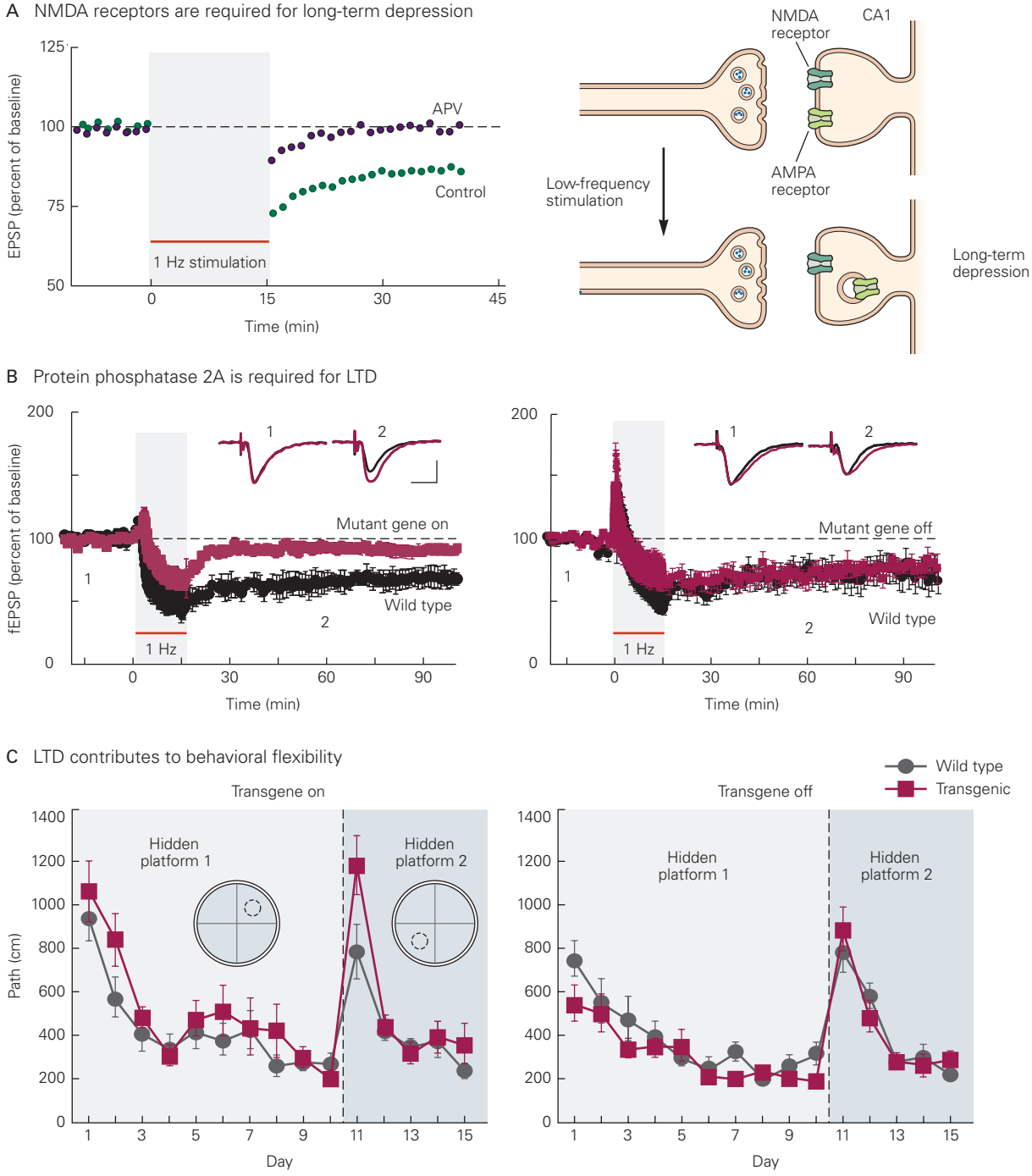


Figure 54–10 Long-term depression of synaptic transmission requires *N*-methyl-D-aspartate (NMDA) receptors and phosphatase activity.

A. Prolonged low-frequency stimulation (1 Hz for 15 minutes) of Schaffer collateral fibers produces a long-term decrease in the size of the field excitatory postsynaptic potential (**fEPSP**) in the hippocampal CA1 region, a decrease that outlasts the period of stimulation (control). Long-term depression (**LTD**) occurs when α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (**AMPA**) receptors are removed from the postsynaptic membrane by endocytosis; it is blocked when the NMDA receptors are blocked by the drug 2-amino-5-phosphonovaleric acid (**APV**). (Adapted from Dudek and Bear 1992.)

B. LTD requires protein dephosphorylation. The plots compare LTD in the hippocampal CA1 region of wild type mice and transgenic mice that express a protein that inhibits phosphoprotein phosphatase 2A. Transgene expression is under control of the tTA system. In the absence of doxycycline, the phosphatase inhibitor is expressed, and induction of LTD is inhibited (*left plot*). When expression of the phosphatase inhibitor is turned off by administering doxycycline, a normal-sized LTD is induced (*right plot*).

C. Inhibition of phosphatase 2A reduces behavioral flexibility. Transgenic mice expressing the phosphatase inhibitor learn the location of a submerged platform in the Morris maze at the same rate as wild-type mice (days 1–10). Thus, LTD is not necessary for learning the initial platform location. At the end of day 10, the platform is moved to a new hidden location and the mice are retested (days 11–15). Now the transgenic mice travel significantly longer paths to find the platform on the first day of retesting (day 11), indicating an impaired learning (reduced flexibility). When transgene expression is turned off with doxycycline, the transgenic mice display normal learning on all phases of the test. (Panels B and C reproduced, with permission, from Nicholls et al. 2008.)

Long-term depression may also depend on a surprising metabotropic action of the ionotropic NMDA receptor-channels. Glutamate binding, in addition to opening the receptor pore, is thought to trigger a conformational change in a cytoplasmic domain of the receptor that directly activates a downstream signaling cascade that increases the activity of phosphoprotein phosphatase 1 (PP1). Activation of PP1 or calcineurin eventually leads to changes in protein phosphorylation that promote endocytosis of AMPA receptors, resulting in a decrease in the size of an EPSP.

Distinctly different forms of LTD can be induced through the activation of G protein–coupled metabotropic glutamate receptors. Such forms of LTD depend on activation of mitogen-activated protein (MAP) kinase signaling pathways (Chapter 14) rather than activation of phosphatases. These types of LTD lead to a reduction in synaptic transmission through a decrease in glutamate release from presynaptic terminals as well as through alterations in the trafficking of AMPA receptors in the postsynaptic cells.

Much less is known about the behavioral role of LTD compared to that of LTP, but some insight has come from studies with mice using a transgene that expresses an inhibitor of protein phosphatase. LTD that depends on NMDA receptors is inhibited when the transgene is expressed but is normal when transgene expression is suppressed (Figure 54–10B). Transgene expression does not affect LTP or forms of LTD that involve metabotropic glutamate receptors. Mice that express the transgene show normal learning the first time they are tested in the Morris maze. However, when the same mice are retested after the hidden platform has been moved to a new location, they show a decreased ability to learn the new location and tend to persevere in searching for the platform near the previously learned location (Figure 54–10C). Thus, LTD may be necessary not only to prevent LTP saturation but also to enhance flexibility in memory storage and specificity in memory recall. Studies on fear conditioning suggest that LTD in the amygdala may be important for reversing learned fear.

Memory Is Stored in Cell Assemblies

While the cumulative evidence for a relationship between long-term synaptic plasticity and memory formation is strong, we know less about how specific cellular processes such as LTP enable memory formation. This reflects limitations in our knowledge of how neural circuits operate and how memories might be embedded in them. Theoretical models for memory storage in neural circuits can be traced to Hebb's concept of a cell assembly—a network of neurons that is activated whenever a function is executed; for example, each time a memory is recalled. Cells within an assembly are bound together by excitatory synaptic connections strengthened at the time the memory was formed.

Today, more than half a century later, Hebb's thoughts still form the framework for how the hippocampus mediates the storage and recall of memory, although experimental proof has been difficult to obtain. A proper test requires recording the activity of thousands of neurons simultaneously, in combination with the experimental excitation or inactivation of selected cell groups. Technological advances are now enabling such experiments. By and large, the results obtained so far confirm Hebb's cell assembly model and implicitly point to LTP as the mechanism for their formation.

In a telling study with mice, Susumu Tonegawa and his colleagues tested whether reactivation of neurons that participated in the storage of a specific memory is sufficient to trigger recall of that memory. The researchers first applied an electric shock to an animal as it explored a novel environment. Reexposure of the animal to the same environment a day or more later elicited a freezing response, indicating that the animal associated the environment or context (the conditioned stimulus) with the shock (the unconditioned stimulus). Using a genetic strategy, Tonegawa caused a subset of dentate gyrus granule neurons that were active during the fear conditioning to express the light-activated cation channel channelrhodopsin-2 (Figure 54–11). The conditioned animals were subsequently placed in a novel environment that did not resemble the conditioned environment and so did not elicit a fear response. However, light activation of the subset of granule cells that were active during fear conditioning was able to elicit a strong freezing response, even though the animals were in a nonthreatening environment. This supports the idea that memories are stored in cell assemblies and, more importantly, demonstrates that reactivation of these assemblies is sufficient to induce recall of an experience.

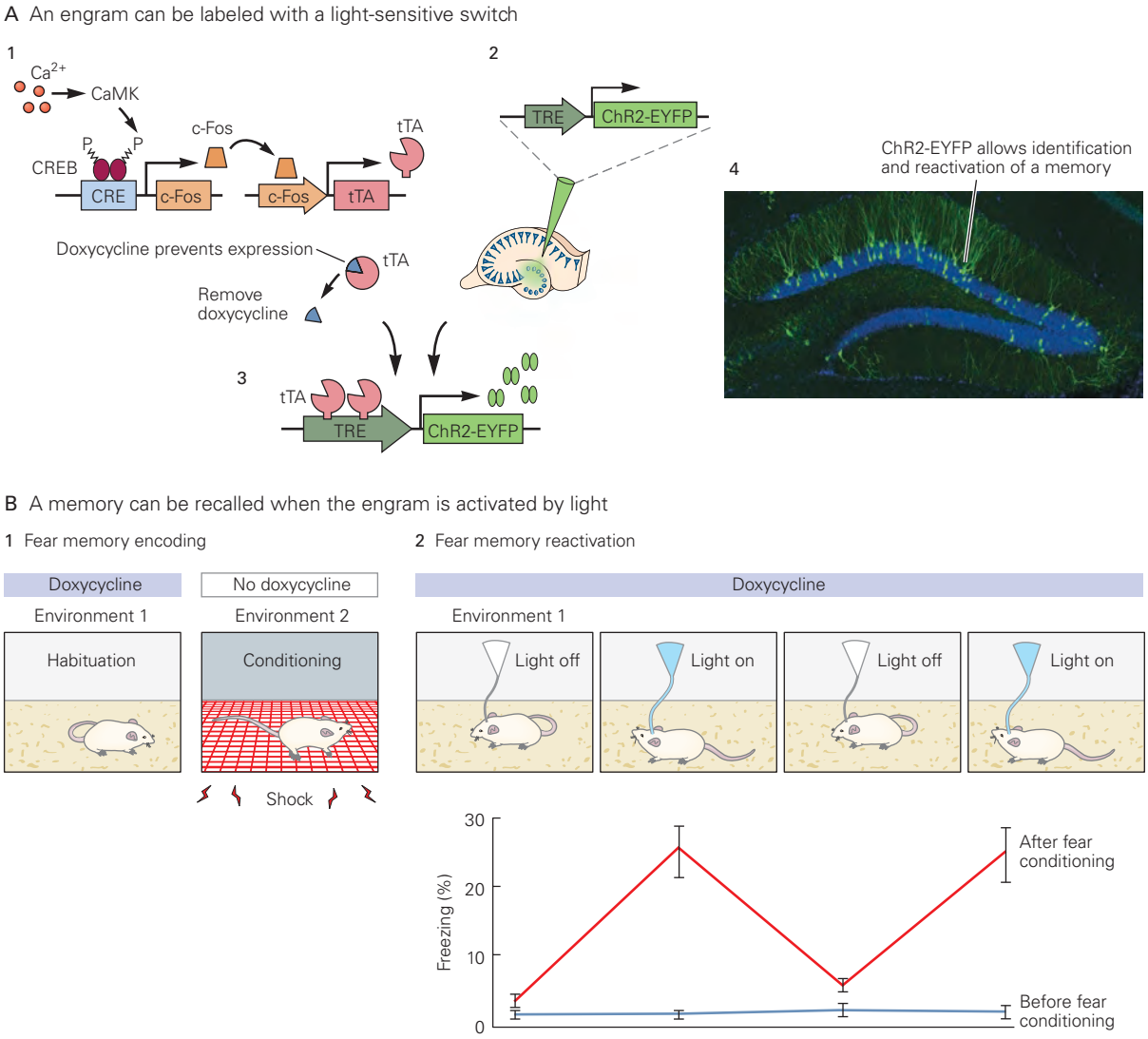
In a complementary experimental approach, a light-activated inhibitory Cl^- transporter was expressed in CA1 cells active at the time of fear conditioning. Later, the labeled cells were inactivated and the animals were placed again in the environment in which they received the shock. Under these conditions, the normal freezing behavior (ie, recall of the memory of fear conditioning) was blocked, suggesting that activity in the labeled CA1 cell population was necessary for memory retrieval. Taken together, these findings

suggest that reactivation of the specific cell assembly pattern that occurred during encoding is both necessary and sufficient for memory retrieval.

Perhaps the most direct test of the ensemble model is the creation of a false memory. Tonegawa and colleagues expressed channelrhodopsin in cells that were active during exploration of a novel environment (context A), except that no shock was delivered this time. At a later time, the labeled cells were reactivated using light stimulation as the mice explored a second novel environment (context B), this time in combination with an electric shock. When the animals were returned to the neutral context A, they froze, although they had never been shocked in this environment. This result indicates that the reactivation of the original engram of context A when paired with an aversive experience in context B is able to create a false memory, causing the animals to fear context A. Thus, it is possible to modify the behavioral significance of a neural representation (a pattern of neural firing in response to a given stimulus) by pairing the assembly with a new experience unrelated to the original experience.

Different Aspects of Explicit Memory Are Processed in Different Subregions of the Hippocampus

Explicit memory stores knowledge of facts (semantic memory), places (spatial memory), other individuals (social memory), and events (episodic memory). As discussed above, successful storage and recall of explicit



memory requires that patterns of activity be formed within local cell assemblies to avoid mix-up between memories. At the same time, an important psychological feature of hippocampal-dependent memory is that a few cues are usually enough to trigger the recall of a complex memory. How does the hippocampus perform all of these diverse functions? Do its subregions have specialized roles, or is memory a unitary function of the hippocampus? In at least some instances, it has been possible to assign key functions to specific areas of the hippocampus.

The Dentate Gyrus Is Important for Pattern Separation

How does the hippocampus store a different pattern of neural activity in response to every experience that needs to be remembered, including patterns that distinguish between two closely related environments? Contemporary ideas about how neural circuits accomplish this task, often referred to as *pattern separation*, dates to the theoretical work of David Marr in the late 1960s and early 1970s. In a landmark paper on the cerebellum, Marr suggested that the extensive divergence of mossy-fiber inputs onto an extraordinarily large number of cerebellar granule cells might allow for pattern separation in this system.

This idea of "expansion recoding," in which distinct firing patterns are formed through the projection of a limited number of inputs onto a larger population of synaptic target cells, was later applied by others to the hippocampus. They proposed that hippocampal pattern separation results from the divergence of entorhinal inputs onto a larger number of granule cells in the dentate gyrus. The findings of subsequent experimental studies are broadly in line with these theoretical suggestions: Neural activity patterns recorded in different environments differ more extensively in the dentate gyrus and CA3 than they do one synapse upstream in the entorhinal cortex. The dentate gyrus is also implicated in pattern separation by the fact that lesions or genetic manipulations targeted to this area impair the ability of rats and mice to discriminate between similar locations and contexts.

The dentate gyrus is the site of one of the most unexpected findings in neuroscience, the discovery that the birth of new neurons, or neurogenesis, is not limited to early stages of development. New neurons

Figure 54–11 (Opposite) Stimulating a neuronal assembly associated with a stored memory of fear conditioning elicits fear behavior. (Panels reproduced or redrawn, with permission, from Liu et al. 2012. Copyright © 2012 Springer Nature.)

A. *Experimental protocol*. 1. Exposure of a mouse to a new environment increases activity in a group of hippocampal neurons (cell assembly) that codes for the environment. The activity increases intracellular Ca^{2+}, which activates a CaM kinase signaling cascade, resulting in phosphorylation of the transcription factor CREB. Phosphorylated CREB increases expression of immediate early genes, including the c-Fos transcription factor. In the *c-fos-tTA* transgenic mouse line, c-Fos binds to the *c-fos* promoter of the transgene and thereby initiates expression of the transcription factor tTA. The antibiotic doxycycline is fed to mice, which binds to and inhibits tTA, until the day of the experiment. 2. The dentate gyrus of the same transgenic mice was previously injected with an adeno-associated virus that contains a DNA sequence encoding ChR2 fused to the fluorescent marker protein EYFP (ChR2-EYFP). The transcription of this sequence is under control of the TRE promoter, which requires tTA (without doxycycline) for expression. 3. Exposure of the mice to a novel environment (after removing doxycycline from the feed) leads to expression of tTA and subsequent expression of ChR2-EYFP in a subset of active dentate gyrus neurons. 4. The ChR2-EYFP remains expressed for several days in the neurons, as seen by the EYFP fluorescence signal in dentate gyrus granule cells in a hippocampal slice. (ChR2-EYPF in **green**, dentate gyrus cell body layer in **blue**.)

B. *Recall of a fear memory*. An optical fiber is implanted above the dentate gyrus. 1. During fear memory encoding, mice were first habituated in one environment while being fed doxycycline (which prevents expression of ChR2-EYFP). The mice were then taken off doxycycline and exposed to a new environment for a few minutes. This turns on gene transcription in the assembly of neurons that are active in the new environment, leading to prolonged expression of ChR2-EYFP in these cells. The mice were then given a series of footshocks while in the new environment to induce fear conditioning: The mice learn to associate the new environment with a fearful stimulus. The mice were then returned to their cage and put back on doxycycline. 2. During fear memory reactivation 5 days after conditioning, mice show a normal defensive freezing behavior when reintroduced to the environment where they received footshocks (not shown). However, when mice are exposed to the environment to which they were initially habituated (no associated foot shock), they normally recognize this as a neutral environment and do not exhibit defensive freezing. However, as the mice explore the neutral environment, delivery of blue light to activate ChR2-expressing neurons in the dentate gyrus causes the mice to freeze. This indicates that activation of the ensemble of ChR2-expressing neurons initially activated in the conditioning environment is sufficient to recall the fear memory associated with that environment. The experimental data show the freezing response in the neutral environment is much greater when light pulses are turned on compared to when the light is off (**red plot**; light delivery indicated in cartoon on top). Delivery of light pulses to an animal that had not undergone fear conditioning does not elicit freezing (**blue plot**).

continue to be born from precursor stem cells throughout adulthood and become incorporated into neural circuits. Nevertheless, adult neurogenesis is limited to granule neurons in two brain regions: inhibitory granule cells in the olfactory bulb and the excitatory granule neurons of the dentate gyrus. Recent experimental findings raise the possibility that newly born granule neurons in the adult are particularly important for pattern separation, even though they represent only a minor fraction of the total number of granule cells. Procedures that stimulate neurogenesis enhance the ability of a mouse to discriminate between closely related environments. Experimental silencing of all dentate gyrus granule neurons except those newly born in the adult does not seem to impair pattern separation, implying that it is the newborn neurons that are most essential to pattern separation. Although some uncertainties remain on the role of neurogenesis in pattern separation and memory encoding, methods that enhance neurogenesis are currently being explored as a means of treating different types of age-related memory loss.

The CA3 Region Is Important for Pattern Completion

A key feature of explicit memory is that a few cues are often sufficient to retrieve a complex stored memory. Marr suggested in a second landmark paper in 1971 that the recurrent excitatory connections of CA3 pyramidal cells might underlie this phenomenon. He proposed that when a memory is encoded, neuronal activity patterns are stored as changes in connections between active CA3 cells. During subsequent retrieval of the memory, the reactivation of a subset of this stored cell assembly would be sufficient to activate the entire original neural ensemble that encodes the memory because of the strong recurrent connections between the cells of the ensemble. This restoration is referred to as *pattern completion*.

The importance of LTP for pattern completion in the CA3 network is seen in studies with mice in which the NMDA glutamate receptor is selectively deleted from the CA3 neurons. These mice experience a selective loss of LTP at the recurrent synapses between CA3 neurons, with no change in LTP at the synapses between mossy fibers and CA3 neurons or at the Schaffer collateral synapses between CA3 and CA1 neurons. Despite this deficit, the mice show normal learning and memory for finding a submerged platform in a water maze using a complete set of spatial cues. However, when the mice are asked to find the platform with fewer spatial cues, their performance is impaired, indicating that LTP at the recurrent synapses between CA3 neurons is important for pattern completion.

The CA2 Region Encodes Social Memory

Studies comparing neuronal representations in the dentate gyrus and CA3 and CA1 areas have indicated that each region has a unique function in the storage and retrieval of hippocampal memory. Recent evidence suggests that the CA2 region plays a crucial role in social memory, the ability of an individual to recognize and remember other members of its own species (conspecifics). Genetic silencing of CA2 disrupts the ability of a mouse to remember encounters with other mice, but does not impair other forms of hippocampal-dependent memory, including memory of objects and places.

The CA2 region is also unique among hippocampal regions in having very high levels of receptors for the hormones oxytocin and vasopressin, important regulators of social behaviors. Selective stimulation of the vasopressin inputs to CA2 neurons can greatly prolong the duration of a social memory. Social memory also depends on CA1 neurons in the ventral region of the hippocampus, an area linked to emotional behavior, which receives important input from CA2.

A Spatial Map of the External World Is Formed in the Hippocampus

How do hippocampal neurons encode features of the external environment to form a memory of spatial locale, enabling an animal to navigate to a remembered goal? At the end of the 1940s, the cognitive psychologist Edward Tolman proposed that somewhere in the brain there must be representations of one's environment. He referred to these neural representations as cognitive maps. They were thought to form not only an internal map of space but also a mental database in which information is stored in relation to an animal's position in the environment, similar to the GPS coordinates of a photograph.

Tolman did not have the opportunity to determine whether a cognitive map actually existed in the brain, but in 1971, John O'Keefe and John Dostrovsky discovered that many cells in the CA1 and CA3 areas of the rat hippocampus fire selectively when an animal is located at a specific position in a specific environment. They called these cells "place cells" and the spatial location in the environment where the cells preferentially fired "place fields" (Figure 54–12A,B). When the animal enters a new environment, new place fields are formed within minutes and are stable for weeks to months.

Different place cells have different place fields, and collectively, they provide a map of the environment, in the sense that the combination of currently active cells is sufficient to read out precisely where the animal is in the environment. A place-cell map is not egocentric in its organization, like the neural maps for touch or vision on the surface of the cerebral cortex. Rather, it is allocentric (or geocentric); it is fixed with respect to a point in the outside world. Based on these properties, John O'Keefe and Lynn Nadel suggested in 1978 that place cells are part of the cognitive map that Tolman had in mind. The discovery of place cells provided the first evidence for an internal representation of the environment that allows an animal to navigate purposefully around the world.

Entorhinal Cortex Neurons Provide a Distinct Representation of Space

How is the hippocampal spatial map formed? What type of spatial information is carried by afferent connections from the entorhinal cortex to the hippocampal place cells? In 2005, a surprising discovery was made about the spatial representation formed by certain neurons in the medial entorhinal cortex, whose axons provide a major part of the perforant pathway input to the hippocampus. These neurons represent space in a manner very different from the hippocampal place cells. Instead of firing when the animal is in a unique location, like the place cells, these entorhinal neurons, termed *grid cells*, fire whenever the animal is at any of several regularly spaced positions forming a hexagonal grid-like array (Figure 54–12C). When the animal moves about in the environment, different grid cells become activated, such that the activity in the entire population of grid cells always represents the animal's current position.

The grid allows the animal to locate itself within a Cartesian-like external coordinate system that is independent of context, landmarks, or specific markings. A grid cell's firing pattern is expressed in all environments that an animal visits, including during complete darkness. The independence of grid-cell firing from visual input implies that intrinsic networks, as well as self-motion cues, may serve as sources of information to ensure that grid cells are activated systematically throughout the environment. The gridded spatial information conveyed by the entorhinal inputs is then transformed within the hippocampus into unique spatial locations represented by the firing of ensembles of place cells, but how this transformation occurs remains to be determined. Since grid cells were discovered in the medial entorhinal cortex of rats in 2005, they have been identified in mice, bats, monkeys, and humans. Recordings from flying bats have shown that grid cells and place cells represent locations in three-dimensional space, suggesting the generality of the cortico-hippocampal spatial navigation system. Finally, it has been proposed that grid cells in primates may encode positions in multiple sensory coordinate systems, including eye fixation coordinates.

Grid cells display a characteristic relation between their firing fields and anatomical organization (Figure 54–13). The x,y coordinates of a cell's grid fields—often called the phase of the grid—differ among cells at the same location of the medial entorhinal cortex. The x,y coordinates of two neighboring cells are often as different as those of widely separated grid cells. In contrast, the size of the individual grid fields and the spacing between them generally increase topographically from the dorsal to the ventral part of the medial entorhinal cortex, expanding from a typical grid spacing of 30 to 40 cm at the dorsal pole to several meters in some cells at the ventral pole (Figure 54–13A). The expansion is not linear but step-like, suggesting that the grid-cell network is modular.

Interestingly, a gradual expansion is seen also in the size of the place fields of hippocampal place cells along the dorsal to ventral axis of the hippocampus (Figure 54–13B). This is consistent with the known pattern of synaptic connectivity: Dorsal entorhinal cortex innervates dorsal hippocampus, whereas ventral entorhinal cortex innervates ventral hippocampus. The finding that place fields are larger in the ventral hippocampus is in accord with results suggesting that the dorsal hippocampus is more important for spatial memory, whereas the ventral hippocampus is more important for nonspatial memory, including social memory and emotional behavior.

Grid cells are not the only medial entorhinal cells with projections to the hippocampus. Others include *head direction cells*, which respond primarily to the direction that the animal is facing (Figure 54–14A). Such cells were originally discovered in the presubiculum, another region of the parahippocampal cortex, but they exist also in the medial entorhinal cortex. Many entorhinal head direction cells also have grid-like firing properties. Like grid cells, such head direction cells are active when an animal traverses the vertices of a triangular grid in a two-dimensional environment. However, within each grid field, these cells fire only if the animal is facing a certain direction. Head direction cells and conjunctive grid and head direction cells are thought to provide directional information to the entorhinal spatial map.

Intermingled among grid cells and head direction cells is yet another type of spatially modulated cell, the *border cell* (Figure 54–14B). The firing rate of a border

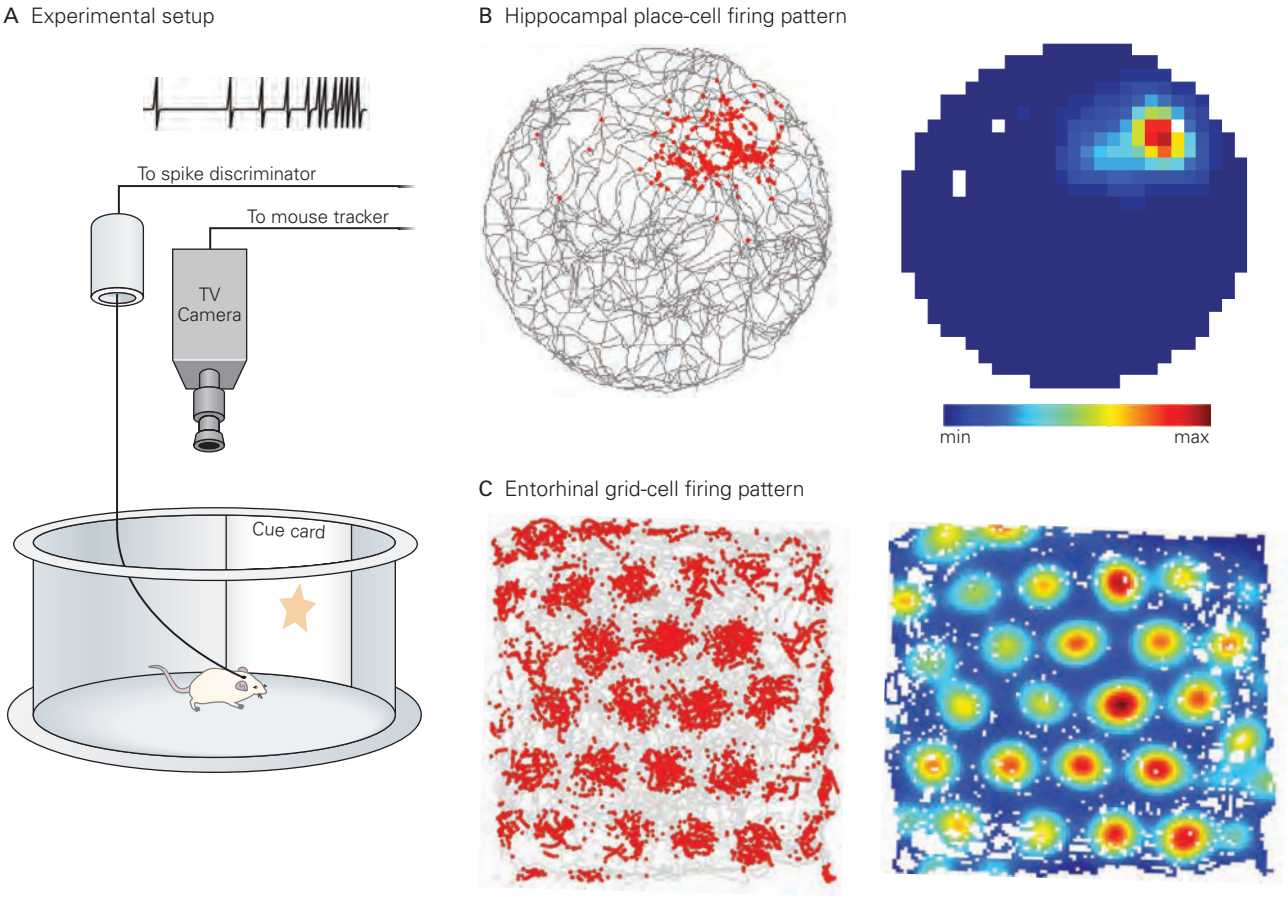


Figure 54–12 The firing patterns of cells in the hippocampus and medial entorhinal cortex signal the animal's location in its surroundings.

A. Electrodes implanted in the hippocampus of a mouse are attached to a recording cable, which is connected to an amplifier attached to a computer-based spike-discrimination program. The mouse is placed in an enclosure with an overhead TV camera that transmits to a device that detects the position of the mouse. The enclosure also contains a visual cue to orient the animal. Spikes in individual hippocampal pyramidal neurons ("place cells") are detected by a spike discrimination program. The firing rate of each cell is then plotted as a function of the animal's location in the cylinder. This information is visualized as a two-dimensional activity map for the cell, from which the cell's firing fields can be determined (shown in part **B**). (Adapted, with permission, from Muller, Kubie, and Ranck 1987. Copyright © 1987 Society for Neuroscience.)

B. Location-specific firing of a hippocampal place cell. A rat is running in a cylindrical enclosure similar to the one shown in part **A**. *Left*: The animal's path in the enclosure is shown in **gray**; firing locations of individual spikes are shown for a single place cell as **red dots**. *Right*: The firing rate of the same cell is color-coded (**blue** = low rate, **red** = high rate). In larger environments, place cells usually have more than one firing field but the fields have no apparent spatial relationship.

C. Spatial pattern of firing of an entorhinal grid cell in a rat during 30 minutes of foraging in a square enclosure 220 cm wide. The pattern shows typical periodic grid firing fields. *Left*: The trajectory of the rat is shown in **gray**; individual spike locations are shown as **red dots**. *Right*: Color-coded firing rate map for the grid cell to the left. Color coding as for the place cell in part **B**. (Adapted, with permission, from Stensola et al. 2012.)

cell increases whenever the animal approaches a local border of the environment, such as an edge or a wall. Border cells may help align the phase and orientation of grid cell firing to the local geometry of the environment. A similar role may be played by recently discovered object-vector cells—cells in medial entorhinal cortex that encode the animal's distance and direction relative to salient landmarks. A final entorhinal cell type is the *speed cell*. Speed cells fire proportionally to the running speed of the animal, irrespective of the animal's location or direction (Figure 54–14C). Together with head direction cells, speed cells can provide grid cells with information about the animal's instantaneous velocity, allowing the ensemble of active grid cells to be updated dynamically in accordance with a moving animal's changing location.

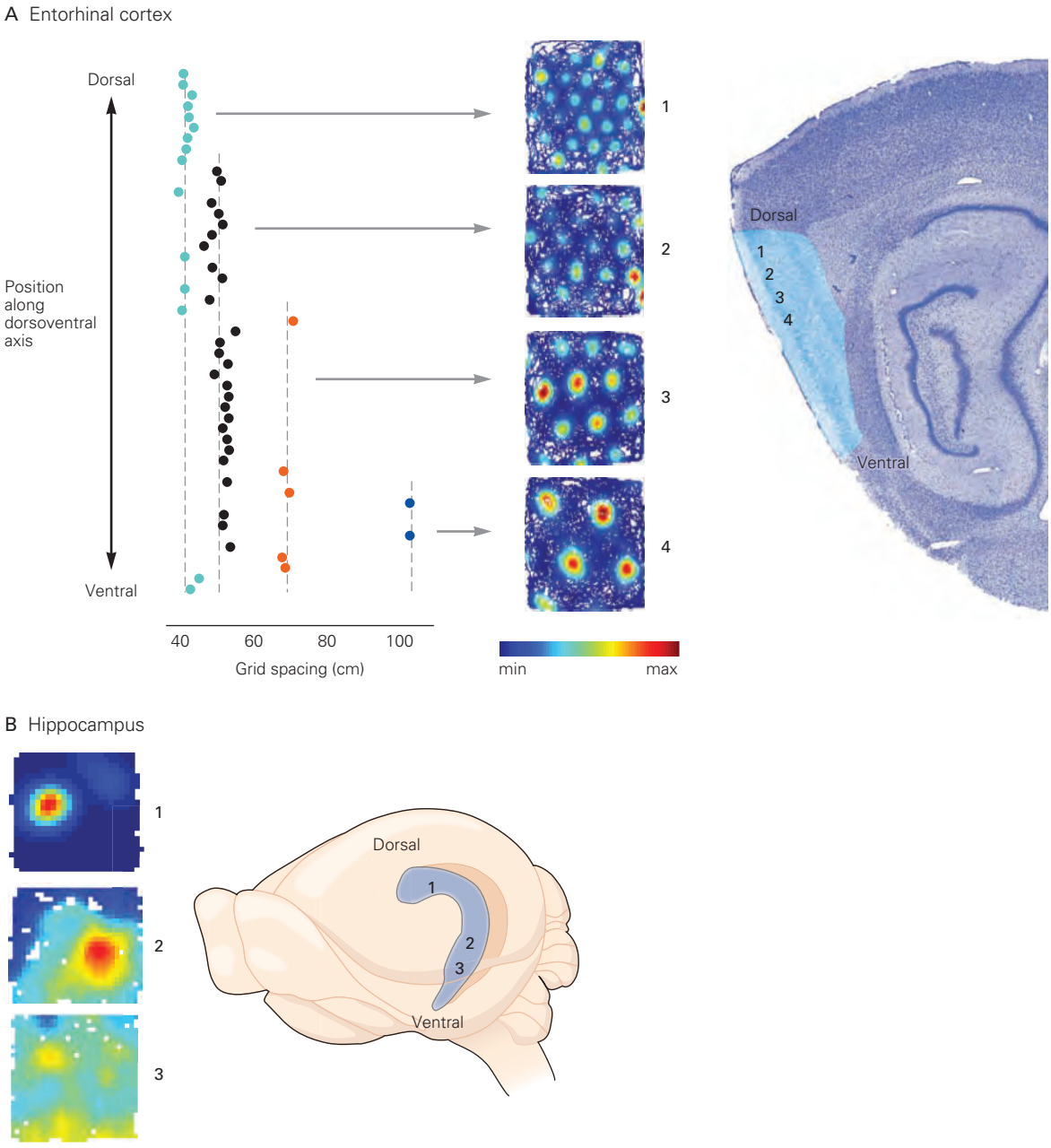


Figure 54–13 Grid fields and place fields expand in size as a function of neuronal location along the dorsoventral axis of the entorhinal cortex and hippocampus.

A. Topographical organization of grid scale in the entorhinal cortex. Grid spacing (distance between grid fields) was determined for 49 grid cells (colored dots) recorded in the same rat at successive dorsal to ventral levels in the medial entorhinal cortex (**green** area in the sagittal brain section on the right). **Dashed lines** indicate mean grid-spacing values, indicating that grid-spacing falls in one of four discrete modules, with points colored according to module. Firing rate maps for four of the cells are shown in the middle (similar to those of Figure 54–12C). Recording locations for these cells are indicated by numbers 1 to 4 to the right. (Adapted, with permission, from Stensola et al. 2012.)

B. Place fields from three different locations along the dorsoventral axis of the hippocampus. *Right*: Recording positions (numbers) in the hippocampal formation are shown at right. *Left*: Color-coded maps show the firing fields of each place cell at the recording locations. The field size expands in cells along the dorsoventral axis of the hippocampus. (Reproduced, with permission, from Kjelstrup et al. 2008.)

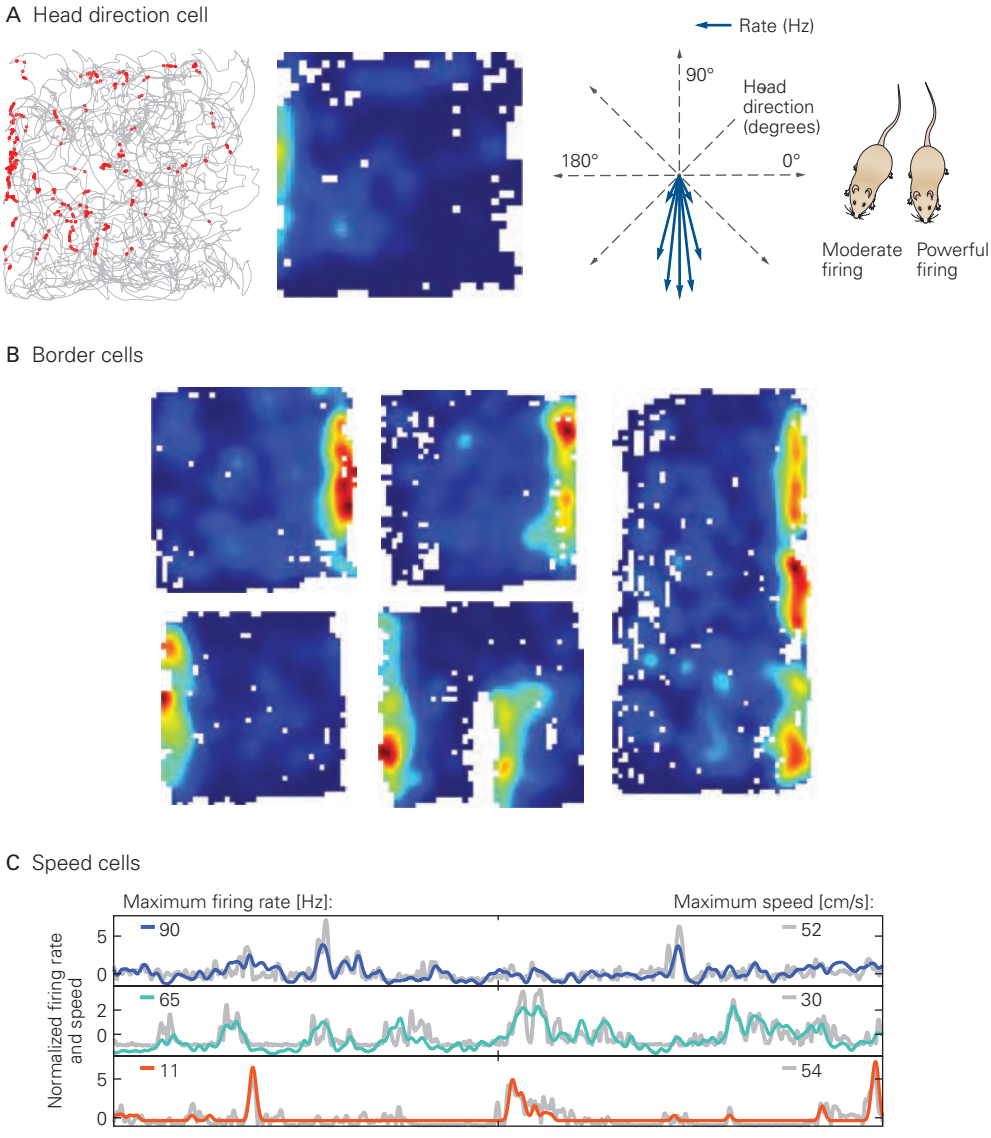


Figure 54–14 The medial entorhinal cortex contains several functional cell types tuned to distinct representations of an animal's navigation.

A. On the left is the trajectory of a rat exploring a 100-cm-wide square enclosure (**red dots** indicate firing locations). A color-coded firing rate map is also shown (color scale as in previous figures). Note that the cell's firing is scattered across the enclosure. The plot on the right shows the same cell's firing rate as a function of head direction, in polar coordinates. The cell fires selectively when the rat faces south, anywhere in the box. (Adapted, with permission, from Sargolini et al. 2006.)

B. Firing rate maps for a representative border cell in enclosures with different geometric shapes (**red** = high rate; **blue** = low rate). **Top row:** The firing field map follows the walls when the enclosure is stretched from a square (left and middle maps) to a rectangle (right map). **Bottom row:** The firing field of the same border cell in another environment. Introduction of a discrete wall (**white pixels**, right map) inside the square enclosure causes a new border field to appear to the right of the wall. (Reproduced, with permission, from Solstad et al. 2008.)

C. Speed cells. Traces show normalized firing rate (**colored traces**) and speed (**gray**) for seven representative entorhinal speed cells during 2 minutes of free foraging. Maximum values of firing rate and speed are indicated (left and right, respectively). Note high correspondence between speed and firing rate in these cells. (Reproduced, with permission, from Kropff et al. 2015.)