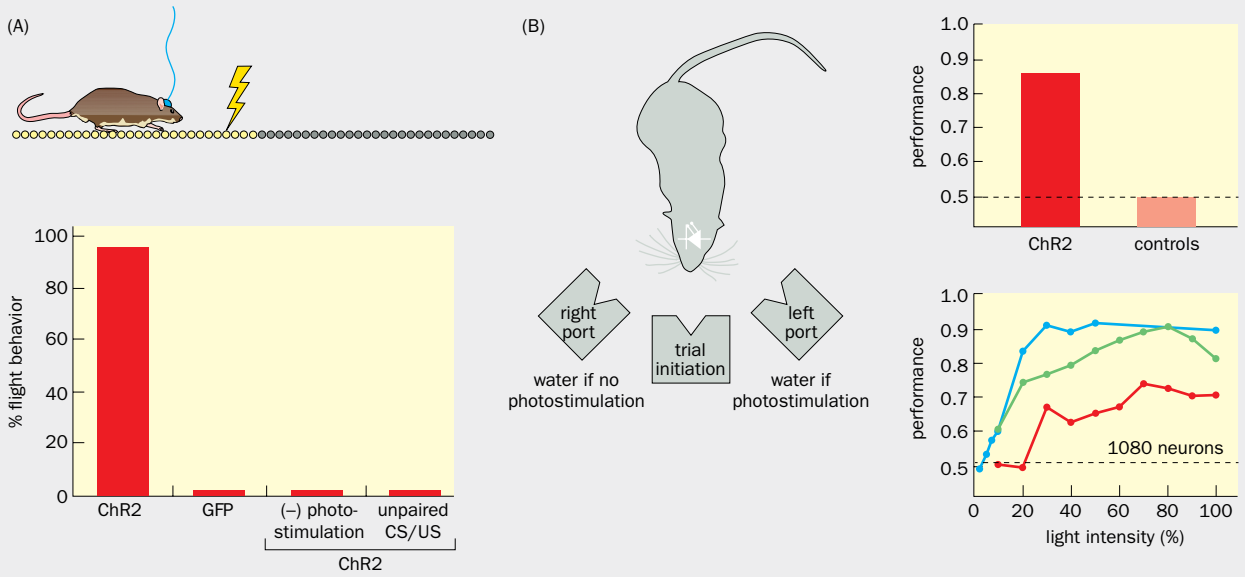

Figure 10–46 Forming a memory by activating a random population of cortical neurons. (A) Top, schematic of the experimental design. During training, when the mouse moved to the left side of the chamber, it received an electrical shock (yellow) while its piriform cortex, which contained channelrhodopsin (ChR2)-expressing neurons, was photostimulated. After two training sessions (each of which consisted of 10 pairings), mice readily exhibited flight behavior in response to photostimulation alone (bottom, left bar). In negative control groups, mice did not exhibit flight behavior when green fluorescent protein (GFP) instead of ChR2 was expressed, when photostimulation was omitted during training, or when photostimulation was not paired with shock. **(B)** Left, experimental design. The mouse was trained to place its snout at the center port to initiate the trial. Water was delivered to the left or the right port depending on whether the mouse received photostimulation or not during the trial. Right, After 4–7 sessions of trials (200–800 trials per session), ChR2-expressing mice reliably reported photostimulation by making proper port choices compared to controls that did not express ChR2; the dotted line indicates port selection at chance levels. Performance on the *y* axis is the ratio of the number of corrected trials, which include turning left in photostimulation trials and turning right in no-photostimulation trials, over the total number of trials. Performance increased as the number of ChR2 neurons increased (shown at bottom is an example of 1080 ChR2-expressing neurons), with light intensity, and with the number of 1-ms pulses of light (red, 1 pulse; green, 2 pulses; blue, 5 pulses; separate experiments indicated that each pulse elicited at most one action potential in ChR2-expressing neuron at 100% light intensity). (A, adapted from Choi GB, Stettler DD, Kallman BR et al. [2011] *Cell* 146:1004–1015. With permission from Elsevier Inc.; B, adapted from Huber D, Petreanu L, Ghitani N et al. [2008] *Nature* 451:61–64. With permission from Macmillan Publishers Ltd.)

Recall that the owl's auditory map can adapt to match a visual map altered by wearing prisms and that this ability declines with age (see Section 1.3). Recall further that if an owl had an earlier experience of auditory map adjustment, its auditory map re-adapted to an altered visual map more easily in adulthood (see Figure 1–7). What is the neural basis for these phenomena? As we learned in Chapter 6, neurons in the nucleus laminaris of the owl's brainstem form a map that identifies sound locations on the horizontal plane based on interaural time differences (ITDs) (see Figure 6–55). This ITD map projects topographically to the central nucleus of the inferior colliculus (ICC). ICC axons project further to the external nucleus of the inferior colliculus (ICX). ICX neurons then project to the optic tectum, where integration of auditory and visual information occurs in a topographically aligned manner (**Figure 10–47**A, top). Anatomical tracing studies indicate that in juvenile prism-reared owls, ICC axonal projections to the ICX expand in the direction that matches the altered visual map in the optic tectum (Figure 10–47A, bottom). The expanded axons bear synaptic terminals and likely make functional connections with the postsynaptic neurons in the new topographic location, thus realigning the auditory map with that prism-altered visual map. Although the mechanisms underlying this axonal expansion have not been examined in detail, it is likely that the connections made by the expanded axons are stabilized by synchronous firing with postsynaptic neurons that process altered visual information, similar to the Hebbian-based synaptic strengthening in visual system wiring that we discussed in Chapter 5.

As discussed in Section 1.3, when the prisms were removed from the juvenile prism-reared owls, the auditory map was restored to normal so that it was realigned with the normal visual map. Indeed, ICC neurons still maintain their normal axonal projections in the ICX during the prism-rearing period (Figure 10-46A bottom); these normal projections, which become topographically mismatched during the prism-wearing period, receive preferential GABAergic inhibition such that they are preferentially silenced. The persistence of these normal connections during prism rearing may account for the rapid restoration of the normal auditory map after the prisms are removed. The ICC axons that expanded into the topographically abnormal area of the ICX due to juvenile prism rearing are also maintained into adulthood (Figure 10–47B), well after prism removal and complete

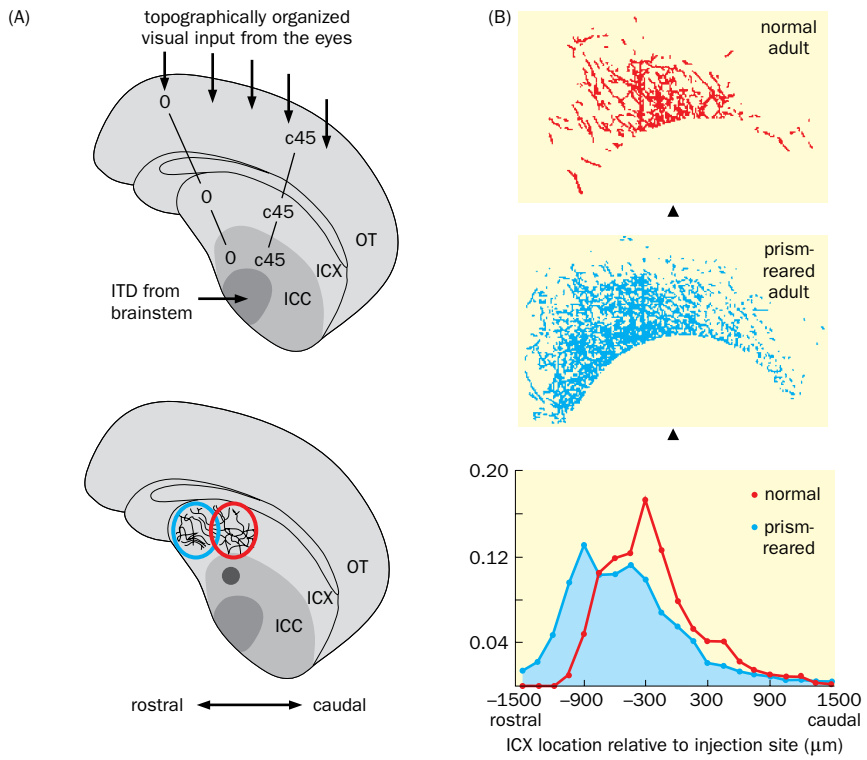

Figure 10–47 Adaptive axonal expansion in the inferior colliculus and auditory map adjustment in juvenile and adult owls. (A) Top, representation of auditory and visual information in the owl's brain. Brainstem inputs to the central (ICC) and external (ICX) nuclei of the inferior colliculus are topographically organized according to interaural time differences (ITDs): 0 represents ITD = 0, c45 represents the contralateral side leading by 45 μs. ICX neurons project to the optic tectum (OT), where they align with topographically organized visual input. Bottom, axons of ICC neurons from the area indicated by the dark dot normally project to a topographically appropriate area of the ICX (red circle), but expand rostrally (blue circle) to match with a visual map altered by prism experience in the juvenile owl. **(B)** Top and middle, anterograde tracing was used to examine ICC → ICX axonal projection in normal (top) and prism-reared (middle) adult owls. Arrowheads indicate the anterograde tracer injection site in the ICC. Bottom, normalized distribution of axonal projections in normal and prism-reared owls. The significant rostral shift in adult owls due to juvenile experience with wearing prisms (prism-reared) likely accounts for the rapid auditory map adjustment during a second prism experience in adulthood (see Figure 1–7B). (Adapted from Linkenhoker BA, con der Ohe CG & Knudsen EI [2005] *Nat Neurosci* 8:93–98. With permission from Macmillan Publishers Ltd. See also DeBello WM, Feldman DE & Knudsen EI [2001] *J Neurosci* 21:3161–3174.)

restoration of the normal auditory map as assayed by behavior. (It is unknown how the activity of these expanded axons is silenced after prism removal so that they do not interfere with behavior.) Thus, adaptive expansion of ICC axons as a consequence of juvenile prism rearing leaves behind an anatomical trace that likely facilitates the readjustment of the auditory map in response to a similar visual displacement event later in adulthood.

A conceptually similar experiment examined traces of structural change in mammalian visual cortical neurons in response to monocular deprivation. As we learned in Chapter 5, monocular deprivation within a critical developmental period has a profound effect on wiring of the visual cortex. In mice, for example, transient monocular deprivation for a few days during the critical period can modify the binocular area of the visual cortex, significantly shifting the relative representation of visual input from the two eyes in favor of the open eye, as assayed by intrinsic signal imaging of cortical responses to visual stimulation (see Figure 4–42). If the deprivation period is short, the normal balance of representation of the two eyes is restored after binocular vision is restored. Monocular deprivation in adult mice can also shift ocular dominance in response to a longer duration of monocular deprivation. Interestingly, ocular dominance shifts in response to monocular deprivation are more rapid in adult mice that previously experienced monocular deprivation than in those experiencing monocular deprivation for the first time, analogous to the finding in the owl.

To examine a structural basis for this monocular-deprivation-induced plasticity, repeated two-photon microscopic imaging was carried out through a window implanted in the binocular area of the mouse visual cortex (**Figure 10–48**A). The dendritic spines of pyramidal neurons were observed and quantified to determine spine gains and losses over time (Figure 10–48B). It was found that the first monocular deprivation resulted in significant addition of new spines, a proxy for new synapse formation (see Section 10.13); these spines are subsequently retained. The second deprivation, which caused a more rapid ocular dominance shift, did not change the spine density (Figure 10–48C). A likely interpretation of these data is that the anatomical traces left behind by the first monocular deprivation—the new spines—were reused for the ocular dominance shift during the second monocular deprivation, thus facilitating the neuron's more rapid adaptation. As with the owl experiments, questions remain as to whether and how the activities of the new spines are silenced during the intervening period between the first and second instances of monocular deprivation, such that the synaptic connections enabled by the new spines do not interfere with binocular vision during the intervening period.

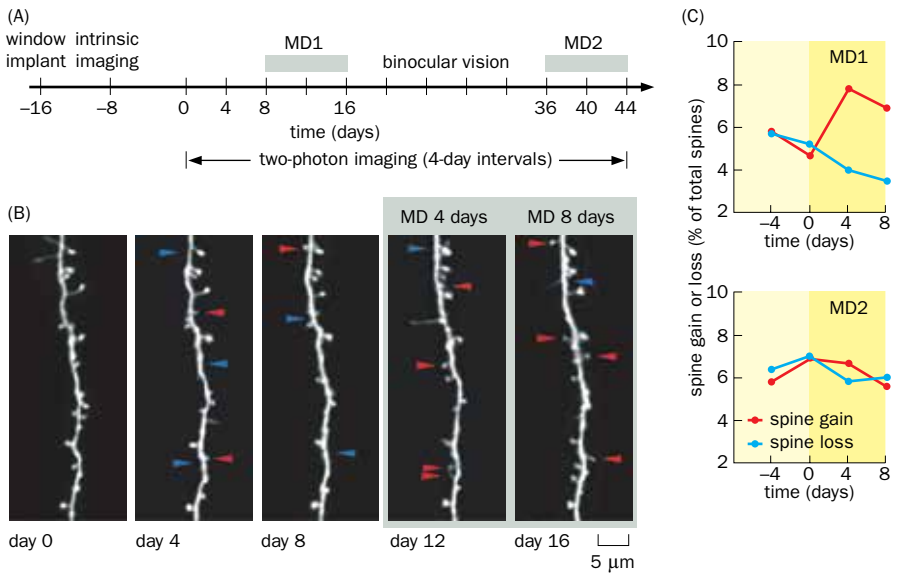

Figure 10–48 Spine dynamics in the adult mouse visual cortex in response to monocular deprivation. (A) Experimental protocol. After window implantation, intrinsic signal imaging (see Figure 4–42B) was carried out to identify the binocular region. Repeated two-photon imaging of dendrites from a transgenic mouse that expresses GFP in a sparse population of neurons (see Section 13.16) in the binocular regions was then carried out every 4 days, covering two 8-day periods of monocular deprivation (MD1 and MD2). **(B)** Representative images of the same apical dendritic segment of a layer 5 pyramidal neuron. Blue and red arrowheads indicate spine loss and spine gain, respectively; these changes were inferred by comparing each image to the image acquired 4 days earlier. **(C)** A significant increase in spine gain is detected only during the MD1 (top) but not the MD2 (bottom), suggesting that new spines gained during MD1 may be used to adjust ocular dominance during MD2. (Adapted from Hofer SB, Mrsic-Flogel TD, Bonhoeffer T et al. [2009] *Nature* 457:313–317. With permission from Macmillan Publishers Ltd.)

In summary, these experiments suggest that structural changes in neural circuits in response to specific experiences—whether changes in axonal arborization in the inferior colliculus or the formation of new dendritic spines in the visual cortex—can provide long-lasting memory traces to facilitate future learning. These structural changes may underlie a widely occurring phenomenon called savings, that is, less effort is required for an animal to re-learn something it has previously learned. Altogether, modern research discussed in this chapter has provided rich neurobiological bases for Descartes' needle-through-the-cloth analogy of memory (see Figure 10–1).

SUMMARY

In this chapter, we have studied memory and learning at multiple levels: molecules, synapses, neurons, circuits, systems, animal behaviors, and theories. From simple invertebrate systems to the complex mammalian brain, diverse experimental models have yielded data that support two central theses: (1) memory is primarily stored as strengths of synaptic connections in neural circuits, and (2) learning modifies synaptic weight matrices through a rich set of plasticity mechanisms.

A reductionist approach in *Aplysia*, using the gill-withdrawal reflex as a model behavior, suggested that depression and potentiation of the synaptic strength between the siphon sensory neurons and gill motor neurons mediate behavioral habituation and sensitization, respectively. Short-term sensitization of the gill-withdrawal reflex by tail shock is mediated by serotonin activation of cAMP/PKA and PKC signaling in the presynaptic terminal of the sensory neuron, modifying ion channels through phosphorylation that results in an elevated membrane potential and broadened spikes. Long-term sensitization involves prolonged activation of cAMP/PKA, causing phosphorylation of the CREB transcription factors, expression of new genes, and growth of new synapses between the sensory and motor neurons. Hence, in *Aplysia* as well as in many other animals, short-term memory does not require new protein synthesis whereas long-term memory requires new protein synthesis. Genetic analysis of *Drosophila* olfactory conditioning independently identified a central role for cAMP signaling in mushroom body neurons. Electric shock and food as the unconditioned stimuli modulate the strengths of synaptic connections between ensembles of mushroom body neurons representing conditioned stimuli (odorants) and output neurons through neuromodulators such as dopamine, whose receptors act through the cAMP cascade. cAMP/PKA also plays an important role in synaptic plasticity and memory in mice. Formation of new explicit memory in humans and spatial memory in rodents relies on the hippocampus, a medial temporal lobe structure that along with the nearby entorhinal cortex also plays a central role in spatial representation in mammals. A rich set of synaptic plasticity mechanisms has been identified in the hippocampus, and strong correlations have been established between hippocampal synaptic plasticity and spatial learning and memory.

Synapses onto the hippocampal CA1 pyramidal neurons in rats and mice have been used as a model to investigate general mechanisms of synaptic plasticity. Long-term potentiation (LTP) of the CA3 → CA1 synapse exhibits cooperativity that follows Hebb's rule: LTP is induced when presynaptic glutamate release coincides with postsynaptic depolarization. The NMDA receptor serves as a coincidence detector to execute Hebb's rule, and its function in CA1 neurons is required for both LTP induction and spatial memory. Ca^{2+} entry through the NMDA receptor activates protein kinases such as PKA and CaMKII. Auto-phosphorylation of the multi-subunit CaMKII can translate a transient Ca^{2+} signal into more persistent kinase activity. A central mechanism for LTP expression is an increase in AMPA receptor numbers at the postsynaptic membrane, which enhances response magnitude to presynaptic glutamate release. The CA3 → CA1 synaptic efficacy can also be regulated by long-term depression, which preferentially activates phosphatases to counteract the kinase activity. LTD, LTP, and spike-timing-dependent plasticity allow bidirectional adjustment of synaptic weights. Activity-dependent

retrograde endocannabinoid signaling from CA1 neurons can regulate the release of neurotransmitters by their presynaptic GABAergic neurons. Finally, long-term changes in the strength of connections between pre- and postsynaptic neurons involve formation of new synapses as a result of long-lasting LTP.

The synaptic plasticity mechanisms in the hippocampus likely apply, with variations according to specific neuronal and circuit properties, to other synapses in the central nervous system where experience-dependent changes underlie many forms of learning and memory. For example, long-term storage of explicit memory may engage specific neocortical areas that process and relay information to the hippocampus during memory acquisition; these cortical circuits likely interact with the hippocampus during memory consolidation. The amygdala is a center for processing emotion-related memory. Auditory fear conditioning engages parallel pathways and plasticity in multiple synapses in the basolateral and central amygdala, whereas contextual fear conditioning engages additional synaptic plasticity in the hippocampus. The amygdala is also required for fear conditioning in humans as a form of implicit memory. Some midbrain dopamine neurons signal reward prediction errors; they exhibit phasic firing when the actual reward exceeds the predicted reward. This property can be used for reinforcement-based learning, in which the synapses between cortical/thalamic input neurons and striatal spiny projection neurons are modulated by dopamine. This reinforcement-based learning plays an important role in motivational behavior as well as motor skill learning and habit formation.

Learning has different forms including simple habituation and sensitization, associative learning such as classical conditioning and operant conditioning, reinforcement-based learning, cognitive learning, and structural plasticity in both developing and adult sensory systems in response to altered experience. Most forms of learning involve changes in the synaptic weight matrices of relevant neural circuits, whether by strengthening or weakening existing synapses, making new synapses, or dismantling old ones; additional forms of learning include changes in the intrinsic properties of neurons. These changes alter neural circuit function in information processing and ultimately cause behavioral changes that enable animals to better adapt to a changing environment.

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