

CHAPTER 10

Memory, Learning, and Synaptic Plasticity

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 René 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.

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.

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

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)

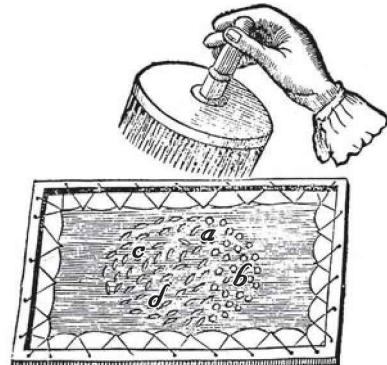


Figure 10-1 Memory as an imprint.

According to René 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.)



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. (From Permanent Present Tense by Suzanne Corkin, copyright © 2013. Reprinted by permission of Basic Books, a member of The Perseus Books Group.)

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 10–3A) 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.

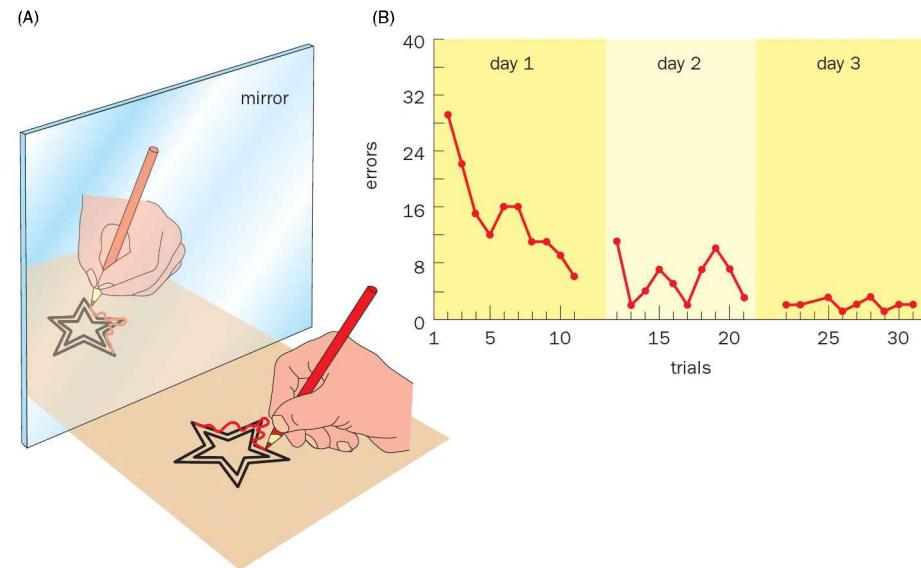
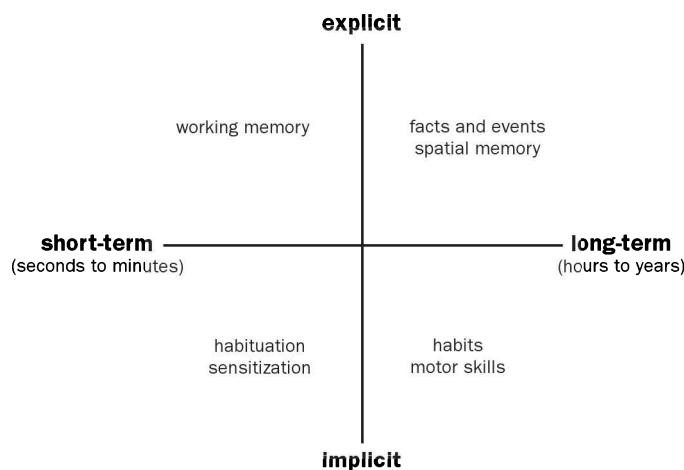


Figure 10–3 Memory of motor-skill learning displayed by H.M. (A) In this task, subjects are asked to view a double-outlined star in a mirror and draw a line in the space between its two borders. Subjects can only see their hands in the mirror. **(B)** With practice (number of trials, x axis), H.M. improved 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

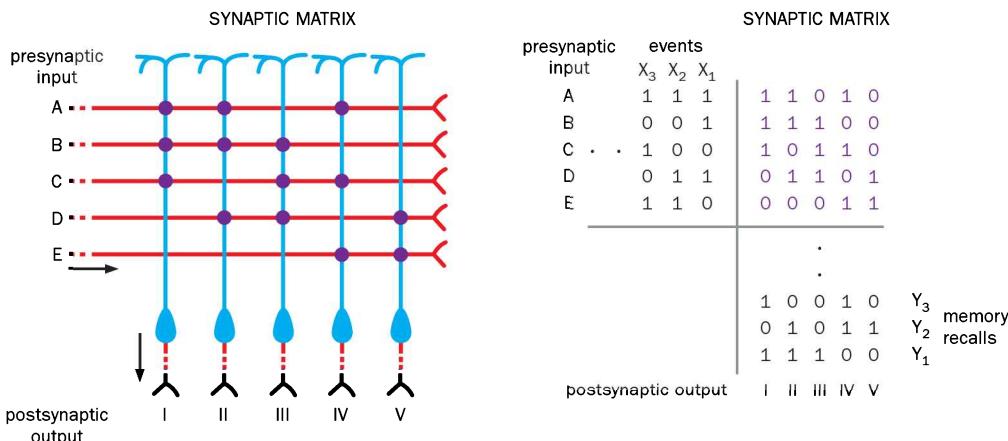


Figure 10–5 The synaptic weight matrix as a memory device. **Left**, a highly simplified model is used to illustrate how a synaptic matrix can store memory. In this synaptic matrix, axons of five presynaptic input neurons (A–E, red) form specific connections with dendrites of five postsynaptic output 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 $2^{(5 \times 5)}$ or ~30 million possible binary codes, a 100×100 matrix has $2^{(100 \times 100)}$ or $\sim 10^{3000}$ 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 $\sim 10^{13}$ 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

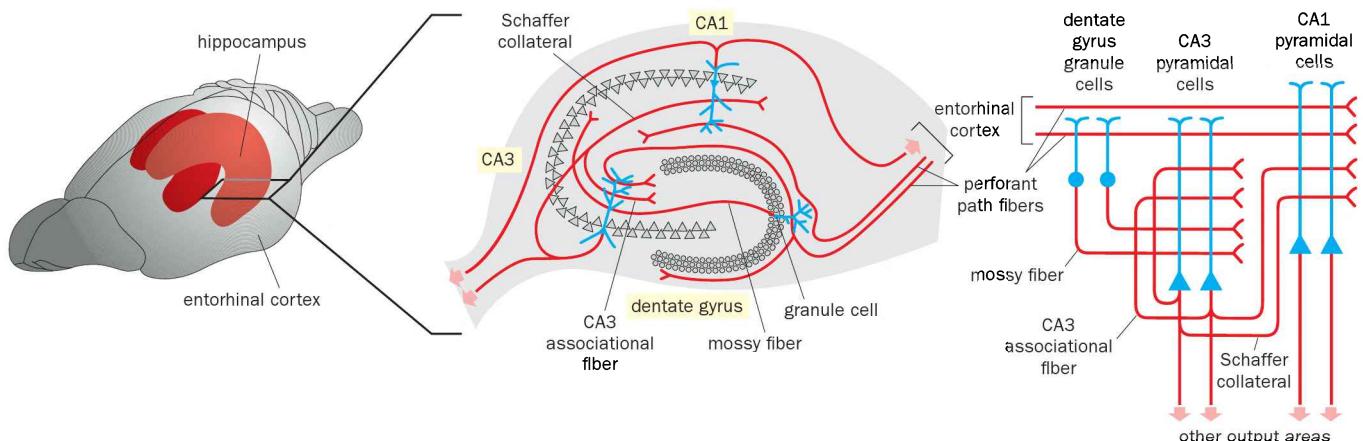


Figure 10–6 The hippocampal circuit. Left, location of the hippocampus and entorhinal cortex in the rat brain. A magnified section of the hippocampus (middle) and a circuit diagram (right) highlight the principal neurons (circles, granule cells; triangles, pyramidal neurons) and their major connections. 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.

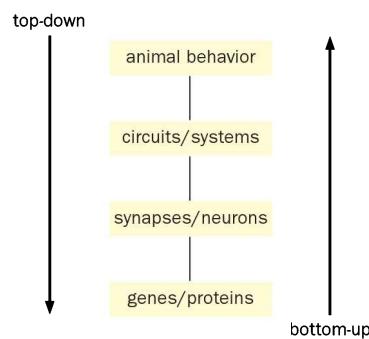


Figure 10–7 Memory and learning can be studied at multiple levels. When researchers start by observing a complex, high-level phenomenon and work to discover its underlying mechanisms, the approach is described as top-down or reductionist. By contrast, when researchers start by examining a low-level phenomenon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

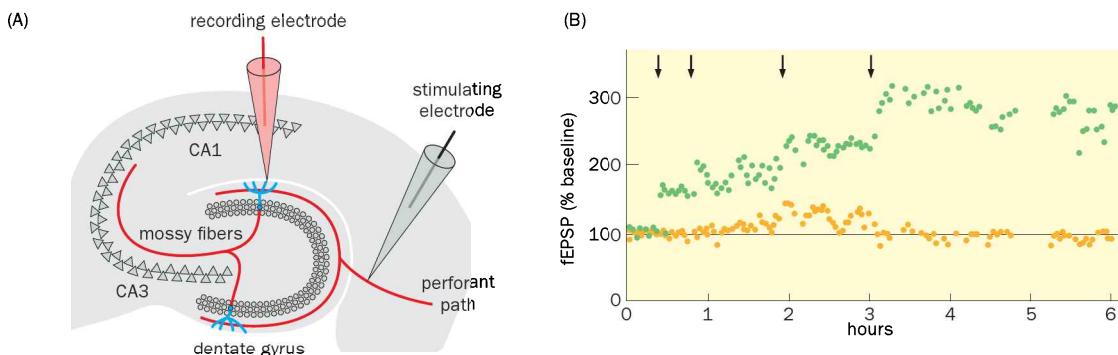


Figure 10-8 Long-term potentiation (LTP) induced by high-frequency stimulation. (A) Experimental setup. The stimulating electrode was placed at the perforant path, which consists of axons that innervate dentate gyrus granule cells. A second electrode was placed near the granule cell bodies to record 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

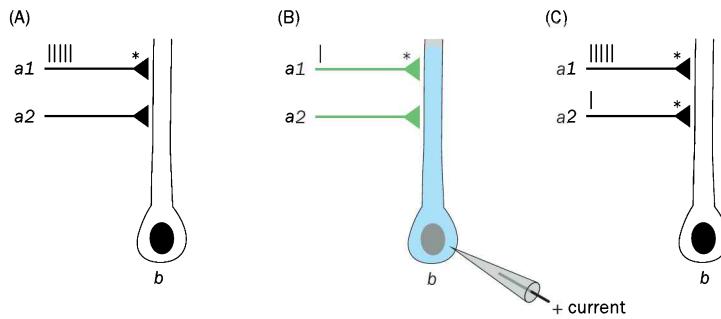
In the early 1970s, it was discovered that the connection strengths of hippocampal neurons could be altered in response to high-frequency stimulation (Figure 10-8). In these experiments, an extracellular recording electrode was implanted in the dentate gyrus of anesthetized rabbits to record the activity 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, a_1 and a_2 , form synapses with the same postsynaptic CA1 neuron b . **(A)** LTP exhibits input specificity. In the schematic shown here, only the $a_1 \rightarrow b$ 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 a_1 to induce LTP. **(C)** LTP exhibits associativity. A weak stimulus at the $a_2 \rightarrow b$ synapses normally would not induce LTP at that synapse. However, when the timing of a weak a_2 stimulus coincides with high-frequency stimulation of a_1 , the $a_2 \rightarrow b$ synapse also becomes potentiated, because local depolarization at the $a_1 \rightarrow b$ synapses spreads to the $a_2 \rightarrow b$ 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), a_1 and a_2 , which synapsed onto the dendrites of cell b , a CA1 postsynaptic neuron that was being recorded. LTP was induced by high-frequency stimulation of a_1 (Figure 10–9A). When the synaptic efficacy was measured afterwards, only the strength of the $a_1 \rightarrow b$ connection was potentiated, whereas the strength of the $a_2 \rightarrow b$ 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 a_1 depolarize cell b at the $a_1 \rightarrow b$ synapses, such that the arrival of action potentials late in the train coincides with a depolarized state of the postsynaptic cell, hence potentiating the $a_1 \rightarrow b$ 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 a_1 to induce LTP at the $a_1 \rightarrow b$ synapses, a_2 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 $a_2 \rightarrow b$ synapses as well (Figure 10–9C). This is because high-frequency stimulation of a_1 causes depolarization in a region of cell b that includes the site of the $a_2 \rightarrow b$ synapses. If a_2 receives a weak stimulus (such as a single shock) during the time b is depolarized at the $a_2 \rightarrow b$ 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 Mg²⁺ (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 10–10A). Second, pharmacological inhibition of the NMDA receptor by a specific NMDA receptor antagonist, **2-amino-5-phosphovaleric 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

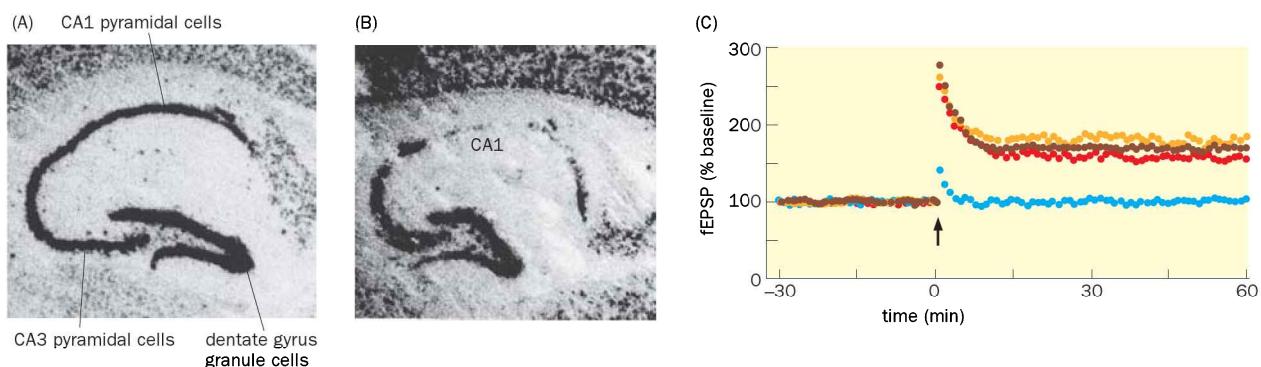


Figure 10–10 The NMDA receptor in the postsynaptic neuron is essential for LTP induction at the CA3 → CA1 synapse. (A) *In situ* hybridization shows that mRNA for the GluN1 subunit of the NMDA receptor is highly expressed in CA3 and CA1 pyramidal neurons as well as dentate gyrus granule cells in the 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 10–11A–C). LTP expression involves both the activation of

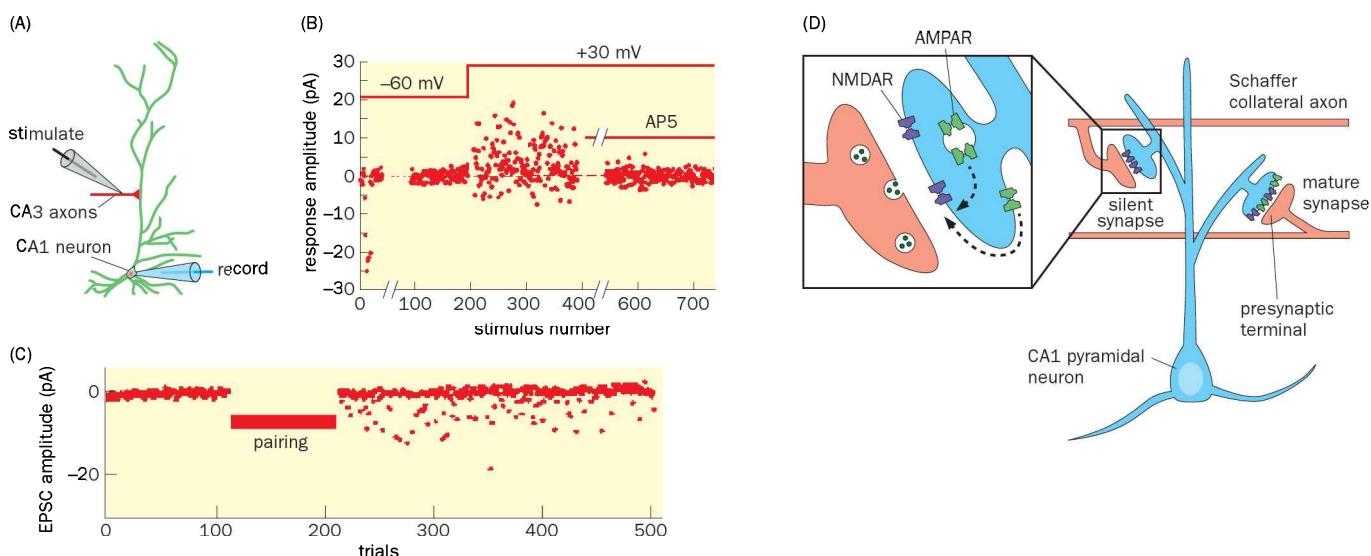


Figure 10–11. Silent synapses and their activation by LTP.

(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 $+30\text{ mV}$, 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 $+30\text{ mV}$ (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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ decreases, Ca^{2+} /calmodulin dissociates, deactivating CaMKII if no further modification occurs to CaMKII (Figure 10–12A, 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

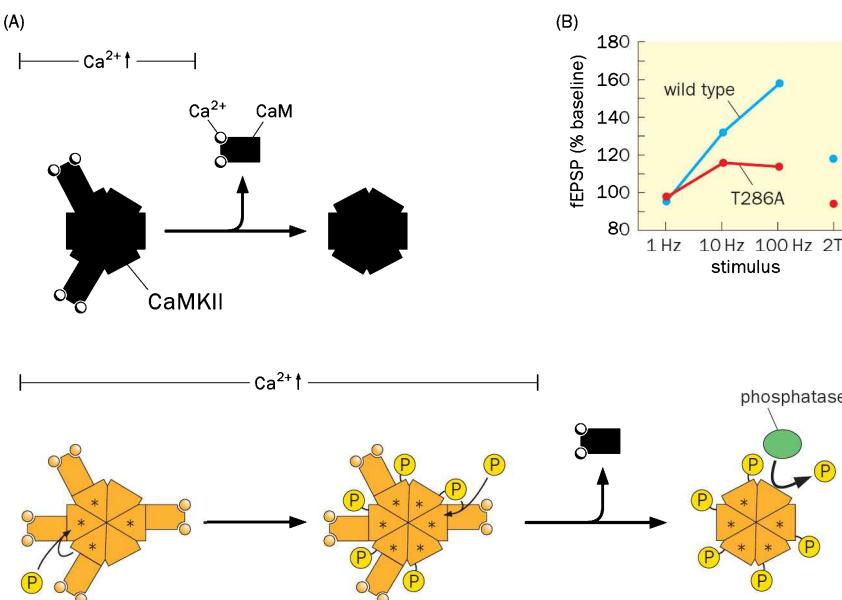


Figure 10–12 Auto-phosphorylation of CaMKII and Its Requirement In LTP.

(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 $[\text{Ca}^{2+}]_i$ drops, the subunit becomes inactive. Bottom, if a sufficient number of CaMKII subunits become activated in response to a prolonged $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ 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.)

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.

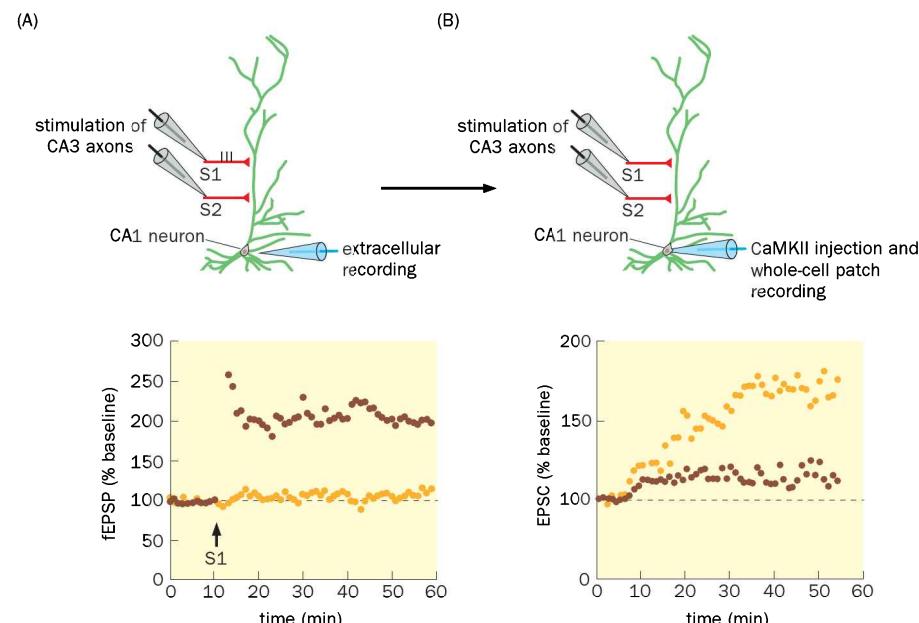
CaMKII activity contributes to the regulation of synaptic transmission strength through multiple mechanisms. For example, CaMKII-catalyzed phosphorylation of AMPA receptors increases their ion conductance and influences their trafficking (see Section 10.9 below). CaMKII also phosphorylates postsynaptic 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

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, Hjemstad GO, Mukherji S et al. [1995] *Proc Natl Acad Sci USA* 92:11175–11179.)

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.



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 10–14A). Like LTP induction, LTD induction is dependent on the NMDA receptor and Ca^{2+} influx. The increase of $[\text{Ca}^{2+}]_i$ resulting from low-frequency stimulation is lower than that resulting from high-frequency stimulation. This lower increase of $[\text{Ca}^{2+}]_i$ 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

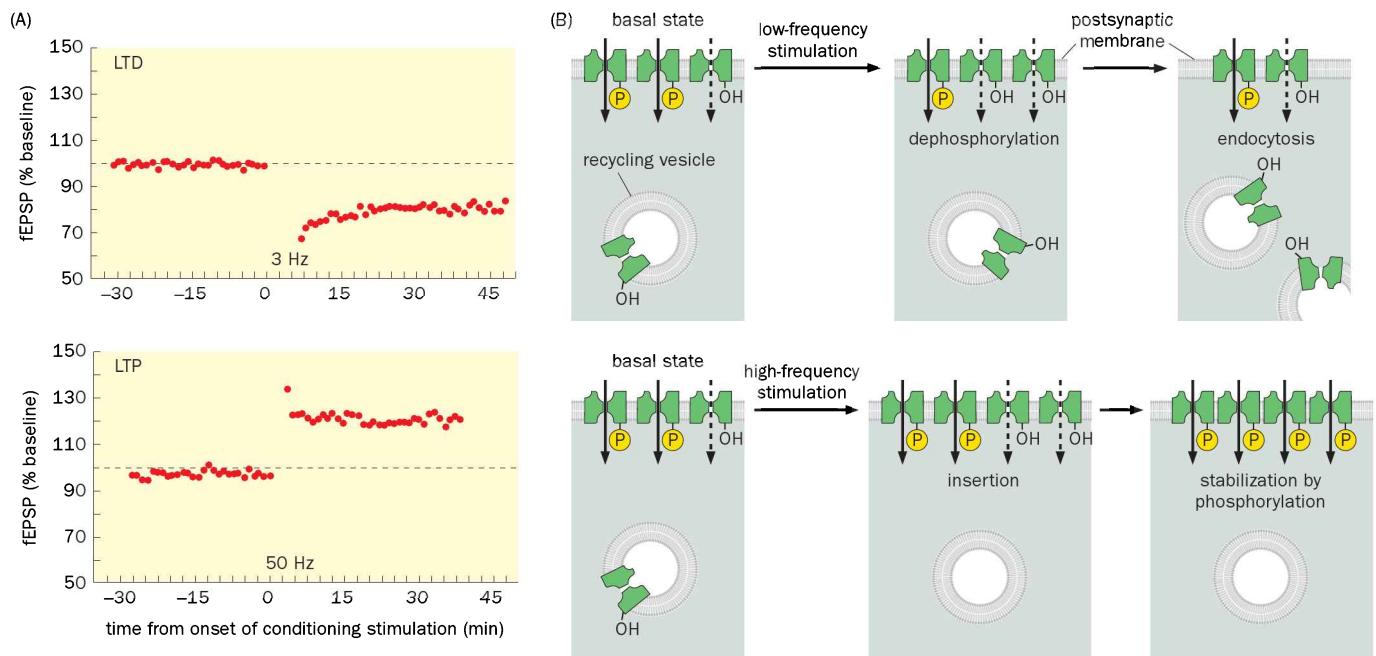


Figure 10–14 Long-term depression at the CA3 → CA1 synapse. (A) Whereas high-frequency (50-Hz) stimulation induces LTP (bottom panel), low-frequency (3-Hz) stimulation of CA3 axons innervating a CA1 neuron causes long-term depression (LTD) of the efficacy of synaptic transmission (top panel). (B) In 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; 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

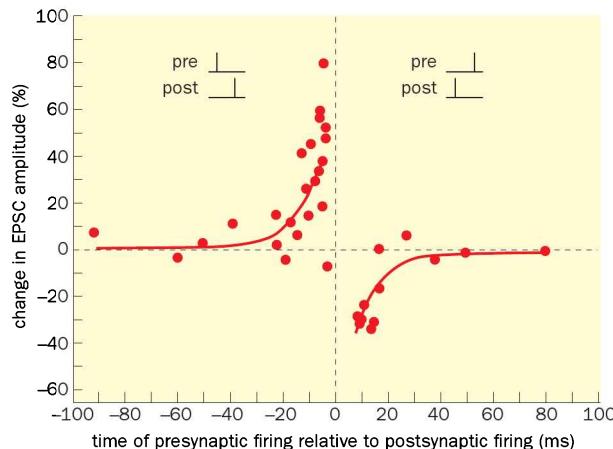


Figure 10–15 Spike timing-dependent plasticity (STDP). If the presynaptic neuron repeatedly fires before the postsynaptic neuron, the synapse is potentiated (left). If the presynaptic neuron repeatedly fires after the postsynaptic neuron, the synapse is depressed (right). Data here were taken from retinotectal 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.)

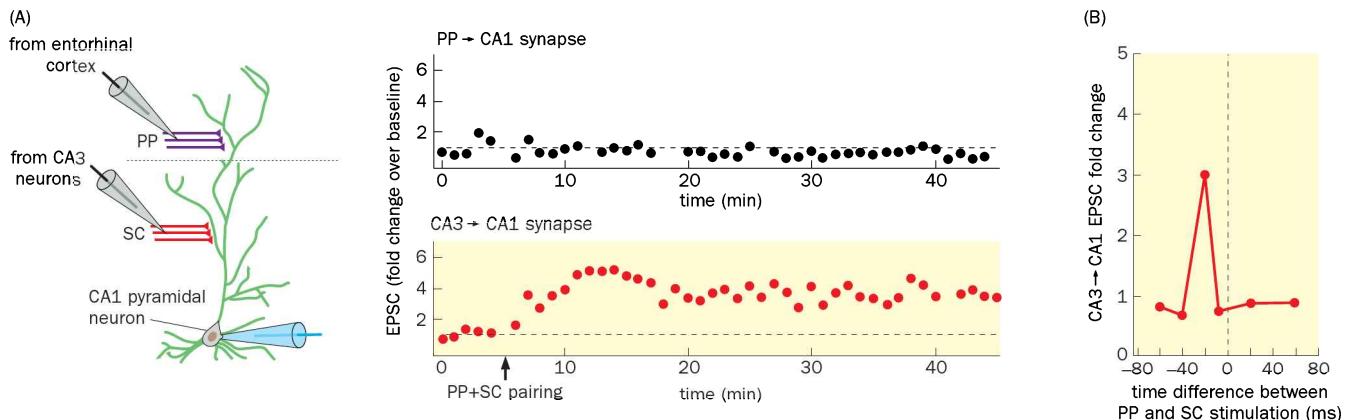


Figure 10–16 Input-timing-dependent plasticity (ITDP) in CA1 neurons. (A) Left, experimental setup. In a hippocampal slice, whole-cell patch clamp recording was performed on a CA1 neuron; stimulating electrodes were placed at the perforant path (PP) and the Schaffer collaterals (SC) that innervate the 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 10–16A, 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 neurotransmitter release by their presynaptic partners

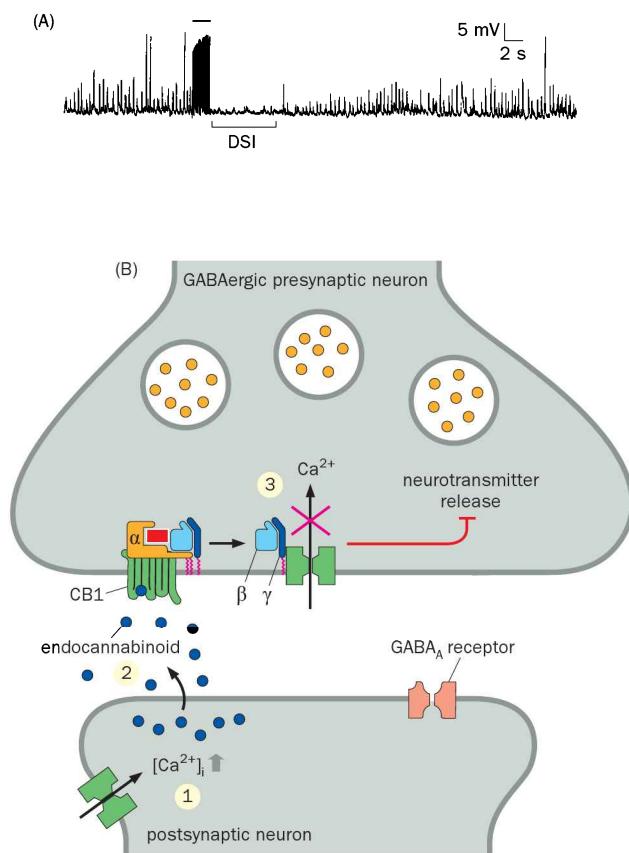
Our discussions thus far have largely focused on postsynaptic mechanisms for modifying the efficacy of synaptic transmission, but synaptic plasticity can also engage presynaptic mechanisms. For example, synapses can be facilitated

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-arachidonoylglycerol, 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 10–17A**).

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 $[\text{Ca}^{2+}]_i$ 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 $\text{G}\beta\gamma$, 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 JA [2002] *Science* 296:678–682.)



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 $\beta\gamma$ 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

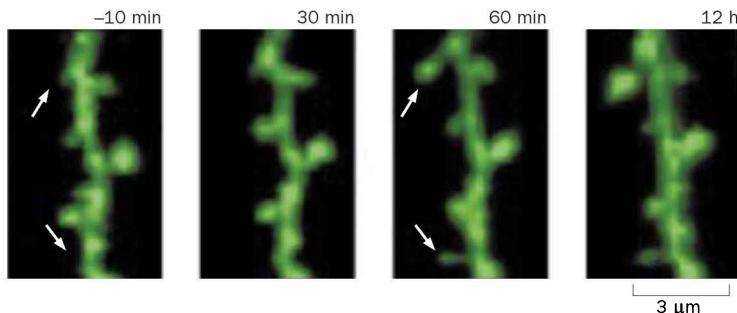


Figure 10–18 Growth of dendritic spines correlates with LTP. LTP is accompanied by the formation of two new spines (arrows) in CA1 pyramidal neurons from a cultured hippocampal slice that was imaged using two-photon microscopy. Time-lapse images were taken at –10, +30, +60 min, and +12 h relative to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

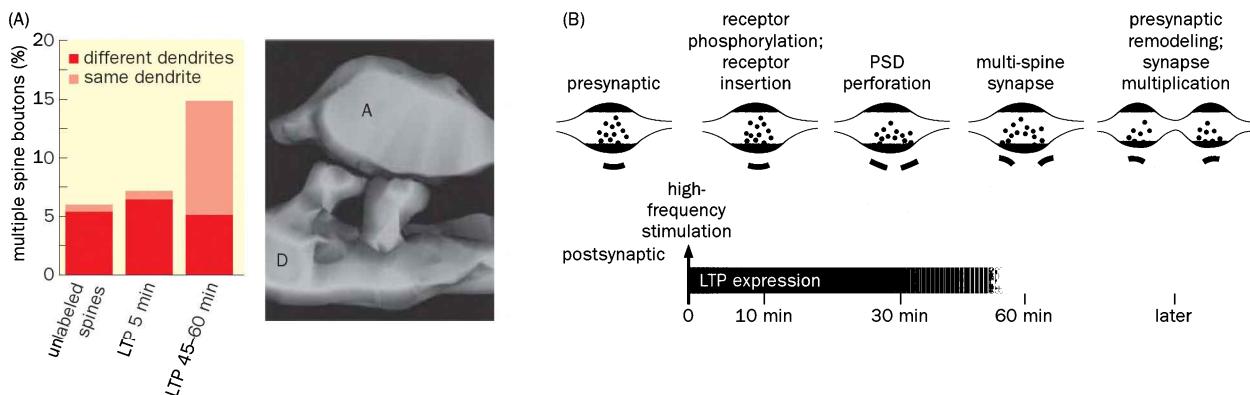


Figure 10–19 LTP correlates with formation of multiple-spine boutons. (A) Left, quantification of the fraction of axon terminals that contact more than one dendritic spine. Dendritic spines activated by LTP were labeled by a staining procedure that produces precipitates in EM micrographs of recently 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 dendrite at a late (60-min) but not early (5-min) phase after the initial stimulation (Figure 10–19A). 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.

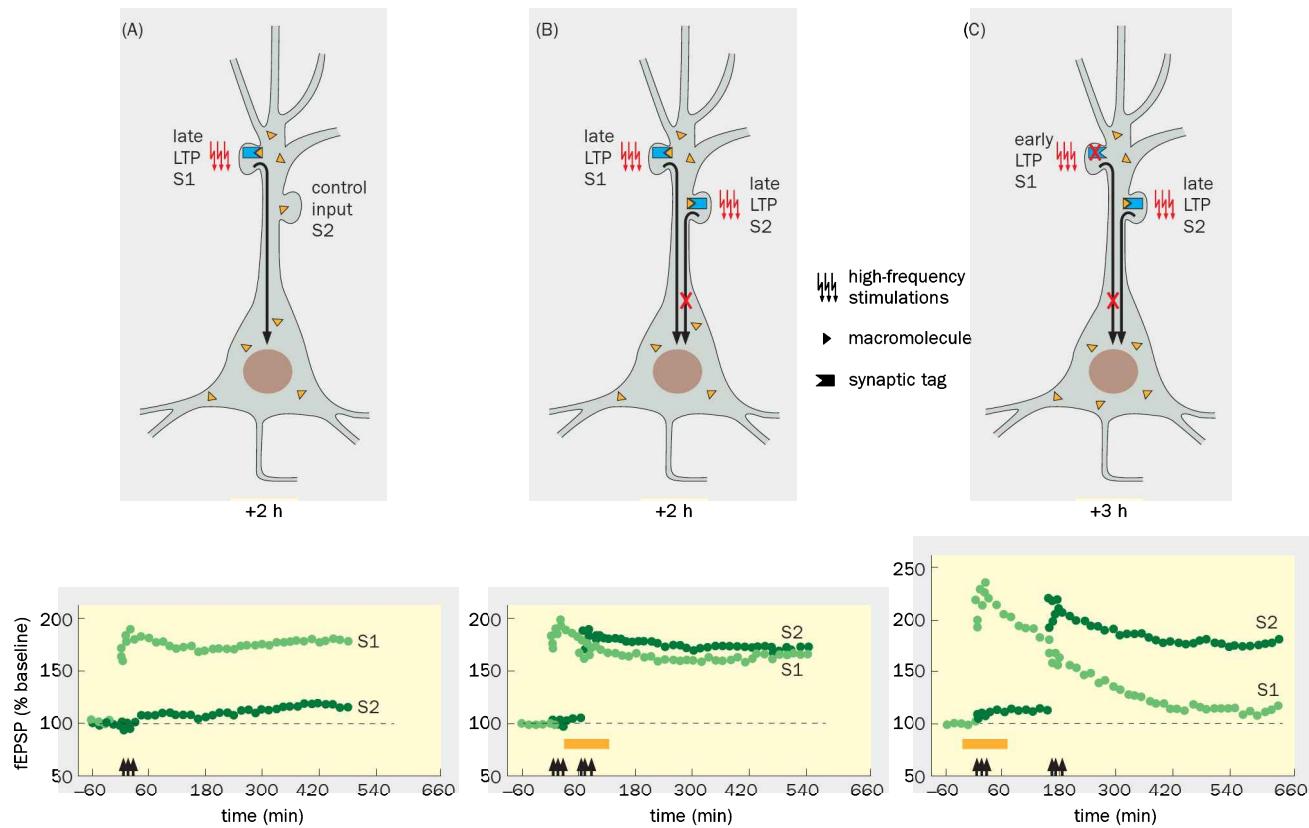


Figure 10–20 Experimental evidence for synaptic tagging hypothesis. The top schematics illustrate the experimental conditions and summarize the results at 2 or 3 hours after the first high-frequency stimulation (HFS) according to the synaptic tagging hypothesis. The bottom panels show the field EPSP 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**.

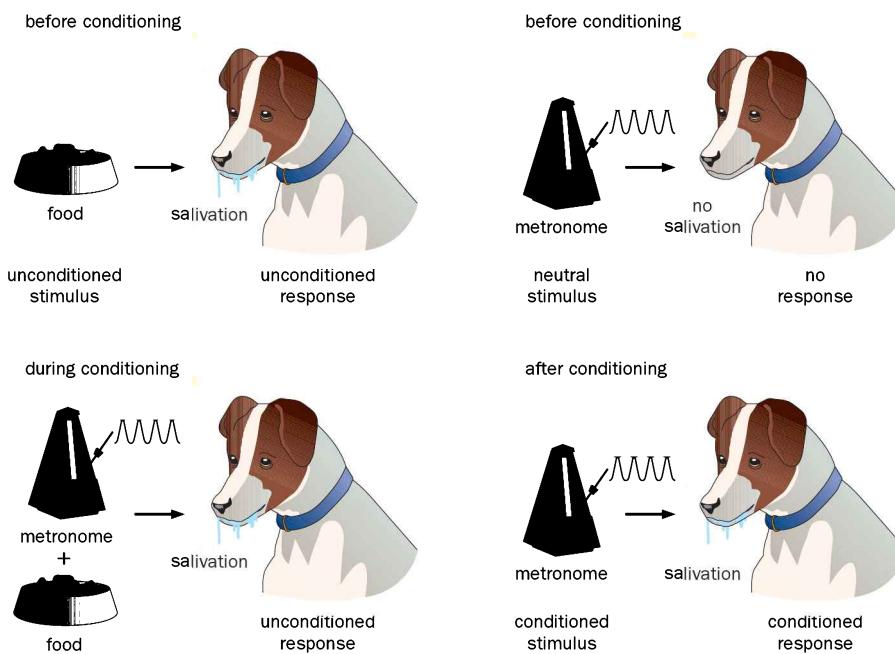


Figure 10–21 Pavlov’s experiment that established the concept of classical conditioning. Before conditioning, the dog salivates in response to food in the mouth (top left), but does not salivate when hearing a sound from a metronome (top right). During conditioning, which consists of repeated pairing 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.

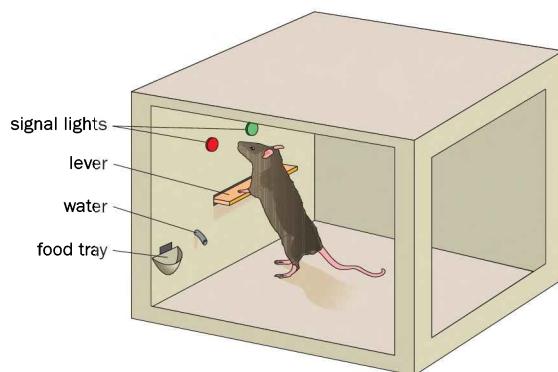


Figure 10–22 Basic design of an operant chamber. A hungry or thirsty rat placed in this chamber can learn through trial and error that pressing the lever results in the dispensing of either food or water (according to the particular experimental design); this reward reinforces the lever-pressing response.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

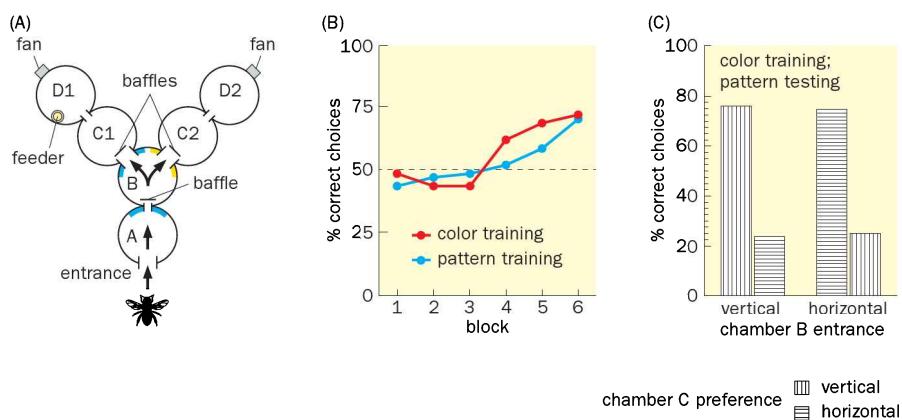
Timing is crucial in operant conditioning—as in classical conditioning—and the effect is greatest when the reinforcer is presented shortly after the behavior. Another property shared by classical and operant conditioning is **extinction**: in classical conditioning, when the CS is repeatedly *not* followed 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 10–23A). 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 10–24A). 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

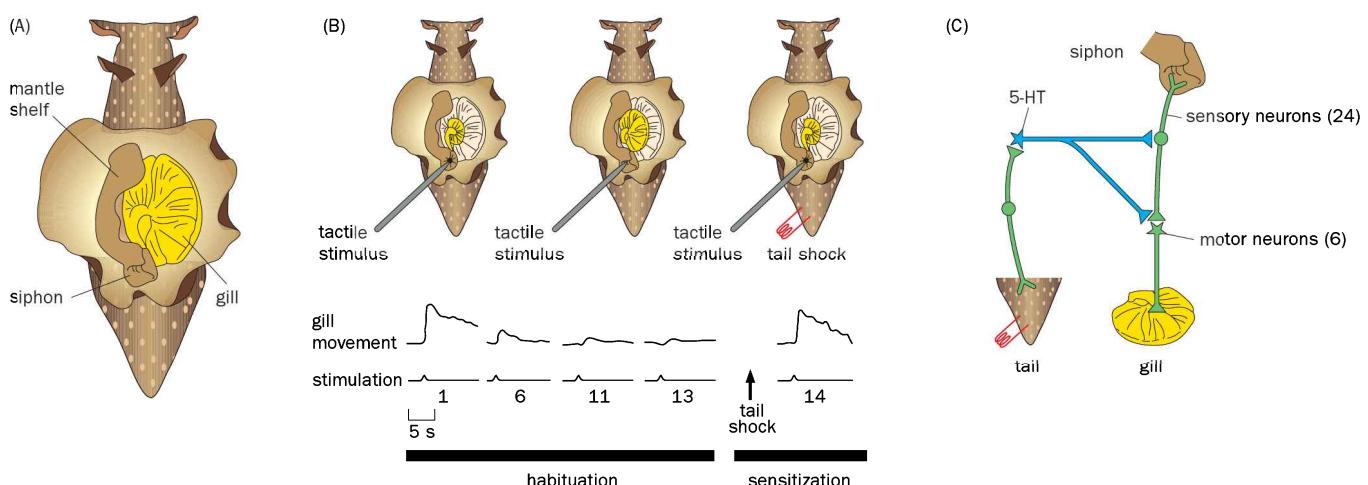


Figure 10–24 The gill-withdrawal reflex in *Aplysia* and the underlying neural circuits. (A) Schematic drawing of *Aplysia* highlighting the structures related to the gill-withdrawal reflex. (B) Top, schematic drawing of the gill-withdrawal reflex and its habituation (middle) and sensitization (right). 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

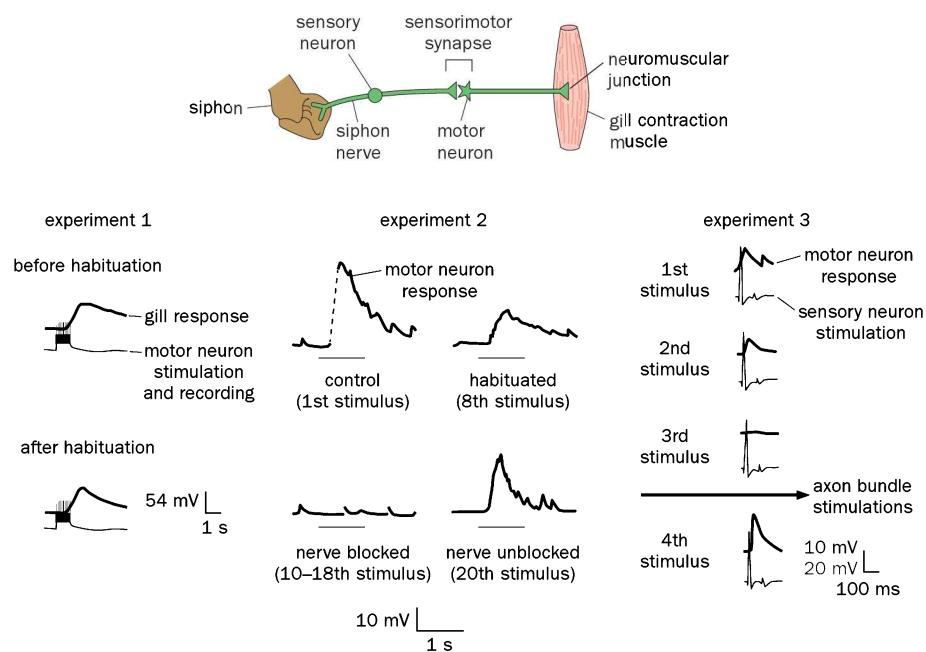
was applied, which caused an increase of response to stimulus 14. (C) Circuit diagram of the gill-withdrawal reflex. The 24 sensory neurons that innervate the siphon connect directly with the six motor neurons that innervate the gill muscle. Sensory neurons activated by tail shock connect with serotonin (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.)

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 10–26A).

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.

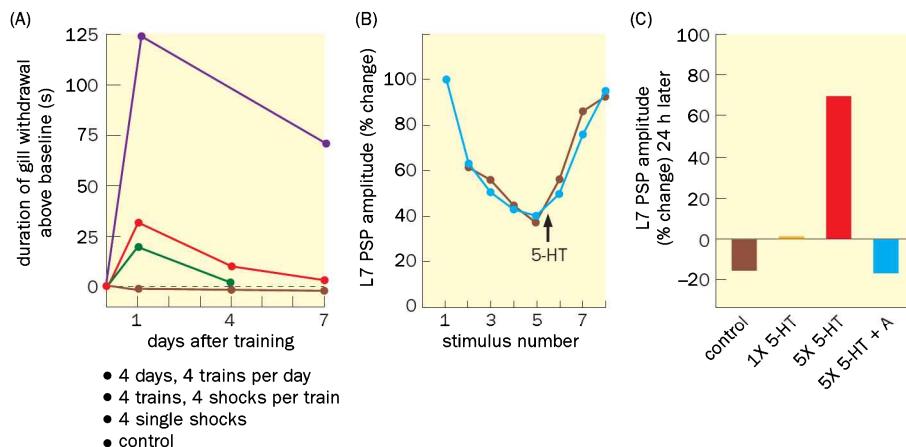


Figure 10–26 Long-term sensitization can be induced by repeated training or serotonin (5-HT) application, and is dependent on protein synthesis.

(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 ($1 \times 5\text{-HT}$) did not produce long-term facilitation measured 24 hours later, whereas a sequence of five 5-HT applications did ($5 \times 5\text{-HT}$). Application of the protein synthesis inhibitor anisomycin during the time of 5-HT application ($5 \times 5\text{-HT} + A$) blocked the long-term facilitation. (A, adapted from Frost WN, Castellucci VF, Hawkins RD et al. [1985] Proc Natl Acad Sci USA 82:8266–8269. With permission from the authors; B & C, adapted from Montarolo PG, Goelet P, Castellucci VF et al. [1986] Science 234:1249–1254.)