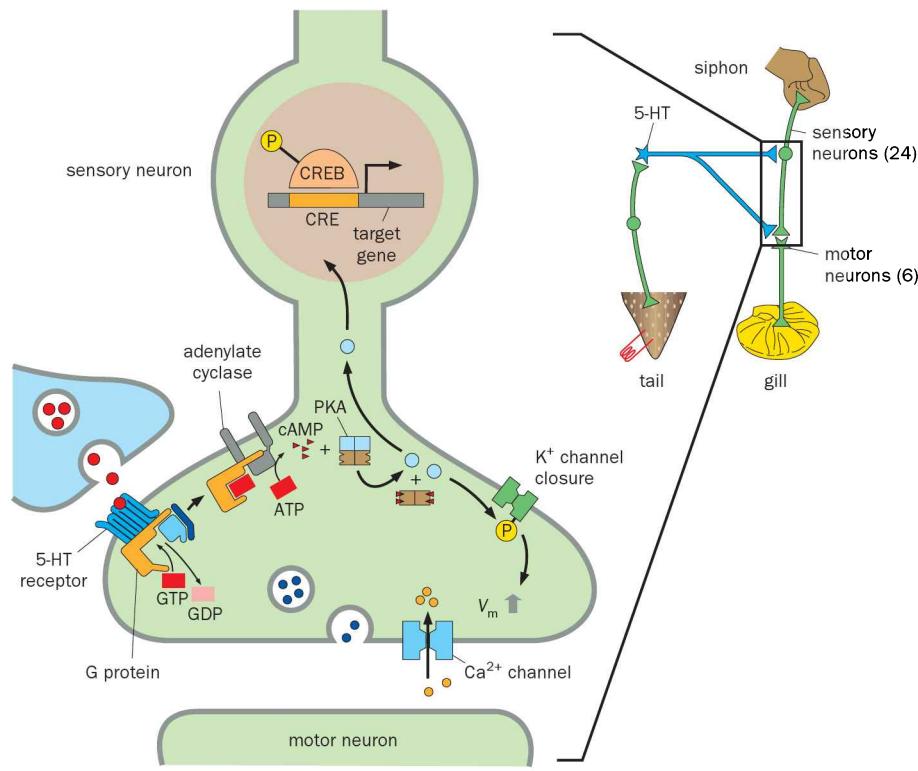


Are there mechanistic differences between short-term and long-term memory? Studies in different animals from mice to goldfish indicated that long-term (but not short-term) memory formation is inhibited by applying drugs that inhibit protein synthesis at the time of training, suggesting that long-term-memory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

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

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

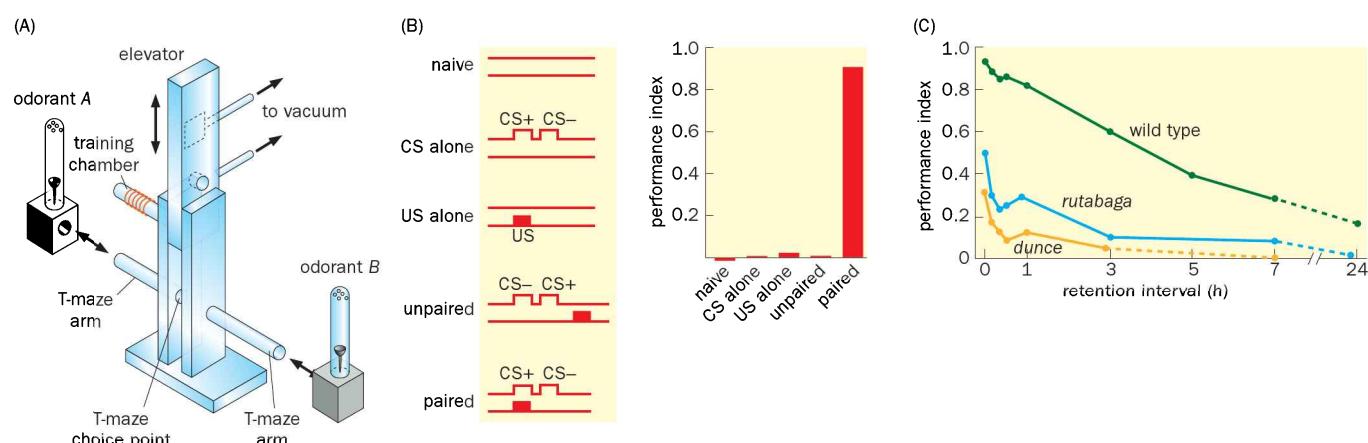


Remarkably, cAMP and PKA are also key components for long-term facilitation (Figure 10–27, top). Here, a widely used signaling pathway involving the transcription factor CREB is engaged (see Figures 3–41 and 7–36B): PKA phosphorylation activates CREB, which binds to the CRE (cAMP response element) sequences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

### 10.17 Olfactory conditioning in *Drosophila* requires cAMP signaling

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

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



**Figure 10–28 Olfactory conditioning in *Drosophila* and its disruption by mutations affecting cAMP metabolism. (A)** Schematic of the olfactory conditioning procedure. About 100 flies in the training chamber were exposed to odorant A (CS+) paired with electric shock (US), and to odorant B without electric shock (CS-). These flies were then transferred via the sliding elevator to the bottom T-maze arms, where flies can freely choose a path to odorant A or odorant B. Performance index = [(number of flies in tube B – number of flies in tube A) / total number of flies] × 100. **(B)** Performance indices of flies under different

training conditions. Flies learn the association only when US is paired with CS+. **(C)** Performance indices of wild-type and mutant flies. Performance indices represent learning when measured immediately after training ( $t = 0$ ) and memory retention when measured as specific times thereafter. *rutabaga* and *dunce* mutant flies are defective in both learning and memory. (Adapted from Tully T & Quinn WG [1985] *J Comp Physiol* 157:263–277. With permission from Springer. See also Dudai Y, Jan Y, Byers D et al. [1976] *Proc Natl Acad Sci USA* 73:1684–1688 for the identification of the first learning mutant, *dunce*.)

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

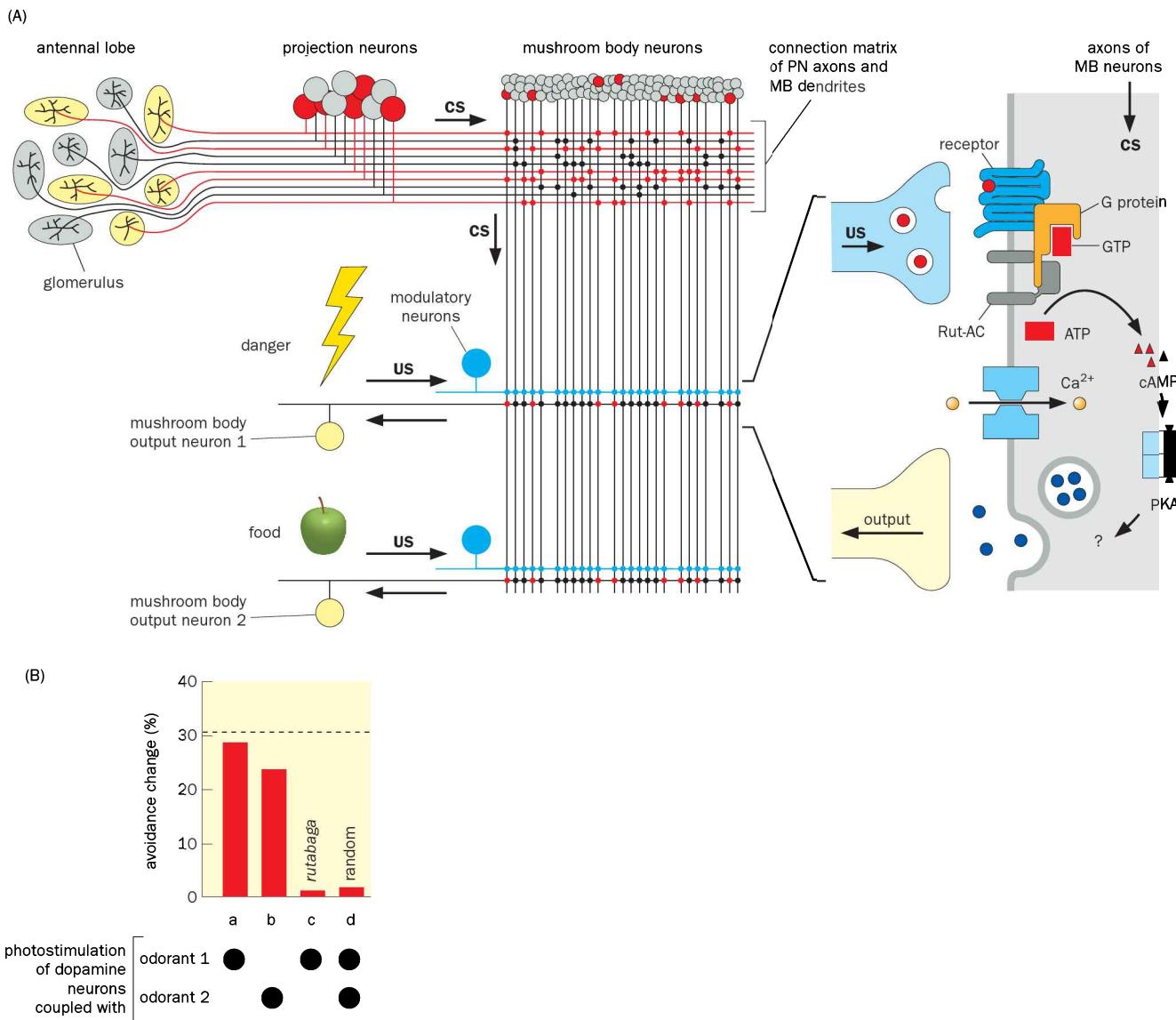
Two of the first mutations identified through genetic screening, named *dunce* and *rutabaga*, affected both learning and memory. Performance of flies carrying either of these two mutations was drastically reduced compared with normal flies immediately after training, indicating a learning defect. In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

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

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

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

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



**Figure 10-29 Neural circuit and mechanisms of *Drosophila* olfactory conditioning.** (A) Left, a circuit model for olfactory conditioning. Odorants activate specific ensembles of glomeruli (ovals) in the antennal lobe, with active glomeruli shown in yellow (see Figure 6-27 for a schematic of the *Drosophila* olfactory system). Projection neurons (PNs) that innervate the active glomeruli become activated (red), which in turn activate a specific subset of mushroom body (MB) neurons. In this representative connection matrix between PN axons and MB neuron dendrites, each MB neuron is connected with three PN axons (dots); only when all three connected PNs are active would the MB neuron become active (red cell on top, with three red dots in the matrix). CS (odorant) information is represented by ensembles of active PNs and subsequently by ensembles of active MB neurons. Synapses between MB axons and dendrites of MB output neurons are modified by nearby input from modulatory neurons, such as dopamine neurons, that signal the presence of an aversive or appetitive US (bottom). Each dot represents a connection and each red or blue dot represents an active synapse. The co-activation of neurons representing the US and the CS modifies the synaptic efficacy between the MB neuron and the MB output neuron. Right, an enlarged diagram of the output synapses of MB neurons. Axons carrying the US information release modulatory neurotransmitters that activate G-protein-coupled receptors, resulting in the activation

of the Rutabaga adenylate cyclase (Rut-AC). This causes an increase in cAMP production and thereby activates PKA, leading to changes in synaptic efficacy through mechanisms yet to be explored.

(B) Photostimulation of dopamine neurons expressing a light-activated ion channel (see Figure 13-44 for details) is used to train flies to avoid a specific odorant in an operant conditioning paradigm. Individual flies were trained to avoid one of the two odorants by giving an electric shock whenever the fly enters a compartment associated with the odorant. The horizontal line indicates the level of avoidance change after electric shock-based training. Photostimulation of specific dopamine neurons can substitute for shock and achieve similar effect. When photostimulation was repeatedly coupled with the fly entering the compartment containing odorant 1 (a) or odorant 2 (b), flies increased avoidance of the stimulus-paired odorant. This effect was abolished in the rutabaga mutant (c), or when photostimulation was random and not uniquely associated with one odorant compartment or the other (d). (A, adapted from Heisenberg M [2003] *Nat Rev Neurosci* 4:266–275. With permission from Macmillan Publishers Ltd. See also Aso Y, Hattori D, Yu Y et al. [2014] *Elife* 3:e04577 and Aso Y, Sitaraman D, Ichinose T et al. [2014] *Elife* 3:e04580; B, adapted from Claridge-Chang A, Roorda RD, Vrontou E et al. [2009] *Cell* 139:405–415. With permission from Elsevier Inc.)

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

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

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

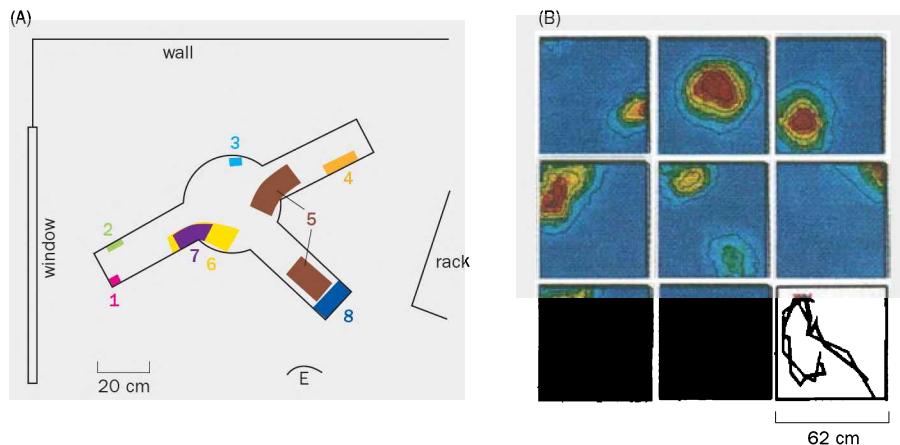
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### Box 10–2: Place cells, grid cells, and representations of space

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

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

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

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


**Figure 10–30 Hippocampal place cells.** **(A)** Map of a maze showing the place fields (numbered and illustrated in different colors) of eight place cells in the hippocampus of a freely moving rat. Each place field represents the regions within the maze in which a given place cell exhibited increased firing rate. E, location of the experimenter. **(B)** The activity of place cells can be used to construct a map of a rat's travels. A multi-electrode array was used to simultaneously record 80 hippocampal cells. The place fields of eight selected place cells are represented here as heat maps in eight squares: for each place field, the colors indicate the firing rate of the cell

when the rat occupied a corresponding position in a 62 cm × 62 cm square arena (red, maximal firing rate; dark blue, no firing). Note that the place fields of different cells vary in size and are situated at different locations in the arena. Bottom right, the vector of firing rates of a neuronal population during a 30-second period was used to reconstruct the spatial trajectory of the rat. The calculated trajectory (red) closely matches the actual trajectory (black). (A, adapted from O'Keefe J [1976] *Exp Neurol* 51:78–109; B, From Wilson MA & McNaughton BL [1993] *Science* 261:1055–1058.)

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

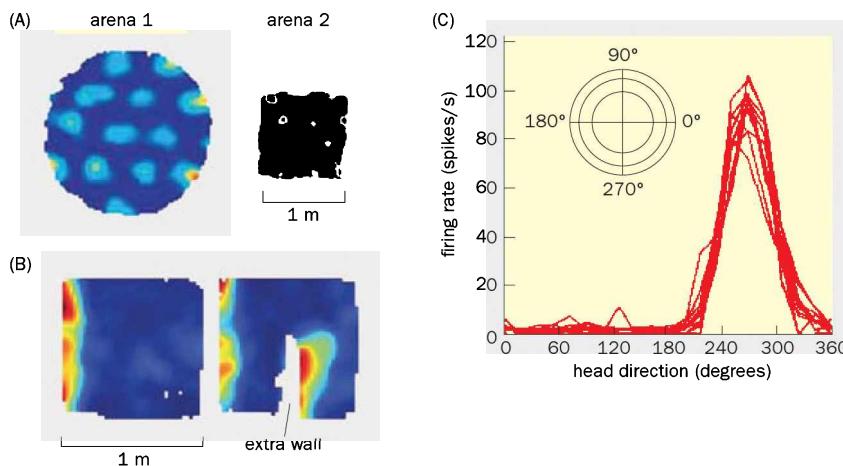
How do hippocampal place cells acquire their firing properties? A partial answer to this question came from another remarkable discovery made in the mid-2000s. A large fraction of layer 2/3 neurons in the medial entorhinal cortex, which provide major input to the hippocampus (see Figure 10–6, right),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

Grid cells and place cells share many similarities. The activities of simultaneously recorded grid cell populations, like those of place cells, can be used to reconstruct trajectories of movement (see Movie 10–2). As with place fields, grid patterns are influenced by external landmarks; when external landmarks are rotated in a circular arena, grid patterns rotate correspondingly. Grid patterns, like place fields, do

not merely mirror sensory cues, since they are maintained when the animal moves in the dark. However, the properties of grid cells and place cells also differ in important ways. Grid cells tile space more efficiently: a few grid cells can cover a space that requires dozens or more place cells. After animals are introduced into a novel environment, grid cells retain their grid size but place fields may remap completely. Populations of grid cells maintain the positions of their grid centers relative to each other across different arenas, whereas place cells remap more randomly. These observations suggest that the grid cells provide a more fundamental metric of space for anchoring the place fields of hippocampal cells.

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

(Continued)

**Box 10-2: Place cells, grid cells, and representations of space****Figure 10-31 Grid cells, border cells, and head direction cells.**

**(A)** Firing patterns of two entorhinal cortex grid cells, one in a circular and another in a square arena. The color of each position within each arena reflects the firing rate of the grid cell when the rat occupied that position (red, maximal firing rate; dark blue, no firing). Periodic peaks in firing rate of each cell form hexagonal grids that tile each arena. **(B)** This entorhinal border cell fired selectively when the rat was located at the left border of a square arena (left). When an extra border was added, a new firing field along the new left border was created (right). **(C)** A head direction cell fired when

the rat's head was facing a specific direction (peaking at ~270°, or when the rat's head was facing south) regardless of where the rat was located in the arena. Each of the 12 traces represents the firing rate of the cell when the rat was at one of the 12 divisions of the circular arena (inset at the top left). (A, from Hafting T, Fyhn M, Molden S et al. [2005] *Nature* 436:801–806. With permission from Macmillan Publishers Ltd; B, from Solstad T, Boccara CN, Kropff E et al. [2008] *Science* 322:1865–1868. With permission from AAAS; C, adapted from Taube JS, Müller RU & Ranck JB [1990] *J Neurosci* 10:420–435. With permission from the Society for Neuroscience.)

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

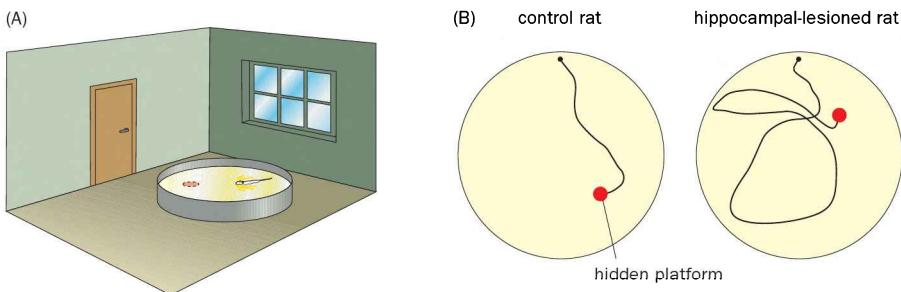
The remarkable properties of place cells and grid cells in the hippocampal–entorhinal network, far removed from the sensory world, have provided a glimpse of how abstract information such as space is represented in the brain. What is the relationship between spatial representation and memory, another important function of the hippocampus? One hypothesis is that the hippocampal–entorhinal network is used in parallel for navigation and memory. Explicit memory often involves the binding of disparate details into a coherent event; this is conceptually similar to the process

by which hippocampal place cells extract spatial information from the activities of grid cells, border cells, and head direction cells. An alternative hypothesis is that the location of an experience is so essential to its explicit memory that the formation of a memory is intimately tied with the representation of space. Indeed, the use of ‘memory palaces,’ that is, the organization of events into imaginary spaces, is an ancient and effective mnemonic technique, and space-based tasks have been among the most effective ways to assay memory in mammals. As researchers learn more about the functions of the hippocampal–entorhinal network in memory and in spatial representation, the connections between these two systems will become clearer.

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

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

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

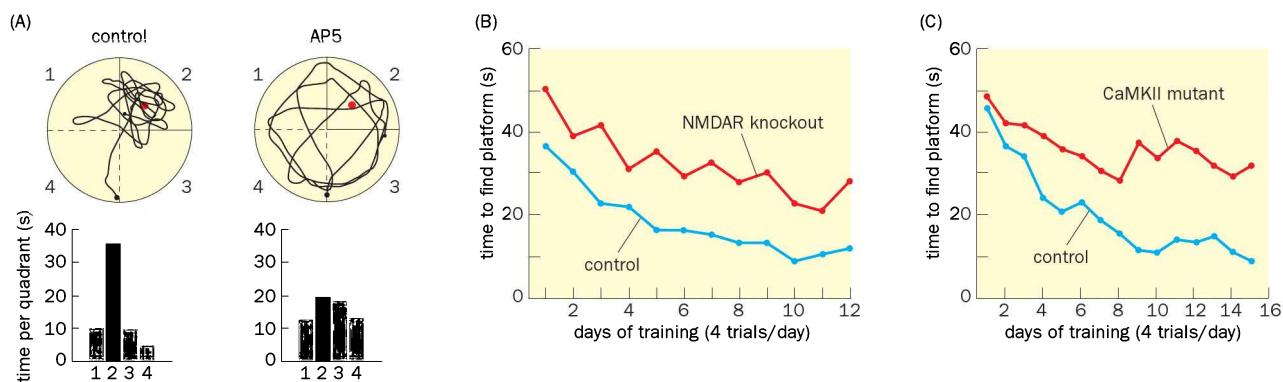


**Figure 10-32 Spatial memory tested in the Morris water maze depends on the hippocampus.** (A) Schematic of the Morris water maze. After training, a rat or a mouse can use distant spatial cues to help find the location of a hidden platform (dashed circle) in a large pool of milky water. (B) After training,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

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

## 10.20 Many manipulations that alter hippocampal LTP also alter spatial memory

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



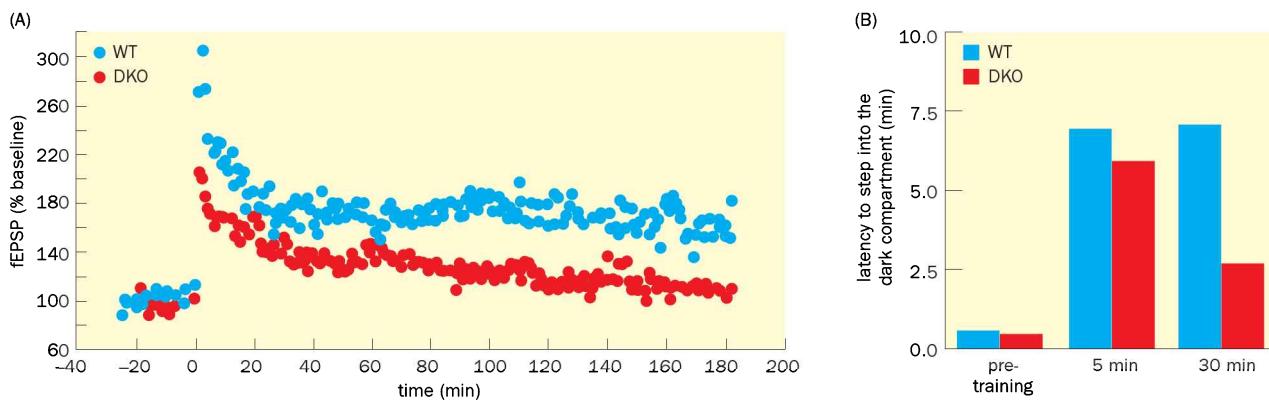
**Figure 10-33 Manipulations that disrupt hippocampal LTP also interfere with performance in the Morris water maze.** (A) Infusion of AP5, an antagonist of the NMDA receptor, disrupts the spatial memory of rats. Rats were trained with the hidden platform (circle) in quadrant 2. During the test, the platform was removed and the trajectory was recorded. Top, while the control rat focused its search near the phantom platform, the rat infused with AP5 during training swam randomly. Bottom, quantification of time spent in four quadrants. (B) Throughout the training regime, mice with CA1-specific knockout of the GluN1 subunit of the NMDA receptor (red trace) were slower to

find the hidden platform than CA1-Cre only control mice (blue trace). (C) Mice in which the CaMKII auto-phosphorylation site is mutated (red trace) also took longer to find the hidden platform compared with controls (blue trace). See Figures 10-10 and 10-12 for LTP defects of the same mice as in panels B and C. (A, adapted from Morris RGM, Anderson E, Lynch GS et al. [1986] *Nature* 319:774–776. With permission from Macmillan Publishers Ltd; B, adapted from Tsien JZ, Huerta PT & Tonegawa S [1996] *Cell* 87:1327–1338. With permission from Elsevier Inc.; C, adapted from Giese KP, Federov NB, Filipkowski RK et al. [1998] *Science* 279:870–873.)

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

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

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



**Figure 10–34** Interfering with the cAMP/PKA pathway affects hippocampal LTP and learning. **(A)** Compared with wild-type (WT) controls, mice in which two  $\text{Ca}^{2+}$ -dependent adenylate cyclase were doubly knocked out (DKO) exhibit reduced magnitude of LTP at the CA3 → CA1 synapse. **(B)** DKO mice also exhibit reduced memory in a passive avoidance task. In this assay, mice are placed in the illuminated compartment of a two-compartment chamber. Before

training, mice quickly move into the dark compartment as a natural tendency to avoid predators. After training (pairing electric shocks with entrance to the dark compartment), DKO mice avoid the dark compartment similarly to controls 5 min after the training, but enter the dark compartment more quickly 30 min after the training, suggesting an impaired memory. (Adapted from Wong ST, Athos J, Figueroa XA et al. [1999] *Neuron* 23:787–798. With permission from Elsevier Inc.)

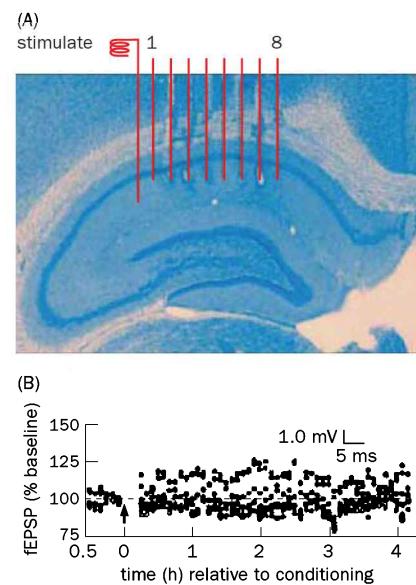
### 10.21 From correlation to causation: the synaptic weight matrix hypothesis revisited

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

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

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

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



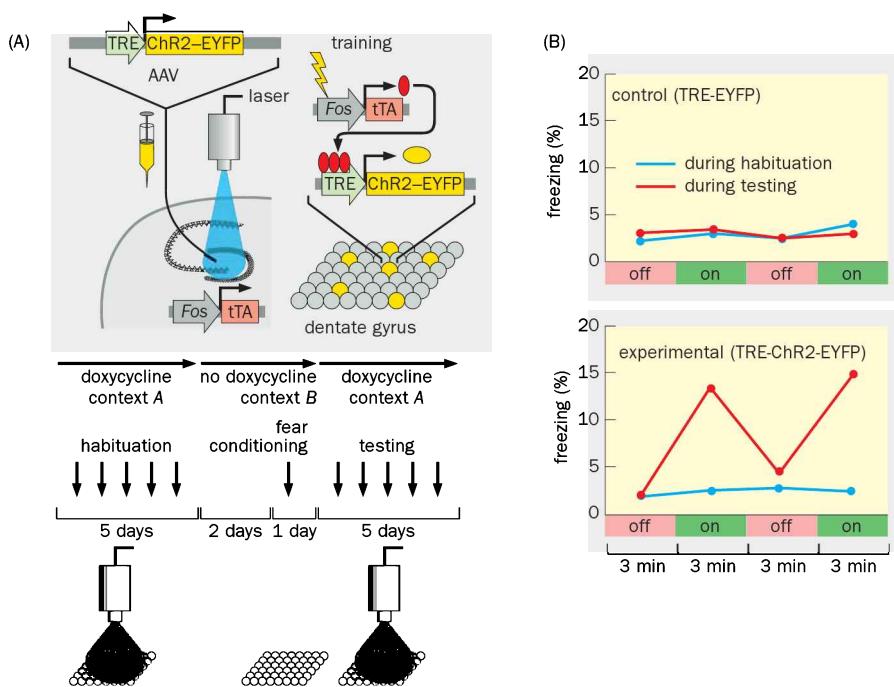
**Figure 10–35 Learning can induce LTP.** (A) A multi-electrode array (electrodes 1–8) was placed in the CA1 area of a rat hippocampus to record the responses of CA1 neurons to stimulation of Schaffer collaterals. (B) After passive avoidance training, recordings from the two electrodes indicated by red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

### Box 10–3: How to find an engram

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

To identify the active population of neurons, the *Fos-tTA* transgenic mouse was used, in which the expression of a **tetracycline-repressible transcriptional activator** (tTA)

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



**Figure 10–36 Optogenetic stimulation of a specific population of dentate gyrus granule cells activates fear memory.**

(A) Experimental design. Adeno-associated virus (AAV) enables the expression of channelrhodopsin (ChR2) fused with an enhanced yellow fluorescent protein (EYFP) under the control of a tetracycline response element (TRE). AAV was injected into the dentate gyrus (indicated by the needle) of a transgenic mouse that express the tetracycline-repressible transcription activator (tTA, red ovals) from the *Fos* promoter so that its expression is induced by neuronal activity. The matrix of circles represents dentate gyrus granule cells. Mice were first exposed to and became habituated to context A and photostimulation in the presence of doxycycline; the dentate gyrus granule cells activated in context A did not express ChR2, because tTA activity is inhibited by doxycycline. Fear conditioning was induced

in context B in the absence of doxycycline such that dentate gyrus granule cells activated by this experience expressed ChR2 (yellow circles); ChR2 expression persisted for several days even after the mice were treated with doxycycline again to prevent further tTA-induced gene expression. Mice were then reintroduced to context A to test whether optogenetic stimulation of ChR2-expressing cells could induce fear memory recall. (B) In control mice, in which tTA induced expression of EYFP, optogenetic activation (light-on period in green) did not induce fear memory, as assayed by the percentage of time spent freezing (top). In experimental mice, optogenetic stimulation induced freezing in a light-dependent manner (bottom) during the testing period but not during the earlier habituation period. (Adapted from Liu X, Ramirez S, Pang PT et al. [2012] *Nature* 484:381–385. With permission from Macmillan Publishers Ltd.)

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**Box 10–3: How to find an engram**

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

To test the effect of reactivation of neurons that were active during fear conditioning in context *B*, mice were given food containing doxycycline to prevent new tTA/TRE-induced ChR2 expression, and were introduced to context *A* with or without optogenetic stimulation (Figure 10–36A). Control mice did not freeze in context *A*. However, ChR2-expressing mice froze in context *A* in response to optogenetic stimulation, as if they were in context *B* (Figure 10–36B). Thus,

activation of a population of cells that were active during contextual fear conditioning was sufficient to induce fear recall in a different context, suggesting that this population of dentate gyrus granule cells contributes to the memory of context *B*.

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

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

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

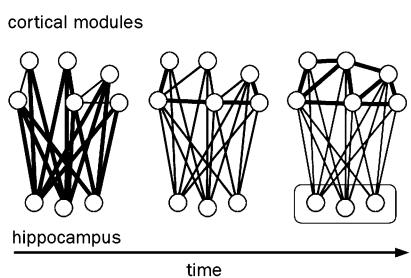
### 10.22 The neocortex contributes to long-term storage of explicit memory

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

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



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



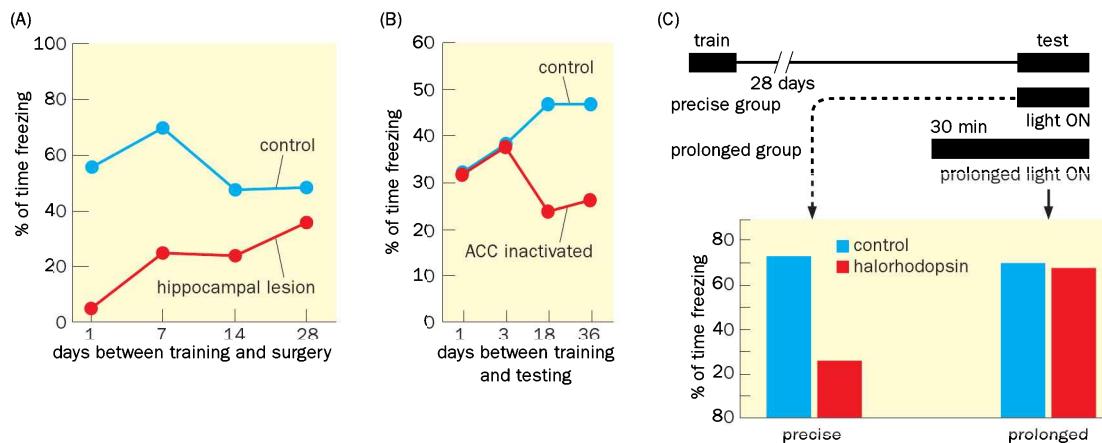
**Figure 10–38 A model illustrating the interactions between the hippocampus and neocortex during long-term memory consolidation.** During the initial memory formation (left), signals that pass through different cortical areas to the hippocampus establish links between the hippocampus and those cortical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

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

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

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

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

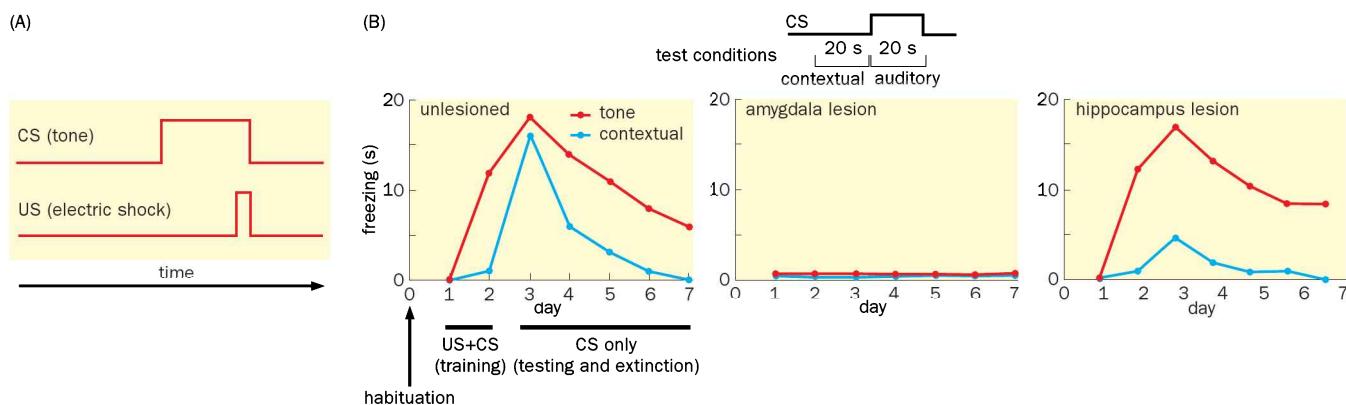


**Figure 10–39 Interplay between the hippocampus and neocortex in contextual fear conditioning.** **(A)** The x axis shows days elapsed between training and the surgery that bilaterally lesioned the hippocampi in experimental rats. All rats were tested for contextual fear conditioning (y axis) 7 days after the surgery. Compared with unlesioned controls that went through the same surgical procedure (blue trace), lesioned rats (red trace) did not exhibit fear memory (quantified by the percentage of time spent freezing) when lesioning was performed 1 day after training. The effect of lesioning became less pronounced as the period between training and lesioning lengthened. **(B)** Injection of lidocaine, an anesthetic that blocks action potentials, into the anterior cingulate cortex (ACC; red trace), reduced contextual fear memory compared with controls (blue trace) when drug administration and testing were performed 18 or 36 days, but not 1 or 3 days, after training. This finding suggests

that the ACC is required for recall of remote memory but not recent memory. **(C)** Compared with controls that expressed a fluorescent protein, optogenetic silencing of mouse hippocampal neurons expressing a modified halorhodopsin significantly reduced contextual fear memory if silencing (light ON) was precisely timed with testing (precise group). If silencing occurred 30 minutes prior to as well as during the testing (prolonged group), the reduction in fear memory disappeared. This suggests that a compensatory mechanism was used for fear memory if hippocampal neurons were silenced under the prolonged condition. (A, adapted from Kim JJ & Fanselow MS [1992] *Science* 256:675–677; B, adapted from Frankland PW, Bontempi B, Talton LE et al. [2004] *Science* 304:881–883; C, adapted from Goshen I, Brodsky M, Prakash R et al. [2011] *Cell* 147:678–689. With permission from Elsevier Inc.)

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

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



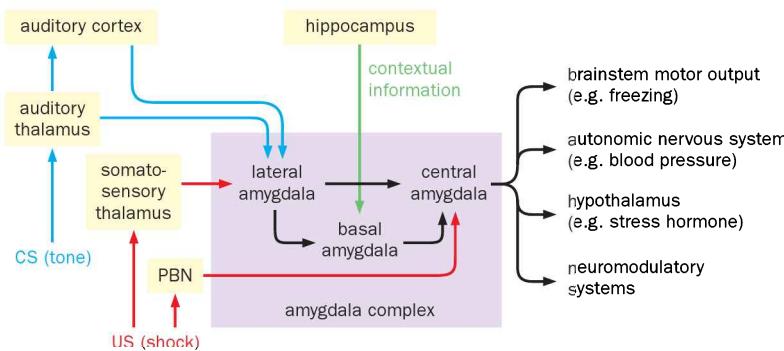
**Figure 10–40 Both auditory and contextual fear conditioning require the amygdala.** (A) Schematic of auditory fear conditioning, a form of classical conditioning in which the tone (~20 s) serves as a conditioned stimulus (CS) and the electric shock (~0.5–1 s) serves as an unconditioned stimulus (US) that co-terminates with the CS. (B) Left, learning curve as measured by average time spent freezing within a 20-s period (y axis) for control rats. On day 0, rats were introduced into the conditioning chamber without shock for habituation. On days 1 and 2, a 20-s tone was paired with a 0.5-s electric shock at the end of the tone, with two pairings per day. On days 3–7, only

the tone was presented in the same conditioning chamber. As shown in the schematic above, contextual conditioning was measured as the freezing time during the 20 s immediately prior to the CS (tone) onset, whereas auditory conditioning was measured during the 20-s tone period. Middle, lesioning of the amygdala prior to training disrupted both contextual and auditory conditioning. Right, lesioning of the hippocampus disrupted contextual conditioning without affecting auditory conditioning. (B, adapted from Phillips RG & LeDoux JE [1992] *Behav Neurosci* 106:274–285. With permission from the American Psychological Association Inc.)

### 10.23 The amygdala plays a central role in fear conditioning

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

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

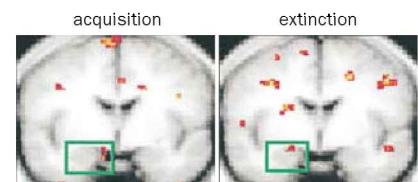


via an indirect pathway from high-order auditory cortex. Information can be sent by lateral amygdala neurons to the central amygdala either directly or indirectly via the basal amygdala. The foot shock (US) also reaches the amygdala by multiple pathways, including projections from the somatosensory thalamic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

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

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

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



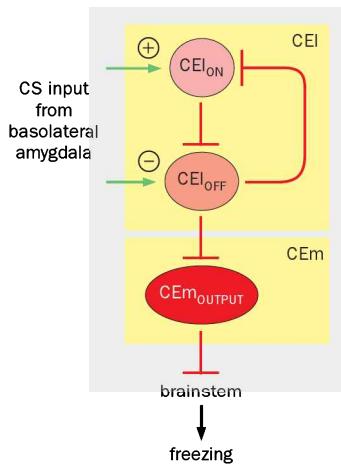
**Figure 10–42 Acquisition and extinction activate the amygdala in humans.** Average fMRI images of 10 healthy human subjects during the acquisition (left) and extinction (right) phases of fear conditioning. The green box in each image highlights the right amygdala, showing activation (yellow to red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

#### Box 10-4: Microcircuits of the central amygdala

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

CEl<sub>ON</sub> cells mostly restrict their projections within the CEl, and most projections from the CEl to the CEm are from

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

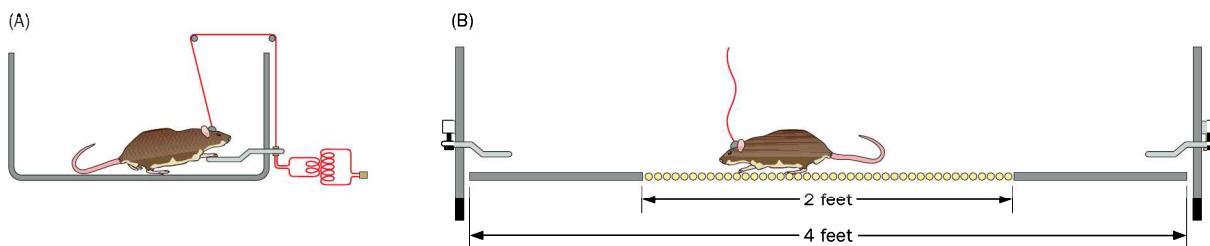


**Figure 10-43 Microcircuits of the central amygdala.** The central amygdala consists of a lateral compartment (CEI) and a medial compartment (CEm). Within the CEI, two separate GABAergic neuronal populations have been identified. CEl<sub>ON</sub> neurons acquire a potentiated conditioned stimulus (CS)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

#### 10.24 Dopamine plays a key role in reward-based learning

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

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



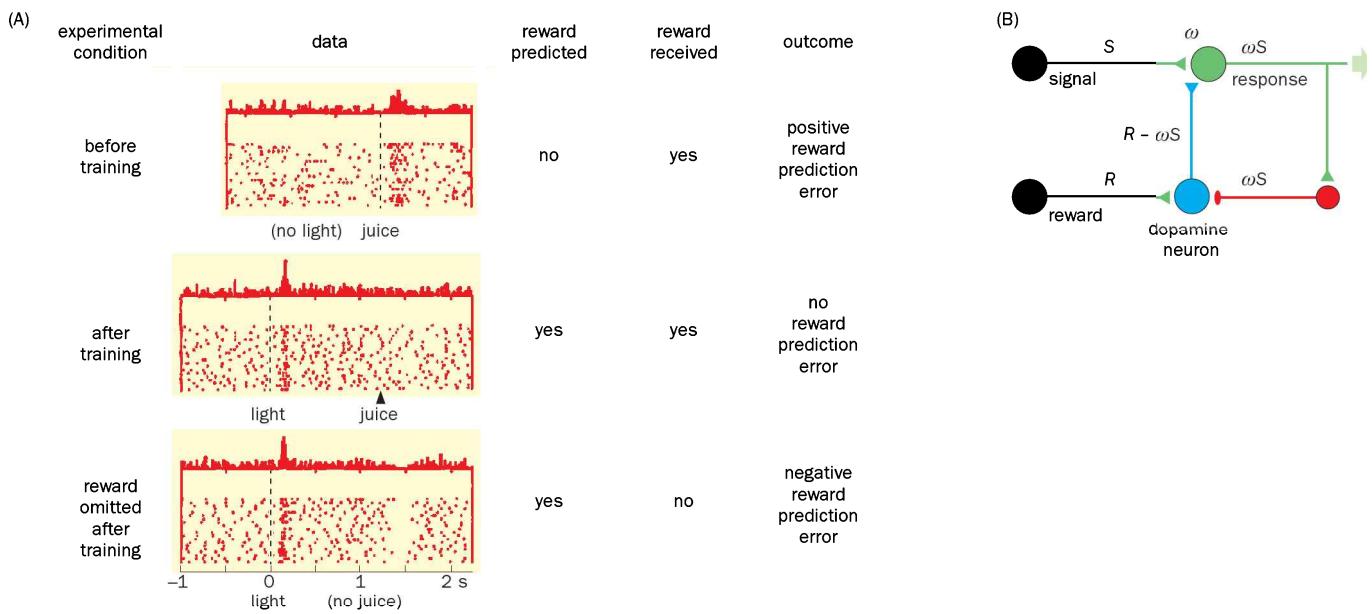
**Figure 10–44 Electrical self-stimulation.** (A) Design of the experiment. When the rat presses the lever, the electrode implanted in its brain connects to a source of current so that the neurons or axon bundles near the electrode tip become excited. If the excited neurons or axons signal reward, the rat will keep pressing the lever. (B) In this experiment, after the rat receives rewarding electric stimulation on one side of the arena, it must run across the center

grid to the other side to receive more stimulation. After that rat had learned the task, electrical shock was applied in the central portion of the arena. If the electrode was implanted in the reward centers, rats withheld more foot shock when crossing the grid to receive electrical brain stimulation than they did when crossing the grid for food after 24 hours of food deprivation. (Adapted from Olds J [1958] *Science* 127:315–324.)

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

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

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



**Figure 10–45 Dopamine neurons, reward prediction error, and reinforcement-based learning.** (A) *In vivo* single-unit recordings of a midbrain dopamine neuron of a monkey trained to associate light onset with a reward (a drop of juice). Within each of the three blocks, each row is a separate trial and each vertical bar is an action potential. Above the individual trials is a histogram that combines the firing rates from all trials. Top, prior to training, phasic firing was triggered by reward (juice) delivery (vertical dashed line). Middle, after training, phasic firing was triggered by light onset (vertical dashed line) but not by reward delivery (arrowhead). Bottom, in trials when reward was omitted after training, tonic firing was depressed around the time reward was expected. Individual trials are aligned with reward delivery (top) or light onset (middle and bottom). Phasic firing of this dopamine neuron can be interpreted to signal the difference between the actual and expected reward, as summarized on the right. (B) An abstract circuit model for reward-based learning. A signal neuron produces a signal with the magnitude  $S$ , and connects with a response neuron through a synapse whose strength  $\omega$  can be adjusted, resulting in a response whose magnitude is the product of  $\omega$  and  $S$ . In addition to

sending information to downstream circuits (arrow), the response is also transmitted to a feedback inhibitory neuron (red), which in turn sends output to a dopamine neuron (blue). The dopamine neuron also receives an excitatory input that delivers a reward signal with a magnitude of  $R$ . Thus, the dopamine neuron positively adjusts  $\omega$  with an output magnitude of  $R - \omega S$  (for simplicity we assume that synaptic transmission is faithful and integration is linear). Before training,  $\omega$  is small and  $R - \omega S$  is large, resulting in a large magnitude of dopamine release to increase  $\omega$ . As training proceeds,  $\omega$  increases and  $R - \omega S$  decreases; when  $R - \omega S$  becomes zero, training is accomplished. Note that although an excitatory neuron (green) is used as the response neuron, in the midbrain dopamine circuit, the response neuron is GABAergic spiny projection neurons (SPNs). SPNs can either signal directly to dopamine neurons to deliver  $\omega S$ , or through an intermediate GABAergic neuron to signal to the feedback inhibitory neuron. (A, adapted from Schultz W, Dayan P & Montague R [1997] *Science* 275:1593–1599. With permission from AAAS; B, adapted from Schultz W & Dickinson A [2000] *Ann Rev Neurosci* 23:473–500.)

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

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

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

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

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

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

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

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

In the first example, a random population of piriform cortical neurons in mice was transduced with an adeno-associated virus to express ChR2, such that they fire action

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

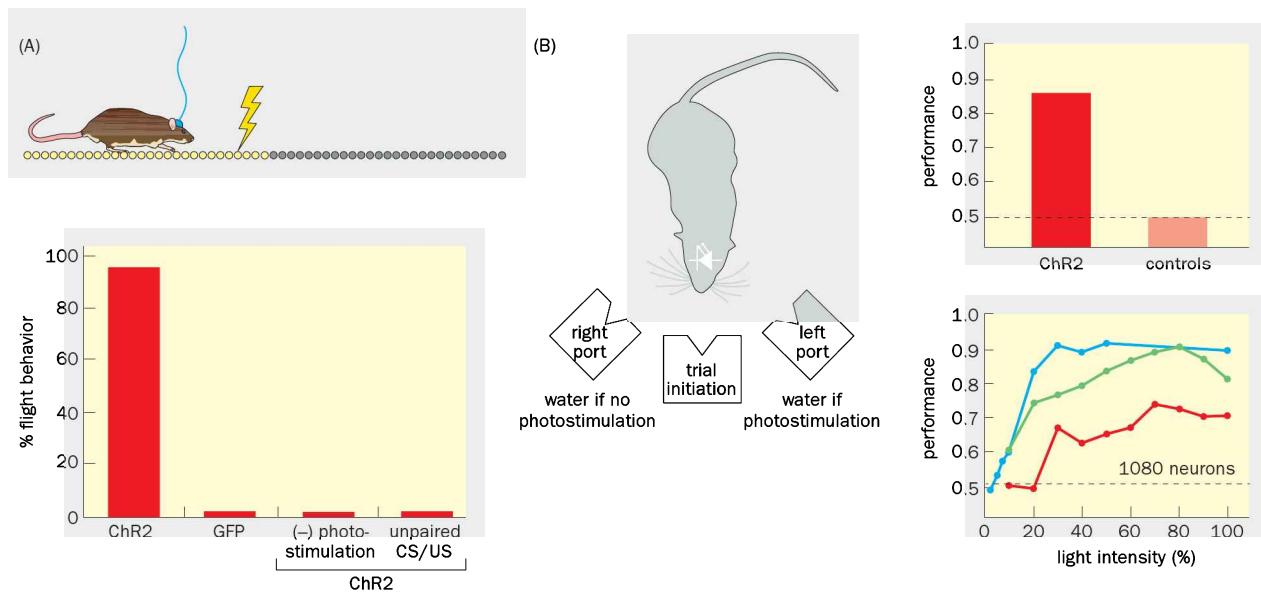
(Continued)

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

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

the water reward. The effectiveness of photostimulation depended on the number of ChR2-expressing neurons and the strength and the duration of photostimulation (Figure 10–46B, right). Single action potentials (elicited by 1-ms photostimulation) in about 300 ChR2-expressing layer 2/3 neurons of the barrel cortex served as a sufficient cue to bias the mouse to the reward port.

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

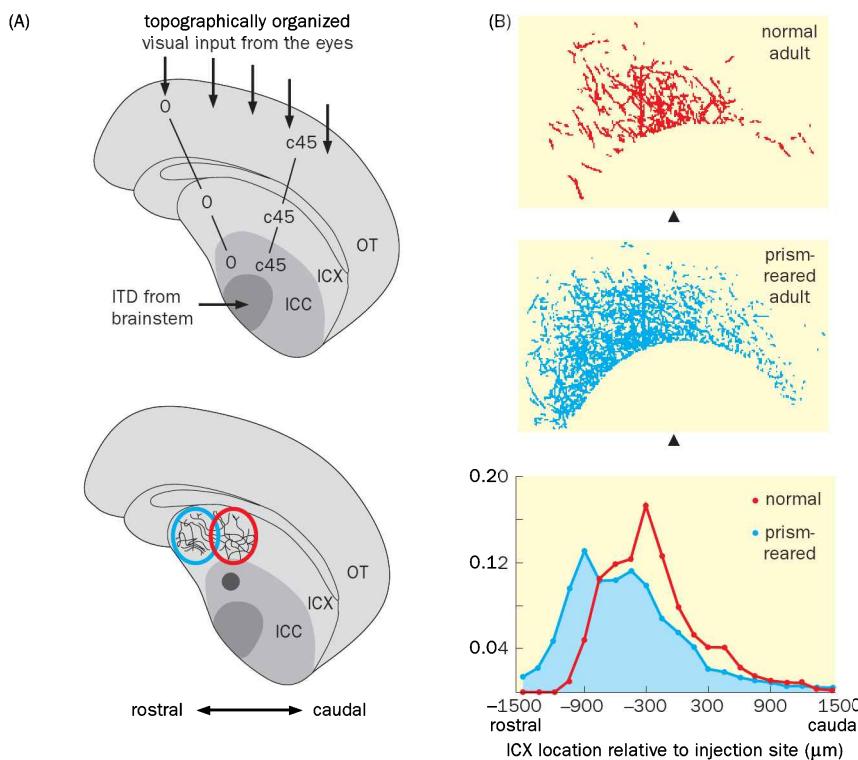


**Figure 10–46 Forming a memory by activating a random population of cortical neurons.** (A) Top, schematic of the experimental design. During training, when the mouse moved to the left side of the chamber, it received an electrical shock (yellow) while its piriform cortex, which contained channelrhodopsin (ChR2)-expressing neurons, was photostimulated. After two training sessions (each of which consisted of 10 pairings), mice readily exhibited flight behavior in response to photostimulation alone (bottom, left bar). In negative control groups, mice did not exhibit flight behavior when green fluorescent protein (GFP) instead of ChR2 was expressed, when photostimulation was omitted during training, or when photostimulation was not paired with shock. (B) Left, experimental design. The mouse was trained to place its snout at the center port to initiate the trial. Water was delivered to the left or the right port depending on whether the mouse received photostimulation or not during the trial. Right, After 4–7 sessions of

trials (200–800 trials per session), ChR2-expressing mice reliably reported photostimulation by making proper port choices compared to controls that did not express ChR2; the dotted line indicates port selection at chance levels. Performance on the y axis is the ratio of the number of corrected trials, which include turning left in photostimulation trials and turning right in no-photostimulation trials, over the total number of trials. Performance increased as the number of ChR2 neurons increased (shown at bottom is an example of 1080 ChR2-expressing neurons), with light intensity, and with the number of 1-ms pulses of light (red, 1 pulse; green, 2 pulses; blue, 5 pulses; separate experiments indicated that each pulse elicited at most one action potential in ChR2-expressing neuron at 100% light intensity). (A, adapted from Choi GB, Stettler DD, Kallman BR et al. [2011] *Cell* 146:1004–1015. With permission from Elsevier Inc.; B, adapted from Huber D, Petreanu L, Ghitani N et al. [2008] *Nature* 451:61–64. With permission from Macmillan Publishers Ltd.)

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

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



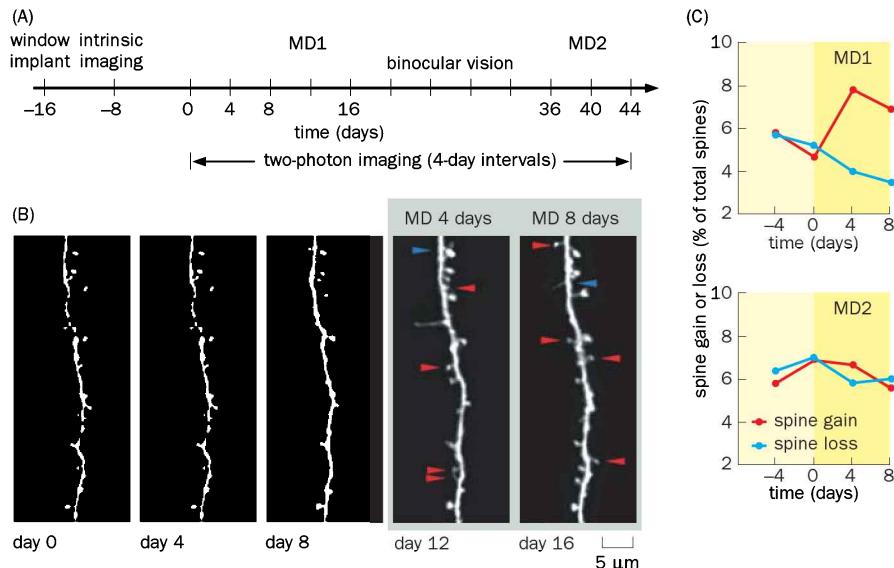
**Figure 10–47 Adaptive axonal expansion in the inferior colliculus and auditory map adjustment in juvenile and adult owls.** (A) Top, representation of auditory and visual information in the owl's brain. Brainstem inputs to the central (ICC) and external (ICX) nuclei of the inferior colliculus are topographically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

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

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

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

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



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

## SUMMARY

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

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

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

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

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

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

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