Chapter 54: The Hippocampus and the Neural Basis of Explicit Memory Storage

Introduction

EXPLICIT MEMORY—THE CONSCIOUS recall of information about people, places, objects, and events—is what people commonly think of as memory. Sometimes called *declarative memory*, it binds our mental life together by allowing us to recall at will what we ate for breakfast, where we ate it, and with whom. It allows us to join what we did today with what we did yesterday or the week or month before that.

Two structures in the mammalian brain are particularly critical for encoding and storing explicit memory: the prefrontal cortex and the hippocampus (Chapter 52). The prefrontal cortex mediates working memory, which can be actively maintained for only very short periods and is then rapidly forgotten, such as a password that is remembered only until it is entered. Information in working memory can be stored elsewhere in the brain as long-term memory for periods ranging from days to weeks to years, and throughout a lifetime. Although long-term storage of explicit memory requires the hippocampus, the ultimate storage site for most declarative memory is thought to be the cerebral cortex.

In this chapter, we focus on the cellular, molecular, and network mechanisms of the hippocampus that underlie the long-term storage of explicit memory. Because the hippocampus receives its major input from a region of the cerebral cortex called the entorhinal cortex, an area that processes many forms of sensory input, we also consider how information from the entorhinal cortex is transformed by the hippocampus. In particular, we examine how neural activity in the entorhinal cortex and hippocampus contributes to spatial memory by encoding a representation of an animal's location in its environment.

Explicit Memory in Mammals Involves Synaptic Plasticity in the Hippocampus

Unlike working memory, which is thought to be maintained by ongoing neural activity in the prefrontal cortex (Chapter 52), the long-term storage of information is thought to depend on long-lasting changes in the strength of connections among specific ensembles of neurons (neural assemblies) in the hippocampus that encode particular elements of memory.

The idea that memory storage involves long-lasting structural changes in the brain, first referred to as an "engram" by the German biologist Richard Semon in the early 20th century, dates back to the French philosopher Rene Descartes. In an attempt to locate an engram, the American psychologist Karl Lashley examined the effects of lesions in different regions of the neocortex on the ability of a rat to learn to navigate a maze. Since the performance in the maze seemed to be directly proportional to the size of the lesion, rather than its precise location, Lashley concluded that any memory trace must be distributed throughout the brain. Although it is now generally accepted that storage of an explicit memory is distributed throughout the neocortex, it is also clear that the process of storing memory requires the hippocampus, as demonstrated by the pioneering studies of Brenda Milner on patient H.M. (Chapter 52) and subsequent studies in animals with targeted lesions of the hippocampus. Thus, understanding how the brain stores explicit memory depends on an understanding of how the cortico-hippocampal circuit processes and stores information.

The nature of the basic mechanisms for memory storage was and remains the subject of much speculation and debate among psychologists and neuroscientists. One influential theory was proposed by the Canadian psychologist Donald Hebb, who suggested in 1949 that memory-encoding neural assemblies may be generated when synaptic connections are strengthened based on experience. According to *Hebb's rule*: "When an axon of cell A … excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased." The key element of Hebb's rule is the requirement for coincidence of pre- and postsynaptic firing, and so the rule has sometimes been rephrased as "Cells that fire together, wire together." A similar Hebbian coincidence principle is thought to be involved in fine-tuning synaptic connections during the late stages of development (Chapter 49). Hebb's ideas were later refined by the theoretical neuroscientist David Marr, based on a consideration of the hippocampal circuit.

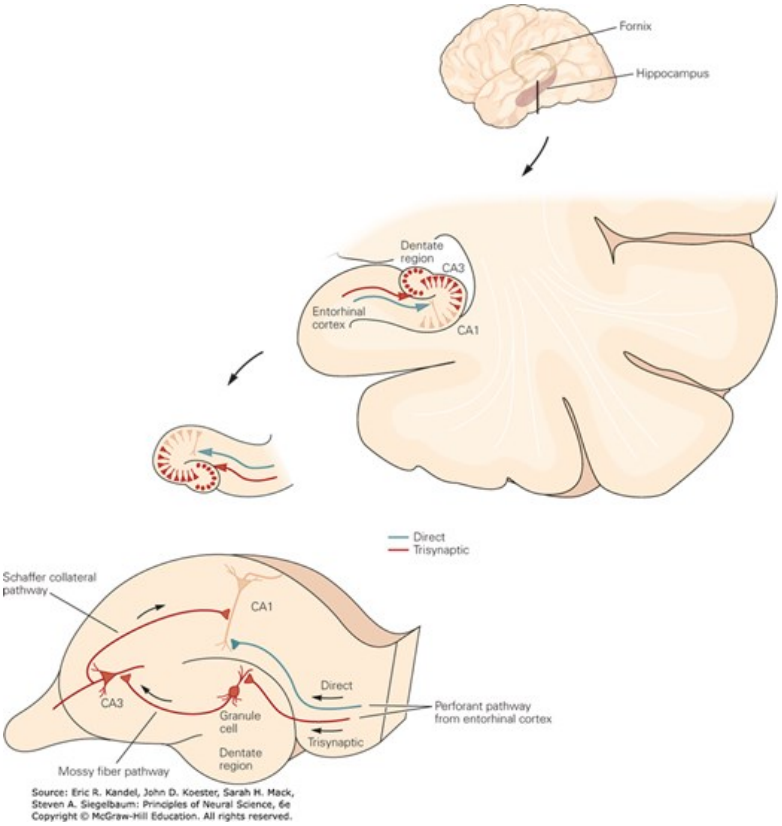
The hippocampus comprises a loop of connections that process multimodal sensory and spatial information from the superficial layers of the nearby

entorhinal cortex. This information passes through multiple synapses before arriving at the hippocampal CA1 region, the major output area of the hippocampus. The critical importance of CA1 neurons in learning and memory is seen in the profound memory loss exhibited by patients with lesions in this region alone, an observation supported by numerous animal studies. Information from the entorhinal cortex reaches CA1 neurons along two excitatory pathways, one direct and one indirect.

In the indirect pathway, the axons of neurons in layer II of the entorhinal cortex project through the *perforant pathway* to excite the granule cells of the dentate gyrus (an area considered part of the hippocampus). Next, the axons of the granule cells project in the *mossy fiber pathway* to excite the pyramidal cells in the CA3 region of the hippocampus. Finally, axons of the CA3 neurons project through the *Schaffer collateral pathway* to make excitatory synapses on more proximal regions of the dendrites of the CA1 pyramidal cells (Figure 54–1). (Because of its three successive excitatory synaptic connections, the indirect pathway is often referred to as the *trisynaptic pathway*). Finally, CA1 pyramidal cells project back to the deep layers of entorhinal cortex and forward to the subiculum, another medial temporal lobe structure that connects the hippocampus with a wide diversity of brain regions.

Figure 54–1

The cortico-hippocampal synaptic circuit is important for declarative memory. Information arrives in the hippocampus from the entorhinal cortex through the perforant pathways, which provide both direct and indirect input to pyramidal neurons in area CA1, the major output neurons of the hippocampus. (**Arrows** denote the direction of impulse flow.) The indirect *trisynaptic pathway* has three component connections. Neurons in layer II of the entorhinal cortex send their axons through the perforant path to make excitatory synapses onto the granule cells of the dentate gyrus. The granule cells project through the mossy fiber pathway and make excitatory synapses with the pyramidal cells in area CA3 of the hippocampus. The CA3 cells excite the pyramidal cells in CA1 by means of the Schaffer collateral pathway. In the *direct pathway*, neurons in layer III of the entorhinal cortex project through the perforant path to make excitatory synapses on the distal dendrites of CA3 and CA1 pyramidal neurons without inter vening synapses (shown only for CA1).



In parallel with the indirect pathway, the entorhinal cortex also projects directly to CA3 and CA1 hippocampal regions. In the direct pathway to CA1, neurons in layer III of the entorhinal cortex send their axons through the *perforant pathway* to form excitatory synapses on the very distal regions of the apical dendrites of CA1 neurons (such projections are also called the *temporoammonic pathway*). Interactions between direct and indirect inputs

at each stage of the hippocampal circuit are likely important for memory storage or recall, although the precise nature of these interactions remains to be determined.

In addition to the above pathways that link different stages of the hippocampal circuit, CA3 pyramidal neurons also make strong excitatory connections with one another. This self-excitation through recurrent collaterals is thought to contribute to associative aspects of memory storage and recall. Under pathological conditions, such self-excitation can lead to seizures.

Finally, neurons in the relatively small CA2 region, located between CA3 and CA1, receive information from entorhinal cortex layer II through both a direct pathway and an indirect pathway via the dentate gyrus and CA3. The CA2 region also receives strong input from hypothalamic nuclei that release oxytocin and vasopressin, hormones important for social behavior. In turn, CA2 sends a strong output to CA1, providing CA1 with a third source of excitatory input (in addition to the direct and trisynaptic routes from the entorhinal cortex).

Long-Term Potentiation at Distinct Hippocampal Pathways Is Essential for Explicit Memory Storage

How is information stored in the hippocampal circuit to provide a long-lasting memory trace? In 1973, Timothy Bliss and Terje Lømo discovered that a brief period of high-frequency synaptic stimulation causes a persistent increase in the amplitude of hippocampal excitatory postsynaptic potentials (EPSPs), a process termed *long-term potentiation* or LTP (Chapter 13). The enhancement in the EPSP, in turn, increases the probability that the postsynaptic cell will fire action potentials.

Bliss and Lømo examined the initial stage of the indirect hippocampal pathway—the synapses formed by the perforant pathway from entorhinal cortex layer II neurons with dentate gyrus granule neurons. Subsequent studies showed that brief high-frequency trains of stimulation can induce forms of LTP at nearly all excitatory synapses of this indirect pathway as well as at the direct perforant path synapses with CA3 and CA1 neurons (Figure 54–2). LTP can last for days or even weeks when induced in intact animals using implanted electrodes and can last several hours in isolated slices of hippocampus and in hippocampal neurons in cell culture.

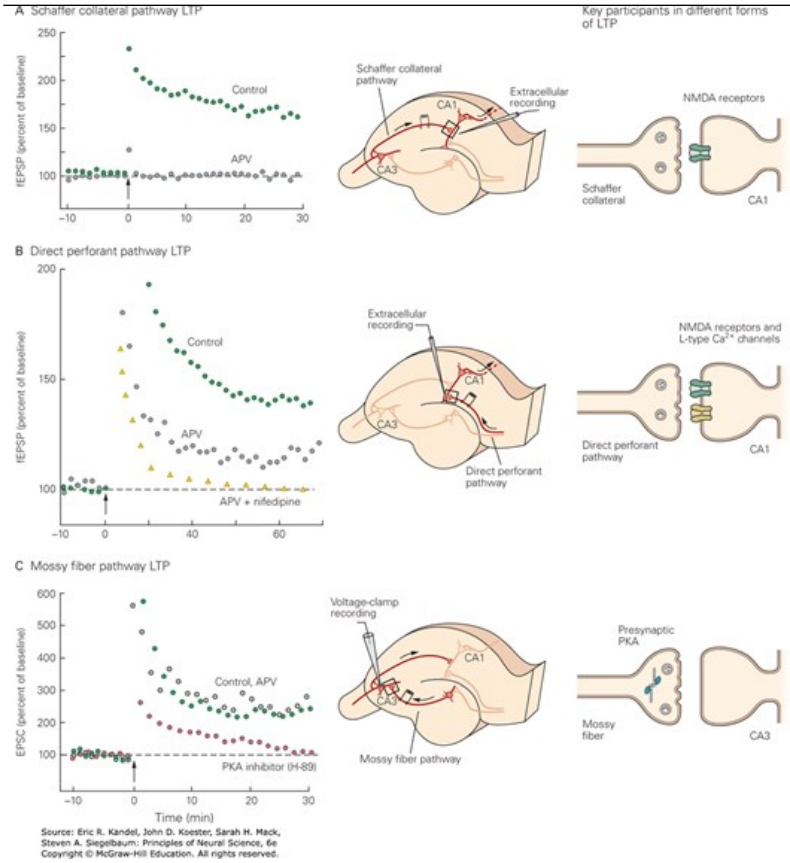
Figure 54–2

Different neural mechanisms underlie long-term potentiation at each of the three synapses in the trisynaptic pathway. Long-term potentiation (**LTP**) occurs at synapses throughout the hippocampus but depends to differing degrees on activation of *N*-methyl-D-aspartate (**NMDA**)-type glutamate receptors.

A. Tetanic stimulation of the Schaffer collateral fibers (at time 0 in the plot) induces LTP at the synapses between presynaptic CA3 pyramidal neurons and postsynaptic CA1 pyramidal neurons. The plot shows the size of the extracellular field excitatory postsynaptic potential (**fEPSP**) as a percentage of the baseline fEPSP prior to induction of LTP. At these synapses, LTP requires activation of the NMDA receptor-channels in the postsynaptic CA1 neurons as it is completely blocked when the tetanus is delivered in the presence of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (**APV**). (Adapted from Morgan and Teyler 2001.)

B. Tetanic stimulation of the direct pathway from entorhinal cortex to CA1 neurons generates LTP of the fEPSP that depends partly on activation of the NMDA receptor-channels and partly on activation of L-type voltage-gated Ca^{2+} channels. It is therefore only partially blocked by APV. Addition of APV and nifedipine, a dihydropyridine that blocks L type channels, is needed to fully inhibit LTP.

C. Tetanic stimulation of the mossy fiber pathway induces LTP at the synapses with the pyramidal cells in the CA3 region. In this experiment, the excitatory postsynaptic current (**EPSC**) was measured under voltage-clamp conditions. This LTP does not require activation of the NMDA receptors and so is not blocked by APV. However, it does require activation of protein kinase A (**PKA**) and so is blocked by the kinase inhibitor H-89. (Reproduced, with permission, from Zalutsky and Nicoll 1990. Copyright © 1990 AAAS.)



Studies in the different hippocampal pathways have shown that LTP at different synapses is not a single process. Rather, it comprises a family of processes that strengthen synaptic transmission at different hippocampal synapses through distinct cellular and molecular mechanisms. Indeed, even at a single synapse, different forms of LTP can be induced by different patterns of synaptic activity, although these distinct processes share many important similarities.

All forms of LTP are induced by synaptic activity in the pathway that is being potentiated—that is, LTP is homosynaptic. In addition, LTP is synapse specific; only those synapses that are activated by the tetanic stimulation are potentiated. However, the various forms of LTP differ in their dependence on specific receptors and ion channels. In addition, different forms of LTP recruit different second-messenger signaling pathways that act at different synaptic sites. Some forms of LTP result from an enhancement of the postsynaptic response to the neurotransmitter glutamate, whereas other forms of LTP result from the enhancement of glutamate release from the presynaptic terminal, and still other forms of LTP engage both the presynaptic and postsynaptic neurons.

The similarities and differences in the mechanisms of different forms of LTP can be seen by comparing LTP at Schaffer collateral, mossy fiber, and direct entorhinal synapses. In all three pathways, synaptic transmission is persistently enhanced in response to a brief tetanic stimulation. However, the contribution of the *N*-methyl-D-aspartate (NMDA) receptor to the induction of LTP differs in the three pathways. At the Schaffer collateral synapses, the induction of LTP in response to a brief 100-Hz stimulation is completely blocked when the tetanus is applied in the presence of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5 or APV). In contrast, APV only partially inhibits the induction of LTP at the direct entorhinal synapses with CA1 neurons and has no effect on LTP at the mossy fiber synapses with CA3 pyramidal neurons (Figure 54–2).

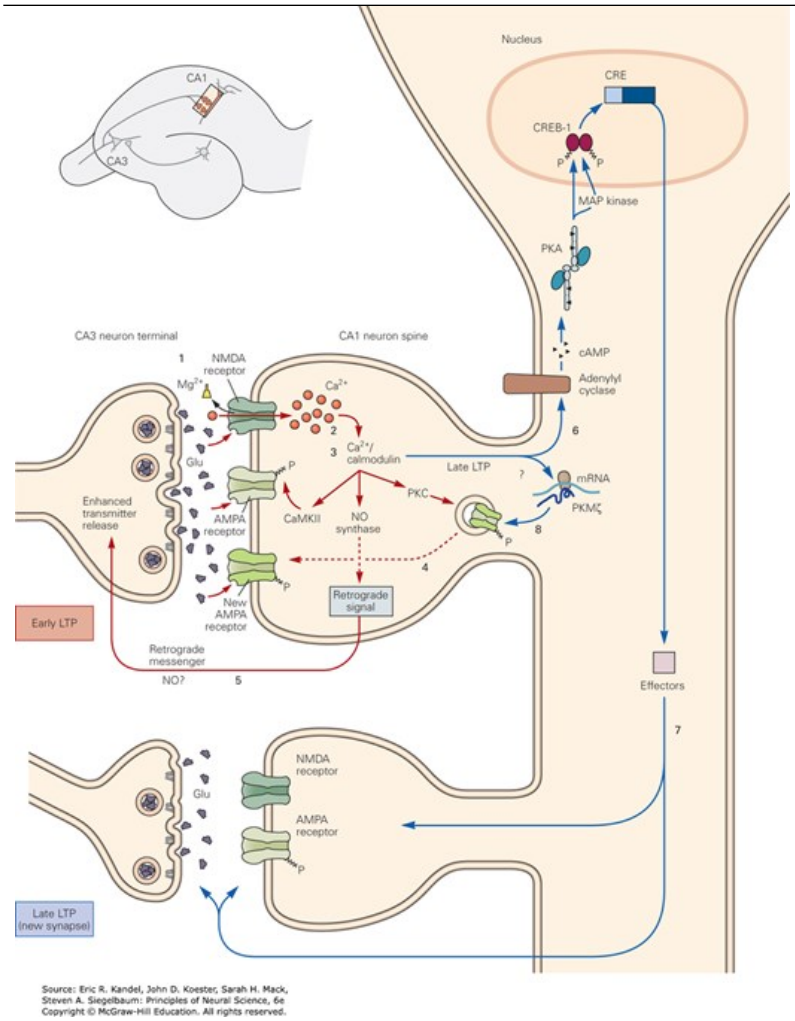
Long-term potentiation in the mossy fiber pathway is largely presynaptic and is triggered by the large Ca^{2+} influx into the presynaptic terminals during the tetanus. The Ca^{2+} influx activates a calcium/calmodulin-dependent adenylyl cyclase, thereby increasing the production of cyclic adenosine monophosphate (cAMP) and activating protein kinase A (PKA; see Chapter 14). This leads to the phosphorylation of presynaptic vesicle proteins that enhance the release of glutamate from the mossy fiber terminals, resulting in an increase in the EPSP. Activity in the postsynaptic cell is not required for this form of LTP. Thus, unlike Hebbian plasticity, mossy fiber LTP is nonassociative.

In the Schaffer collateral pathway, however, LTP is associative, largely as a result of the properties of the NMDA receptors (Figure 54–3; see also

Chapter 13). As is the case with most excitatory synapses in the brain, glutamate released from the Schaffer collateral terminals activates both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptor-channels in the postsynaptic membrane of CA1 pyramidal neurons. However, unlike the AMPA receptors, activation of the NMDA receptors is associative because it requires simultaneous presynaptic and postsynaptic activity. This is because the pore of the NMDA receptor-channel is normally blocked by extracellular Mg^{2+} at typical negative resting potentials, which prevents these channels from conducting ions in response to glutamate. For the NMDA receptor-channel to function efficiently, the postsynaptic membrane must be depolarized sufficiently to expel the bound Mg^{2+} by electrostatic repulsion. In this manner, the NMDA receptor-channel acts as a coincidence detector: It is functional only when (1) the action potentials in the presynaptic neuron release glutamate that binds to the receptor *and* (2) the membrane of the postsynaptic cell is sufficiently depolarized by strong synaptic activity to relieve the Mg^{2+} block. Thus, the NMDA receptor is able to associate presynaptic and postsynaptic activity to recruit plasticity mechanisms that strengthen connections between pairs of cells, fulfilling Hebb's coincidence requirement for synaptic modification.

Figure 54–3

A model for the induction of long-term potentiation (LTP) at Schaffer collateral synapses. A single high-frequency tetanus induces early LTP. The large depolarization of the postsynaptic membrane (caused by strong activation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [**AMPA**] receptors) relieves the Mg^{2+} blockade of the *N*-methyl-D-aspartate (**NMDA**) receptor-channels (**1**), allowing Ca^{2+}, Na^{+}, and K^{+} to flow through these channels. The resulting increase of Ca^{2+} in the dendritic spine (**2**) triggers calcium-dependent kinases (**3**)—calcium/calmodulin–dependent kinase (**CaMKII**) and protein kinase C (**PKC**)—leading to induction of LTP. Second-messenger cascades activated during induction of LTP have two main effects on synaptic transmission. Phosphorylation through activation of protein kinases, including PKC, enhances current through the AMPA receptor-channels, in part by causing insertion of new receptors into the spine synapses (**4**). In addition, the postsynaptic cell releases retrograde messengers, such as nitric oxide (**NO**), that activate protein kinases in the presynaptic terminal to enhance subsequent transmitter release (**5**). Repeated bouts of tetanic stimulation induce late LTP. The prolonged increase in Ca^{2+} influx recruits adenylyl cyclase (**6**), which generates cyclic adenosine monophosphate (**cAMP**) that activates protein kinase A (**PKA**). This leads to the activation of MAP kinase, which translocates to the nucleus where it phosphorylates CREB-1. CREB-1 in turn activates transcription of targets (containing the CRE promoter) that are thought to lead to the growth of new synaptic connections (**7**). Repeated stimulation also activates translation of mRNA encoding PKMζ, a constitutively active isoform of PKC (**8**). This leads to a long-lasting increase in the number of AMPA receptors in the postsynaptic membrane.



What are the functional consequences of the activation of NMDA receptors by strong synaptic excitation? Whereas most AMPA receptor-channels conduct only monovalent cations (Na^+ and K^+), the NMDA receptor-channels have a high permeability to Ca^{2+} (Chapter 13). Thus, the opening of these channels leads to a significant increase in the Ca^{2+}concentration in the postsynaptic cell. The increase in intracellular Ca^{2+} activates several downstream signaling pathways—including calcium/calmodulin–dependent protein kinase II (CaMKII), protein kinase C (PKC), and tyrosine kinases—that lead to changes that enhance the magnitude of the EPSP at Schaffer collateral synapses (Figure 54–3).

Different Molecular and Cellular Mechanisms Contribute to the Forms of Expression of Long-Term Potentiation

Neuroscientists often find it useful to distinguish between the *induction* of LTP (the biochemical reactions activated by the tetanic stimulation) and the *expression* of LTP (the long-term changes responsible for enhanced synaptic transmission). The mechanisms for the induction of LTP at the CA3-CA1 synapse are largely postsynaptic. Is the expression of LTP at this synapse caused by an increase in transmitter release, an increased postsynaptic response to a fixed amount of transmitter, or some combination of the two?

A number of lines of experiments suggest that the form of expression of LTP depends on the type of synapse and precise pattern of activity that induces LTP. In many cases, the expression of LTP in CA1 neurons in response to Ca^{2+} influx through NMDA receptor-channels depends on an increase in the response of the postsynaptic membrane to glutamate. But stronger patterns of stimulation can elicit forms of LTP at the same synapse whose expression depends on presynaptic events that enhance transmitter release.

One of the key pieces of evidence for a postsynaptic contribution to the expression of LTP at Schaffer collateral synapses comes from an examination of so-called “silent synapses.” In some recordings from pairs of hippocampal pyramidal neurons, stimulation of an action potential in one neuron fails to elicit a response in the postsynaptic neuron when that neuron is at its resting potential (approximately −70 mV). This result is not surprising, as each

hippocampal presynaptic neuron is connected to only a small number of other neurons. What *is* surprising is that in some neuronal pairs that appear unconnected when the postsynaptic membrane is initially at −70 mV, stimulation of the same presynaptic neuron is able to elicit a large excitatory postsynaptic current in the second neuron when the second neuron is depolarized under voltage clamp to +30 mV. In such neuronal pairs, the postsynaptic membrane appears to lack functional AMPA receptors so that the excitatory postsynaptic current (EPSC) is mediated solely by NMDA receptors-channels. As a result, there is no measurable EPSC when the membrane is held at the cell's resting potential (−70 mV) because of the strong Mg^{2+} block of these receptor-channels (the synapse is effectively silent). However, a large EPSC can be generated at +30 mV because the depolarization relieves the block (Figure 54–4).

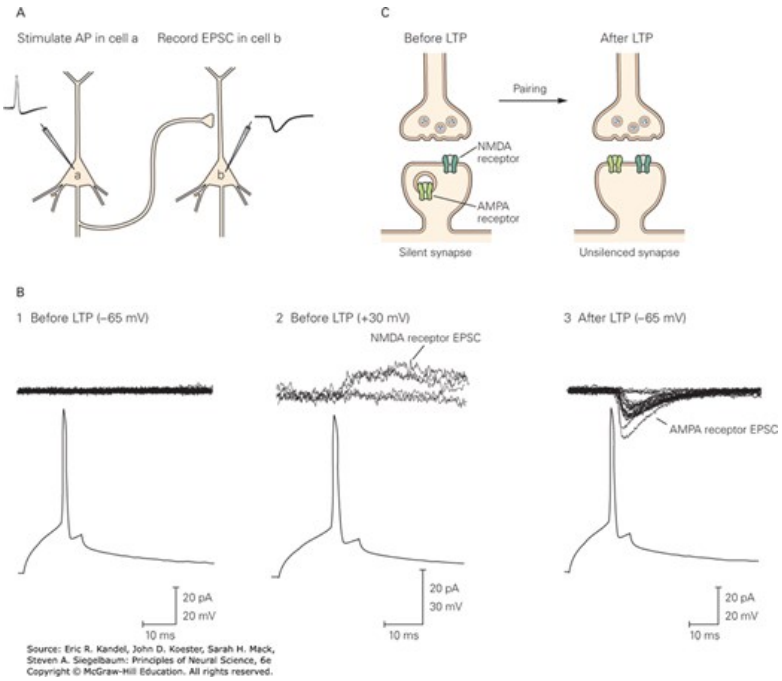
Figure 54–4

Adding α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to silent synapses during long-term potentiation (LTP).

A. Intracellular recordings are obtained from a pair of hippocampal pyramidal neurons. An action potential (**AP**) is triggered in neuron *a* by a depolarizing current pulse, and the resultant excitatory postsynaptic current (**EPSC**) produced in neuron *b* is recorded under voltage-clamp conditions.

B. Before induction of LTP, there is no EPSC in cell *b* (top traces) in response to an action potential in cell *a* (bottom traces) when the membrane potential of neuron *b* is at its resting value of −65 mV (**1**). However, when neuron *b* is depolarized by the voltage clamp to +30 mV, the *N*-methyl-D-aspartate (**NMDA**) receptors are activated and slow EPSCs characteristic of these receptors are observed (**2**). LTP is then induced by pairing action potentials in neuron *a* with postsynaptic depolarization in neuron *b* to relieve the Mg^{2+} block of the NMDA receptors. After this pairing, fast EPSCs initiated by activation of AMPA receptors are seen in cell *b* (**3**). (Reproduced, with permission, from Montgomery, Pavlidis, and Madison 2001. Copyright © 2001 Cell Press.)

C. Mechanism of the unsilencing of silent synapses. Prior to LTP, the dendritic spine contacted by a presynaptic CA3 neuron contains only NMDA receptors. Following induction of LTP, intracellular vesicles containing AMPA receptors fuse with the plasma membrane at the synapse, adding AMPA receptors to the membrane.



The key finding from these experiments is seen following the induction of LTP using strong synaptic stimulation. Pairs of neurons initially connected solely by silent synapses now often exhibit large EPSPs at the negative resting potential, and these EPSPs are mediated by AMPA receptors. The simplest interpretation of this result is that LTP somehow recruits new functional AMPA receptors to the silent synapse membrane, a process Roberto Malinow refers to as "AMPAfication."

How does the induction of LTP increase the response of AMPA receptors? The strong synaptic stimulation used to induce LTP triggers glutamate release at both silent and nonsilent synapses on the same postsynaptic neuron. This leads to the opening of a large number of AMPA receptor-channels at the nonsilent synapses, which in turn produces a large postsynaptic depolarization. The depolarization then propagates throughout the neuron, thus relieving Mg^{2+} block of the NMDA receptor-channels at both the nonsilent and silent synapses. At the silent synapses, the Ca^{2+} influx through the NMDA receptor-channels activates a biochemical cascade that ultimately leads to the insertion of clusters of AMPA receptors in the postsynaptic membrane. These newly inserted AMPA receptors are thought to come from a reserve pool stored in endosomal vesicles within dendritic spines, the site of all excitatory input to pyramidal neurons (Chapter 13). Calcium influx through the NMDA receptor-channels elevates spine Ca^{2+} levels, triggering a postsynaptic signaling cascade that leads to phosphorylation of the cytoplasmic tail of the vesicular AMPA receptors by PKC (Chapter 14), leading to their insertion in the postsynaptic membrane (Figure 54–3).

Because the induction of almost all forms of postsynaptic LTP requires Ca^{2+} influx into the postsynaptic cell, the finding that transmitter release is enhanced during some forms of LTP implies that the presynaptic cell must receive a signal from the postsynaptic cell that LTP has been induced. There is now evidence that calcium-activated second messengers in the postsynaptic cell, or perhaps Ca^{2+} itself, cause the postsynaptic cell to release one or more chemical messengers, including the gas nitric oxide, that diffuse to the presynaptic terminals to enhance transmitter release (Figure 54–3 and Chapter 14). Importantly, these diffusible retrograde signals appear to affect only those presynaptic terminals that have been activated by the tetanic stimulation, thereby preserving synapse specificity.

Long-Term Potentiation Has Early and Late Phases

Long-term potentiation has two phases, early and late, that provide a means of regulating the duration of the enhancement of synaptic transmission. The phase we have focused on up to now lasts for only 1 to 3 hours and is termed early LTP; this phase is typically induced by a single train of 100-Hz tetanic stimulation for 1 second. More prolonged periods of activity (using three or four trains of 100-Hz tetanic stimulation, each lasting 1 second) induce a late phase of LTP that can last 24 hours or even longer. Unlike early LTP, late LTP requires the synthesis of new proteins (Figure 54–5). Whereas the early phase of LTP is mediated by changes at existing synapses, late LTP is thought to result from the growth of new synaptic connections between pairs of co-activated neurons.

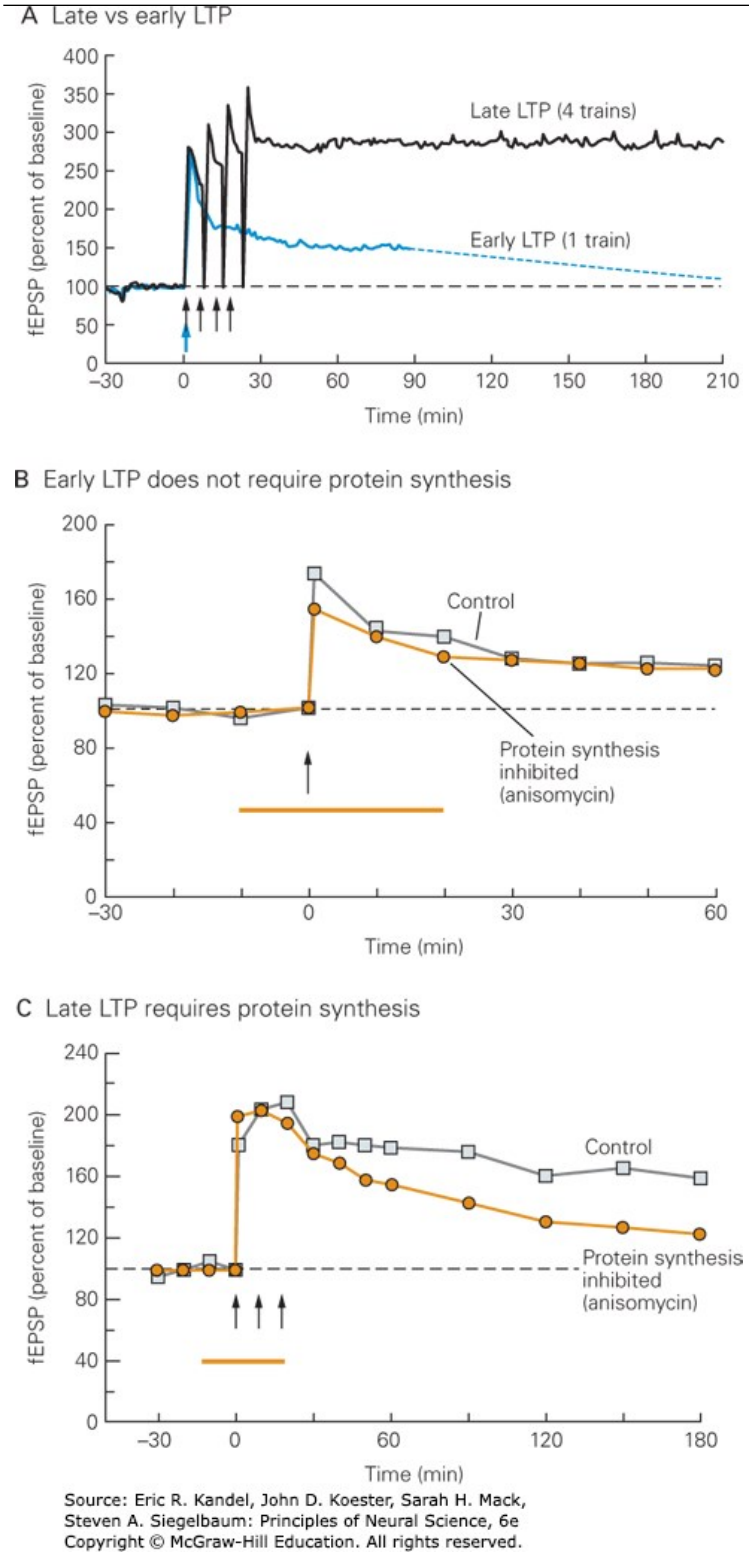
Figure 54–5

(Right) Long-term potentiation (LTP) in the CA1 region of the hippocampus has early and late phases.

A. Early LTP is induced by a single tetanus lasting 1 second at 100 Hz, whereas late LTP is induced by four tetani given 10 minutes apart. Early LTP of the field excitatory postsynaptic potential (**fEPSP**) lasts only 1 to 2 hours, whereas the late LTP lasts more than 8 hours (only the first 3.5 hours are shown).

B. Early LTP induced by one tetanus is not blocked by anisomycin (**bar**), an inhibitor of protein synthesis.

C. Late LTP, normally induced by three trains of stimulation, is blocked by anisomycin. (Three or four trains can be used to induce late LTP.) (Panels B and C reproduced, with permission, from Huang and Kandel 1994.)



Source: Eric R. Kandel, John D. Koester, Sarah H. Mack, Steven A. Siegelbaum: Principles of Neural Science, 6e

Although the mechanisms for early LTP in the Schaffer collateral and mossy fiber pathways are quite different, the mechanisms for late LTP in the two pathways appear similar (Figure 54–3). In both pathways, late LTP recruits the cAMP and PKA signaling pathway to activate by phosphorylation the cAMP response element binding protein (CREB) transcription factor, leading to the synthesis of new mRNAs and proteins. Like sensitization of the gill-withdrawal reflex in *Aplysia*, which also involves cAMP, PKA, and CREB (Chapter 53), late LTP in the Schaffer collateral pathway is synapse specific. When two independent sets of synapses in the same postsynaptic CA1 neuron are stimulated using two electrodes spaced some distance apart, the application of four trains of tetanic stimulation to one set of synapses induces late LTP only at the activated synapses; synaptic transmission is not

altered at the second set of synapses that were not tetanized.

How can late LTP achieve synapse specificity given that transcription and most translation occurs in the cell body, such that newly synthesized proteins should be available to all synapses of a cell? To explain synapse specificity, Uwe Frey and Richard Morris proposed the synaptic capture hypothesis, in which synapses that are activated during the tetanus are tagged in some way, perhaps by protein phosphorylation, that enables them to make use of ("capture") the newly synthesized proteins. Frey and Morris tested this idea using the two-pathway protocol described above. They delivered four tetani to induce late LTP at one set of synapses with one electrode and delivered a single tetanus to a second set of synapses with the other electrode. Although a single tetanus on its own induces only early LTP, it is able to induce late LTP when delivered within 2–3 hours of the four tetani from the first electrode. This phenomenon is similar to the synapse-specific capture of long-term facilitation at the sensory-motor neuron synapses in *Aplysia* (Chapter 53).

According to Frey and Morris, the single train of tetanic stimulation, although not sufficient to induce new protein synthesis, is sufficient to tag the activated synapses, allowing them to capture the newly synthesized proteins produced in response to the prior delivery of the four trains of tetanic stimulation. The increased synaptic plasticity that this tagging mechanism affords, and its limitation to the period when newly synthesized proteins are around, may explain the recent finding that hippocampal cell assemblies that store memories of events closely spaced in time have a larger number of common neurons than do cell assemblies for events widely separated in time.

How can a few brief trains of synaptic stimulation produce such long-lasting increases in synaptic transmission? One mechanism proposed by John Lisman depends on the unique properties of CaMKII. After a brief exposure to Ca^{2+}, CaMKII can be converted to a calcium-independent state through its autophosphorylation at threonine-286 (Thr286). This ability to become persistently active in response to a transient Ca^{2+} stimulus has led to the suggestion that CaMKII may act as a simple molecular switch that can extend the duration of LTP following its initial activation.

Studies from Todd Sacktor have suggested that longer-lasting changes that maintain late LTP may depend on an atypical isoform of PKC termed PKMζ (PKM zeta). Most isoforms of PKC contain both a regulatory domain and a catalytic domain (Chapter 14). Binding of diacylglycerol, phospholipids, and Ca^{2+} to the regulatory domain relieves inhibitory domain binding to the catalytic domain, allowing PKC to phosphorylate its protein substrates. In contrast, PKMζ lacks a regulatory domain and so is constitutively active.

Levels of PKMζ in the hippocampus are normally low. Tetanic stimulation that induces LTP leads to an increase in synthesis of PKMζ through enhanced translation of its mRNA. Because this mRNA is present in the CA1 neuron dendrites, its translation can rapidly alter synaptic strength. Blockade of PKMζ with a peptide inhibitor during the tetanic stimulation blocks late LTP but not early LTP. If the blocker is applied several hours after LTP induction, the late LTP that had been established will be reversed. This result indicates that the maintenance of late LTP requires the ongoing activity of PKMζ to maintain the increase in AMPA receptors in the postsynaptic membrane (Figure 54–3). A second atypical PKC isoform may substitute for PKMζ under certain conditions, which may explain the surprising finding that genetic deletion of PKMζ has little effect on late LTP.

Constitutively active forms of protein kinases may not be the only mechanism for maintaining long-lasting synaptic changes in the hippocampus. Repeated stimulation may lead to the formation of new synaptic connections, just as long-term facilitation leads to the formation of new synapses during learning in *Aplysia.* In addition, long-lasting synaptic changes likely involve epigenetic changes in chromatin structure. During late LTP, phosphorylated CREB activates gene expression by recruiting the CREB binding protein (CBP), which acts as a histone acetylase, transferring an acetyl group to specific lysine residues on histone proteins, and thereby producing long-lasting changes in gene expression. Mutations in CBP impair late LTP and learning and memory in mice. In humans, de novo mutations in the CBP gene underlie Rubinstein-Taybi syndrome, a developmental disorder associated with intellectual impairment. Other studies implicate a second epigenetic mechanism, DNA methylation, in long-lasting synaptic plasticity and learning and memory.

Spike-Timing-Dependent Plasticity Provides a More Natural Mechanism for Altering Synaptic Strength

Under most circumstances, hippocampal neurons do not produce the high-frequency trains of action potentials typically used to induce LTP experimentally. However, a form of LTP termed spike-timing-dependent plasticity (STDP) can be induced by a more natural pattern of activity in which a single presynaptic stimulus is paired with the firing of a single action potential in the postsynaptic cell at a relatively low frequency (eg, one pair per second over several seconds). However, the presynaptic cell must fire just before the postsynaptic cell. If instead the postsynaptic cell fires just before the EPSP, a long-lasting decrease in the size of the EPSP occurs. Such long-term depression of synaptic transmission represents a distinct form of synaptic plasticity from LTP and is described more fully below. If the postsynaptic action potential occurs more than a hundred milliseconds before or

after the EPSP, the synaptic strength will not change.

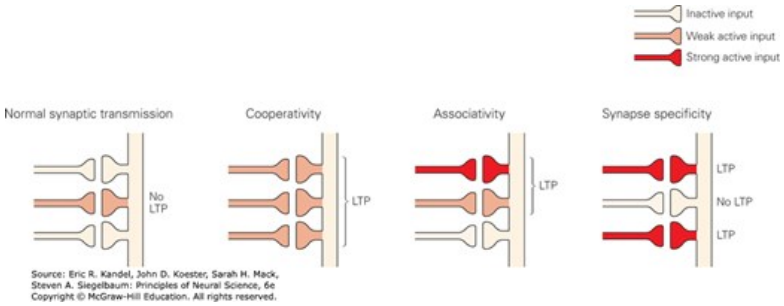
The pairing rules of STDP thus follow Hebb's postulate and result in large part from the cooperative properties of the NMDA receptor-channel. If the postsynaptic spike occurs during the EPSP, it is able to relieve the Mg^{2+} blockade of the channel at a time when the NMDA receptor has been activated by the binding of glutamate. This leads to a large influx of Ca^{2+} through the receptor and the induction of STDP. However, if the postsynaptic action potential occurs prior to the presynaptic release of glutamate, any relief from the Mg^{2+} block will occur when the gate of the receptor-channel is closed (because of the absence of glutamate). As a result, there will be only a small influx of Ca^{2+} through the receptor that is insufficient to induce STDP.

Long-Term Potentiation in the Hippocampus Has Properties That Make It Useful as A Mechanism for Memory Storage

NMDA receptor–dependent LTP at the Schaffer collateral pathway and other hippocampal pathways has three properties with direct relevance to learning and memory (Figure 54–6). First, LTP in such pathways requires the near-simultaneous activation of a large number of afferent inputs, a feature called *cooperativity* (Figure 54–6). This requirement stems from the fact that relief of Mg^{2+} block of the NMDA receptor-channel requires a large depolarization, which is achieved only when the postsynaptic cell receives input from a large number of presynaptic cells.

Figure 54–6

Long-term potentiation (LTP) in CA1 pyramidal neurons of the hippocampus shows cooperativity, associativity, and synapse specificity. With normal synaptic transmission, a single action potential in one or a few axons (weak input) leads to a small excitatory postsynaptic potential (EPSP) that is insufficient to expel Mg^{2+} from the *N*-methyl-D-aspartate (NMDA) receptor-channels and thus cannot induce LTP. This ensures that irrelevant stimuli are not remembered. The near-simultaneous activation of several weak inputs during strong activation (cooperativity) produces a suprathreshold EPSP that triggers an action potential, resulting in LTP in all pathways. Stimulation of strong and weak inputs together (associativity) causes LTP in both pathways. In this way, a weak input becomes significant when paired with a powerful one. An unstimulated synapse does not undergo LTP despite the strong stimulation of neighboring synapses. This ensures that memory is selectively stored at active synapses (synapse specificity).



Second, LTP at synapses with NMDA receptor-channels is *associative.* A weak presynaptic input normally does not produce enough postsynaptic depolarization to induce LTP. However, if the weak input is paired with a strong input that produces a suprathreshold depolarization, the resulting large depolarization will propagate to the synapses with weak input, leading to relief of the Mg^{2+} blockade of the NMDA receptors and induction of LTP at those synapses.

Third, NMDA receptor-dependent LTP is *synapse specific.* If a particular synapse is not activated during a period of strong synaptic stimulation, the NMDA receptors at that site will not be able to bind glutamate and thus will not be activated despite the strong postsynaptic depolarization. As a result, that synapse will not undergo LTP.

Each of these three properties—cooperativity, associativity, and synapse specificity—underlies a key requirement of memory storage. Cooperativity ensures that only events of a high degree of significance, those that activate sufficient inputs, will result in memory storage. Associativity, like associative Pavlovian conditioning, allows an event (or conditioned stimulus) that has little significance in and of itself to be endowed with a higher degree of meaning if that event occurs just before or simultaneously with another more significant event (an unconditioned stimulus). In a network with strong recurrent connections, such as CA3, associative LTP enables a pattern of activity in one group of cells to become linked to a distinct pattern of activity in a separate, but partially overlapping, group of synaptically coupled cells. Such linkages of cell assemblies are thought to enable related

events to become associated with one another and to be important for storing and recalling large varieties of experiences, as occurs with explicit memory. Finally, synapse specificity ensures that inputs that convey information not related to a particular event will not be strengthened. Synapse specificity is critical when large amounts of information must be stored in one network, because much more information can be stored in a cell through functional alterations at individual synapses than through blanket changes in a property of the cell, such as its excitability.

Spatial Memory Depends on Long-Term Potentiation

Long-term potentiation is an experimentally induced change in synaptic strength produced by strong direct stimulation of neural pathways. Does this or a related form of synaptic plasticity occur physiologically during explicit memory storage? If so, how important is it for explicit memory storage in the hippocampus?

To date, a large number of experimental approaches have shown that inhibiting LTP interferes with spatial memory. In one approach, a mouse is placed in a pool filled with an opaque fluid (the Morris water maze); to escape from the liquid, the mouse must swim to find a platform submerged in the fluid and completely hidden from view. The animal is released at random locations around the pool and initially encounters the platform by chance. However, in subsequent trials, the mouse quickly learns to locate the platform and then remembers its position based on spatial information—distal markings on the walls of the room in which the pool is located. This task requires the hippocampus. In a nonspatial, or cued, version of this test, the platform is raised above the water surface or marked with a flag so that it is visible, permitting the mouse to navigate directly to it using brain pathways that do not require the hippocampus.

When NMDA receptors are blocked by a pharmacological antagonist injected into the hippocampus immediately before an animal is trained to navigate the Morris water maze, the animal cannot remember the location of the hidden platform using spatial information but can find it in the version of the task with the visible marker. These experiments thus suggest that some mechanism involving NMDA receptors in the hippocampus, perhaps LTP, is involved in spatial learning. However, if the NMDA receptor blocker is injected into the hippocampus *after* an animal has learned a spatial memory task, it does not inhibit subsequent memory recall for that task. This is consistent with findings that NMDA receptors are required for the induction, but not the maintenance, of LTP.

More direct evidence correlating memory formation and LTP comes from experiments with mutant mice that have genetic lesions that interfere with LTP. One interesting mutation is produced by the genetic deletion of the NR1 subunit of the NMDA receptor. Neurons lacking this subunit fail to form functional NMDA receptors. Mice with a general deletion of the subunit die soon after birth, indicating the importance of these receptors for neural function. However, it is possible to generate lines of conditional mutant mice in which the NR1 deletion is restricted to CA1 pyramidal neurons and occurs only 1 or 2 weeks after birth (see Chapter 2, Figure 2–8, for a description of how this mouse line is generated). These mice survive into adulthood and show a loss of LTP in the Schaffer collateral pathway. Although this disruption is highly localized, the mutant mice have a serious deficit in spatial memory (Figure 54–7).

Figure 54–7

Long-term potentiation (LTP) and spatial learning and memory are impaired in mice that lack the *N*-methyl-D-aspartate (NMDA) receptor in the CA1 region of the hippocampus. (Reproduced, with permission, from Tsien, Huerta, and Tonegawa 1996.)

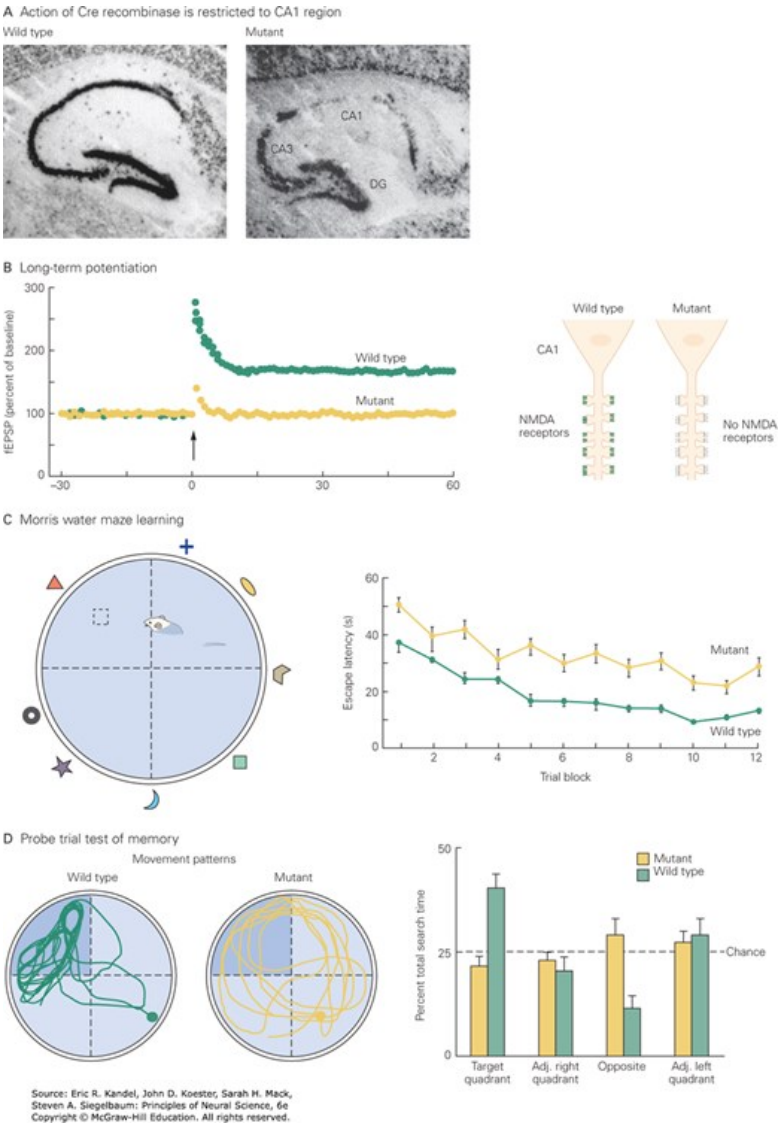
A. A line of mice is bred in which the gene encoding the NR1 subunit of the NMDA receptor is selectively deleted in CA1 pyramidal neurons. In situ hybridization is used to detect mRNA for the NR1 subunit in hippocampal slices from wild type and mutant mice that contain two floxed NR1 alleles and express Cre recombinase under the control of the *CaMKIIα* promoter. Note that NR1 mRNA expression (**dark staining**) is greatly reduced in the CA1 region of the hippocampus but not in CA3 and the dentate gyrus (**DG**).

B. LTP at the CA1 Schaffer collateral synapses is abolished in these mice. Field excitatory postsynaptic potentials (**fEPSPs**) were recorded in response to Schaffer collateral stimulation. Tetanic stimulation at 100 Hz for 1 second (**arrow**) caused a large potentiation in wild type mice but failed to induce LTP in the NMDA receptor knockout (mutant) mice.

C. Mice that lack the NMDA receptor in CA1 pyramidal neurons have impaired spatial memory. A platform (**dashed square**) is submerged in an opaque fluid in a circular tank (a Morris water maze). To avoid remaining in the water, the mice have to find the platform using spatial (contextual) cues on the walls surrounding the tank and then climb onto the platform. The graph shows escape latency or the time required by mice to find the hidden platform in successive trials. The mutant mice display a longer escape latency in every block of trials (four trials per day) than do the wild type mice.

Also, mutant mice do not reach the optimal performance attained by the control mice after 12 training days, even though they show some improvement with training.

D. After the mice have been trained in the Morris maze, the platform is taken away. In this probe trial, the wild type mice spend a disproportionate amount of time in the quadrant that formerly contained the platform (the target quadrant), indicating that they remember the location of the platform. Mutant mice spend an equal amount of time (25%) in all quadrants; that is, they perform at chance level, indicating deficient memory.



In some cases, genetic changes can actually enhance both hippocampal LTP and spatial learning and memory. One of the first examples of such an enhancement comes from studies of a mutant mouse that overexpresses the NR2B subunit of the NMDA receptor. This subunit is normally present at hippocampal synapses in the early stages of development but is downregulated in adults. Receptors that include this subunit allow more Ca^{2+} influx than those without the subunit. In mutant mice that overexpress the NR2B subunit, LTP is enhanced, presumably because of an enhancement in Ca^{2+} influx. Importantly, learning and memory for several different tasks are also enhanced (Figure 54–8).

Figure 54–8

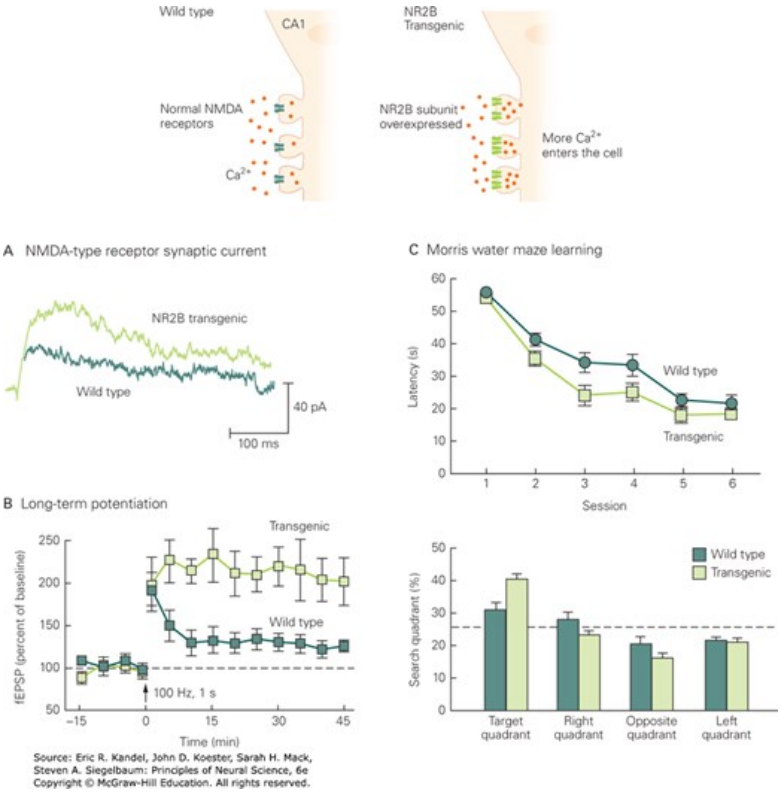
Learning and memory are enhanced in mice that overexpress a subunit of the *N*-methyl-D-aspartate (NMDA) glutamate receptor.
(Reproduced, with permission, from Tang et al. 1999. Copyright © 1999 Springer Nature.)

A. The amplitude of the current generated by the NMDA receptors in response to a brief pulse of glutamate is enhanced and its time course prolonged

in hippocampal neurons obtained from mice that contain a transgene that expresses higher levels of the receptor's NR2B subunit compared to wild type mice.

B. Long-term potentiation produced by tetanic stimulation of the Schaffer collateral synapses is greater in the transgenic mice than in wild type mice. (Abbreviation: **fEPSP**, field excitatory postsynaptic potential.)

C. Spatial learning is enhanced in the transgenic mice (**upper plot**). The rate of learning in a Morris water maze (the reduction in time to find the hidden platform, or escape latency) is faster in transgenic mice than in wild type mice. Spatial memory is also enhanced in the transgenic mice (**lower plot**). In the probe trial, the transgenic mice spend more time in the target quadrant, which previously contained the hidden platform, than do wild-type mice.



One concern with gene knockouts or transgene expression is that such mutations might lead to subtle developmental abnormalities. That is, changes in the size of LTP and spatial memory in the mutant animals could be the result of an early developmental alteration in the wiring of the hippocampal circuit rather than a change in the basic mechanisms of LTP. This possibility can be addressed by reversibly turning on and off a transgene that interferes with LTP.

Reversible gene expression has been used to explore the role of CaMKII, whose autophosphorylation properties and function in LTP were discussed earlier in this chapter (see also Chapter 2, Figure 2–9, for a description of the methodology). Mutation of the autophosphorylation Thr286 site to the negatively charged amino acid aspartate mimics the effect of autophosphorylation at Thr286 and converts the CaMKII to a calcium-independent form. Transgenic expression of this dominant mutation of CaMKII (CaMKII-Asp286) results in a systematic shift in the relation between the frequency of a tetanus and the resultant change in synaptic strength during long-term plasticity.

In the transgenic mice, tetanic stimulation at an intermediate frequency of 10 Hz, which normally induces a small amount of LTP, induces long-term depression of synaptic transmission in the Schaffer collateral pathway (Figure 54–9A). In contrast, the transgenic mice showed normal LTP to a 100-Hz tetanus. The defect in synaptic plasticity with 10-Hz stimulation is associated with an inability of the mutant mice to remember spatial tasks. However, the defects in the induction of LTP and in spatial memory can be fully extinguished when the mutant gene is switched off in the adult, showing that the memory defect is not due to a developmental abnormality (Figure 54–9).

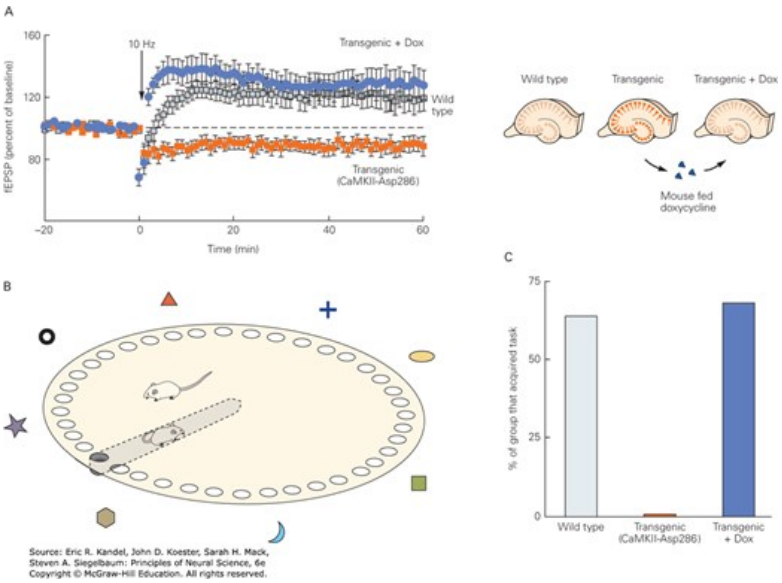
Figure 54–9

Deficits in long-term potentiation (LTP) and spatial memory due to a transgene are reversible. (Reproduced, with permission, from Mayford et al. 1996.)

A. An LTP deficit is seen in hippocampal slices from transgenic mice that overexpress CaMKII-Asp286 kinase, a constitutively active mutant form of CaMKII. Expression of this transgene is driven by a second transgene, the tTA bacterial transcription factor, which is inhibited by the antibiotic doxycycline (**Dox**) (see Chapter 2, Figure 2–9, for a complete description). Four groups of mice were tested: transgenic mice that were fed doxycycline, which blocks expression of the kinase; transgenic mice without doxycycline, in which the kinase is expressed; and wild type mice with and without doxycycline. In wild type mice, a 10-Hz tetanus induces LTP; doxycycline has no effect (data are not shown). In the transgenic mice, the tetanus fails to induce LTP but causes a small synaptic depression. In the transgenic mice that were fed doxycycline, the deficit in LTP is reversed. (Abbreviation: **fEPSP**, field excitatory postsynaptic potential.)

B. The effect of the kinase on spatial memory was tested in a Barnes maze. The maze consists of a platform with 40 holes, one of which leads to an escape tunnel that allows the mouse to exit the platform. The mouse is placed in the center of the platform. Mice do not like open, well-lit spaces and therefore try to escape from the platform by finding the hole that leads to the escape tunnel. The most efficient way of learning and remembering the location of the hole (and the only way of meeting the criteria set for the task by the experimenter) is for the mouse to use distinctive markings on the four walls as spatial cues, thus demonstrating hippocampal spatial memory.

C. Transgenic mice that receive doxycycline perform as well as wild type mice in learning the Barnes maze task (approximately 65% of animals learn the task), whereas transgenic mice without the doxycycline, which thus express CaMKII-Asp286, do not learn the task.



These several experiments using restricted knockout and overexpression of the NMDA receptor and regulated overexpression of CaMKII-Asp286 make it clear that the molecular pathways important for LTP at Schaffer collateral synapses are also required for spatial memory. However, such results do not directly show that spatial learning and memory are actually associated with an enhancement in hippocampal synaptic transmission. Mark Bear and his colleagues addressed this question by monitoring the strength of synaptic transmission at the Schaffer collateral synapses in vivo in rats.

Recordings were made of synaptic strength using an array of extracellular electrodes to stimulate the Schaffer collateral inputs and another array to record the extracellular field EPSPs at various locations. Rats were then trained to avoid one side of a box through administration of a foot shock; the field EPSPs were remeasured after training, showing a small but significant increase in the amplitude of synaptic transmission at a subset of the recording electrodes. Does the increase in synaptic transmission during learning result from LTP or some other mechanism? Because the amount of LTP at a given synapse is finite, if learning does indeed recruit an LTP-like process, then the ability to induce LTP by tetanic stimulation after learning should be reduced. Indeed, Bear and his colleagues found that the magnitude of LTP is diminished at those recording sites where the behavioral training produced the greatest enhancement in the field EPSP. This result is similar to findings in the amygdala, where fear learning reduces the magnitude of LTP induced by subsequent tetanic stimulation.

If LTP-like changes take place during memory formation in the hippocampus, such changes would be expected only in a small subset of synapses,

namely those that participate in the storage of the particular memory. Different memories probably correspond to different assemblies of cells with strengthened synaptic interconnections. If this is true, however, hippocampal memories should be vulnerable to disruption by manipulations that indiscriminately alter synaptic strength within the network as a whole. To test this idea, investigators induced LTP throughout the dentate gyrus *after* hippocampal-dependent spatial training in the water maze task. This protocol indeed impairs the animal's memory of the goal location in the water maze. Control animals that are given NMDA receptor antagonists after learning but prior to high-frequency stimulation exhibit normal spatial memory. These results indicate that the memory impairment was generated specifically as a consequence of the generation of indiscriminate LTP, which likely disrupts the specific pattern of strong and weak synapses that encode memory of the goal location.

Finally, although most behavioral tests of LTP have used spatial learning tasks to assess memory, studies have also shown that NMDA receptors, and by inference LTP, are necessary for a variety of hippocampal-dependent explicit memories. When NMDA receptors in the CA1 area are blocked, mice are not able to master a nonspatial object recognition task, learn complex odor discrimination, or undergo the social transmission of a food preference, in which an animal learns to accept a novel food by observing a conspecific (another animal of its species) consume that same food. Thus, NMDA receptor–dependent LTP is likely required for many, if not all, forms of explicit memory in the hippocampus (most of which include a spatial recognition element).

Explicit Memory Storage Also Depends on Long-Term Depression of Synaptic Transmission

If synaptic connections could only be enhanced and never attenuated, synaptic transmission might rapidly saturate—the strength of the synaptic connections might reach a point beyond which further enhancement is not possible. Moreover, uniform synaptic strengthening may lead to a loss of memory specificity, with one memory interfering with another. Yet individuals are able to learn, store, and recall new memories throughout a lifetime. This paradox led to the suggestion that neurons must have mechanisms to downregulate synaptic function to counteract LTP.

Such an inhibitory mechanism, termed *long-term depression* (LTD), was first discovered in the cerebellum, where it is important for motor learning. Since then, LTD has also been characterized at a number of synapses within the hippocampus. Whereas LTP is typically induced by a brief high-frequency tetanus, LTD is induced by prolonged low-frequency synaptic stimulation (Figure 54–10A). As mentioned above, it can also be induced by a spike pairing protocol in which an EPSP is evoked *after* an action potential in the postsynaptic cell. This suggests a corollary to Hebb's learning rule: Active synapses that do not contribute to the firing of a cell are weakened. Like LTP, a number of molecular and synaptic mechanisms are engaged during the induction and expression of LTD.

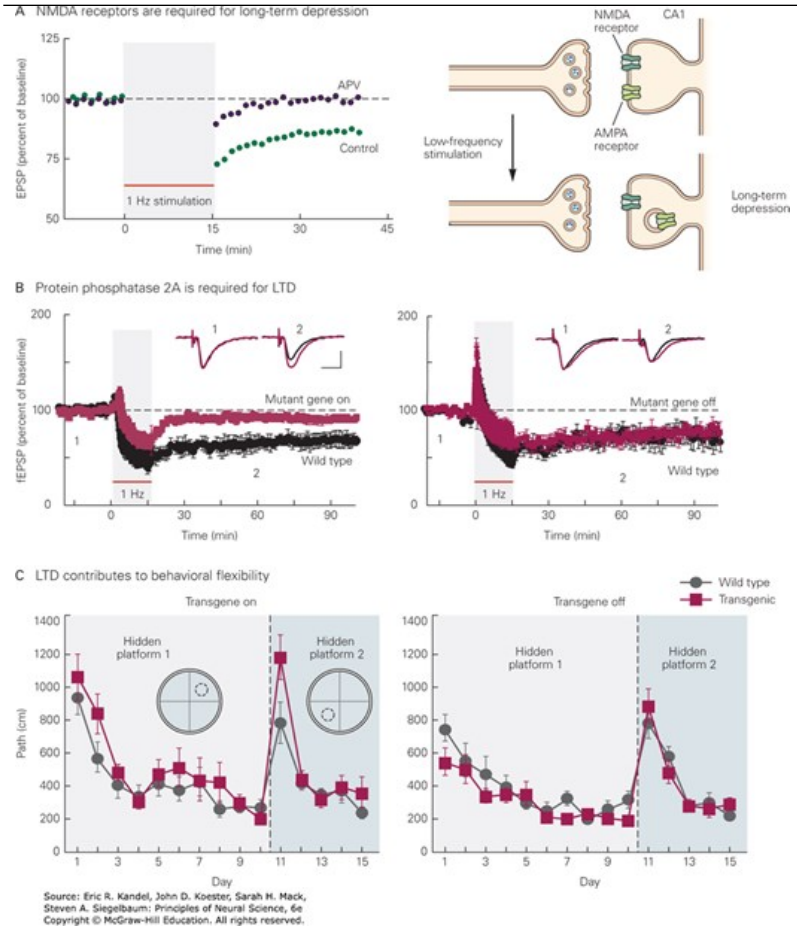
Figure 54–10

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.)



Surprisingly, many forms of LTD require activation of the same receptors involved in LTP, namely the NMDA receptors (Figure 54–10A). How can activation of a single type of receptor produce both potentiation and depression? A key difference lies in the experimental protocols used to induce LTP or LTD. Compared to the high-frequency stimulation used to induce LTP, the low-frequency tetanus used to induce LTD produces a relatively modest postsynaptic depolarization and thus is much less effective at relieving the Mg^{2+} block of the NMDA receptors. As a result, any increase in Ca^{2+} concentration in the postsynaptic cell is much smaller than the increase observed during induction of LTP and therefore insufficient to activate CaMKII, the enzyme implicated in LTP. Rather, LTD may result from activation of the calcium-dependent phosphatase calcineurin, an enzyme complex that has a higher affinity for Ca^{2+} compared to that of CaMKII (Chapter 14).

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.

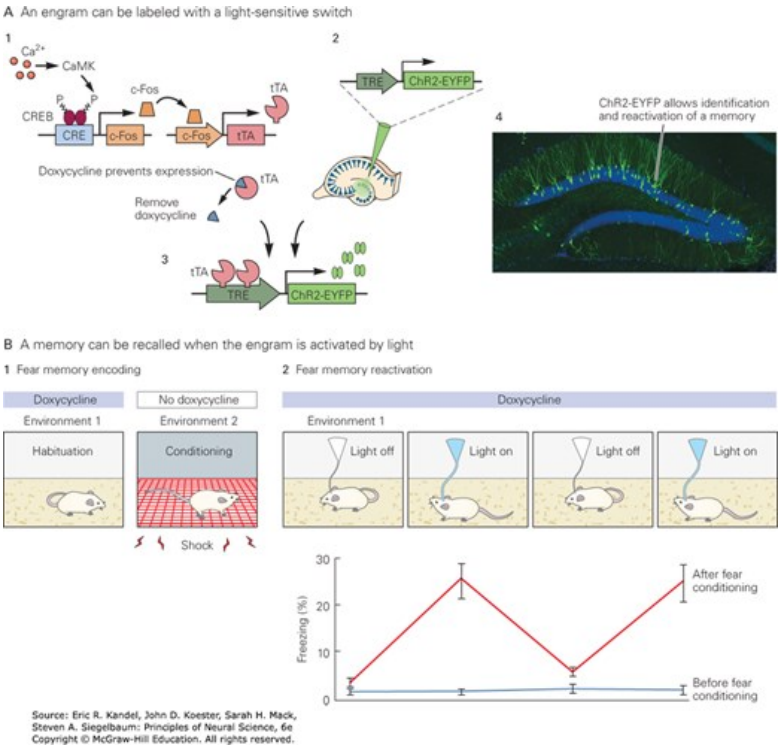
Figure 54–11

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**).



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 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.

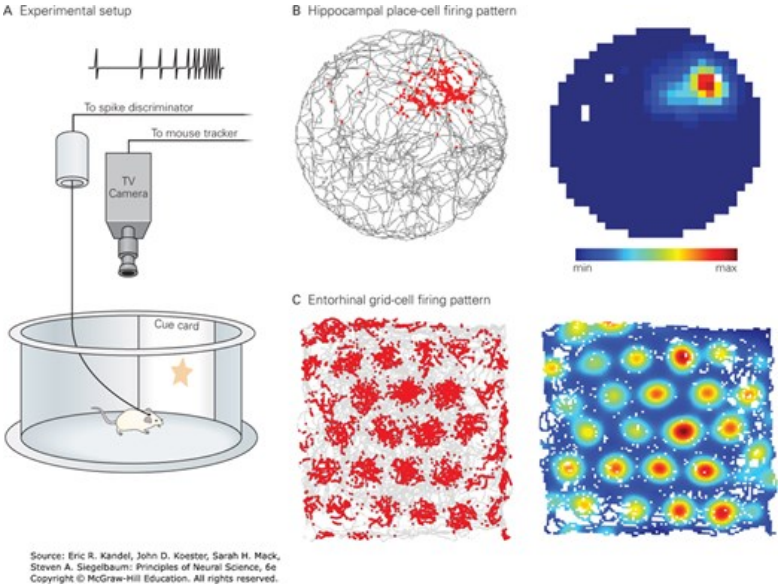
Figure 54–12

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.)



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.

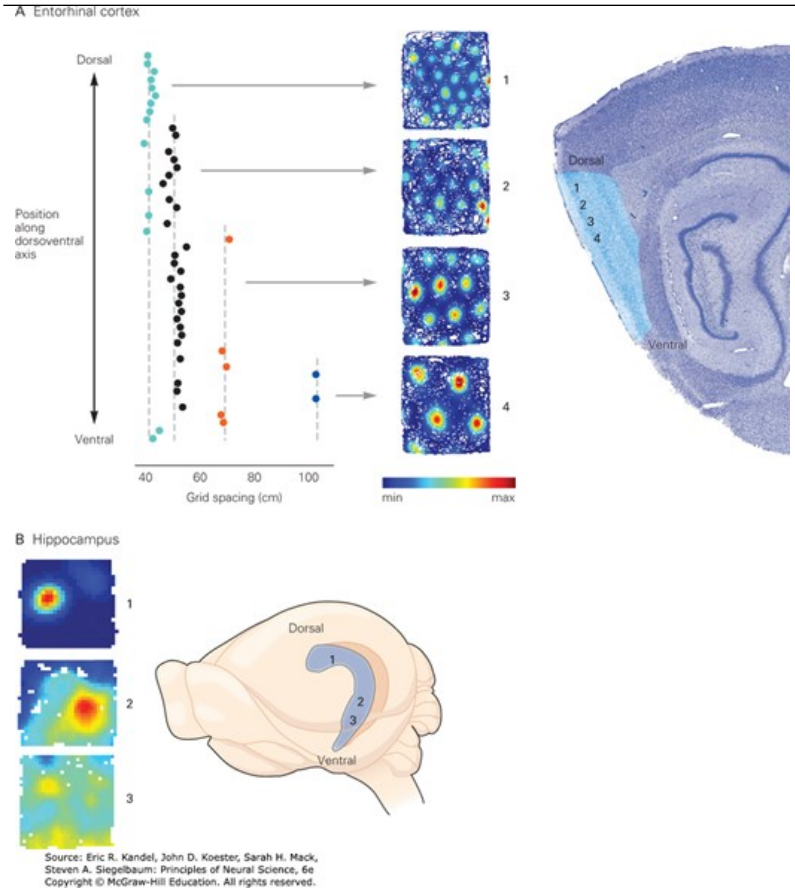
Grid cells display a characteristic relation between their firing fields and anatomical organization (Figure 54–13). The x,y coordinates of a cell's grid fields—often called the phase of the grid—differ among cells at the same location of the medial entorhinal cortex. The x,y coordinates of two neighboring 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.

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.)



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.

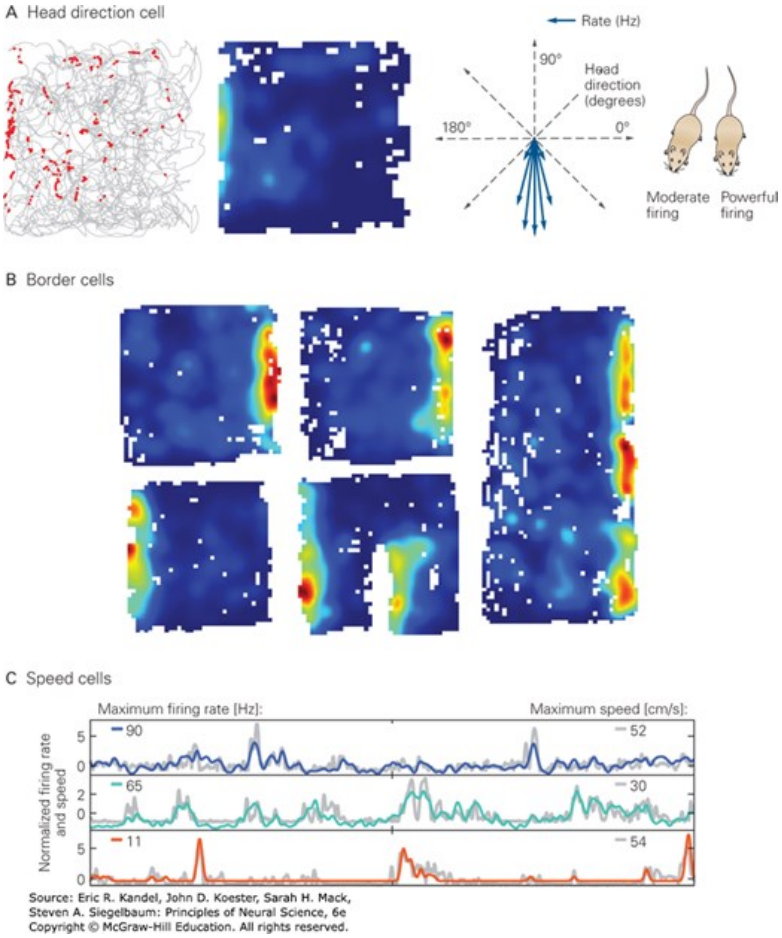
Figure 54–14

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.)



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 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.

Taken together, these discoveries point to a network of functionally dedicated cells in the medial entorhinal cortex reminiscent of the feature detectors of the sensory cortices. The functional specificity of each cell type stems from the cell's representation of a specific feature of behavior. In this sense, the entorhinal cell types differ from cells in most other association cortices, which integrate information from many sources in ways that are not straightforward to decode.

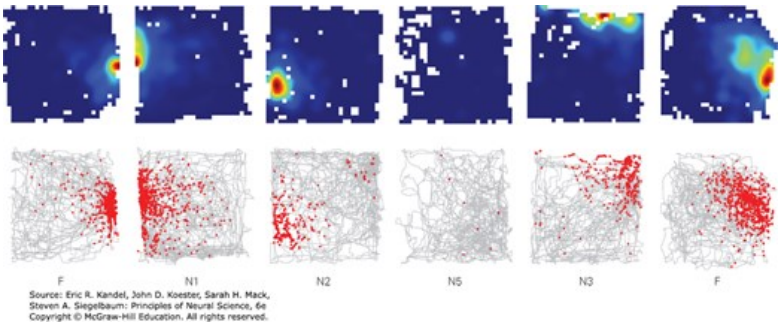
What are the key differences between space-coding cells in the hippocampus and the medial entorhinal cortex? A striking property of all entorhinal cell types is the rigidity of their firing patterns. Ensembles of co-localized grid cells maintain the same intrinsic firing pattern regardless of context or environment. When a pair of grid cells has overlapping grid fields in one environment, their grid fields overlap also in other environments. If their grid fields are opposite, or "out of phase," they will be opposite in other environments as well. A similar rigidity is seen in head direction cells and border cells: Cells with similar orientation in one environment have similar orientations in other environments. Speed cells also maintain their unique tuning to running speed across environments. These findings suggest that the medial entorhinal cortex, or modules of this cortical circuit, may operate like a

universal map of space that disregards the details of the environment. By doing so, the entorhinal map differs strongly from the place-cell map of the hippocampus.

The firing pattern of a hippocampal place cell is very sensitive to changes in the environment. The place fields of a given place cell in the hippocampus often switch to encode a completely different spatial locale when an animal's environment undergoes a major change, a process referred to as "remapping." Sometimes even minor changes in sensory or motivational inputs are sufficient to elicit remapping. The lack of correlation of hippocampal place maps for different environments (Figure 54–15) is thought to facilitate storage of discrete memories and minimize the risk that one memory will be confused with another, a process termed interference. For an explicit memory system like the hippocampus, with millions of events to be stored, this may be a huge advantage. For accurate and fast representation of an animal's position in space, as occurs in the medial entorhinal cortex, it may instead be beneficial to use a more stereotyped code that is less sensitive to environmental context or nonspatial sensory stimuli.

Figure 54–15

Place cells form independent maps for different environments. Rate maps showing firing patterns of a single hippocampal place cell in different square enclosures, each located in a different room. The rat was tested in one familiar (**F**) and 11 novel (**N**) rooms (recordings only shown for four of the novel rooms). The top row shows firing rate maps, whereas the bottom row shows trajectories of the animal's movement with firing locations in **red**. The cell was active only in some of the rooms (F, N1, N2, N3), where the firing locations were different. When the rat was returned to the familiar room at the end of the experiment, the cell's firing field had a similar location to the initial recording in the familiar room, indicating that a given cell's spatial firing pattern in the same environment is stable. (Adapted, with permission, from Alme et al. 2014.)



Place Cells Are Part of the Substrate for Spatial Memory

In addition to representing the animal's current location, place cells are thought to also store the memory of a location in position-related firing patterns that are evoked in the absence of the sensory inputs that originally elicited the firing. For example, as an animal sleeps after running repeated laps along a linear maze, place cells spontaneously fire in the same order that they did in the maze, a phenomenon called "replay." Similarly, past trajectories and experiences may influence firing rates at particular locations in the environment. The ability of place cells to represent events and locations experienced in the past likely underlies the ability of the hippocampus to encode complex memories of events.

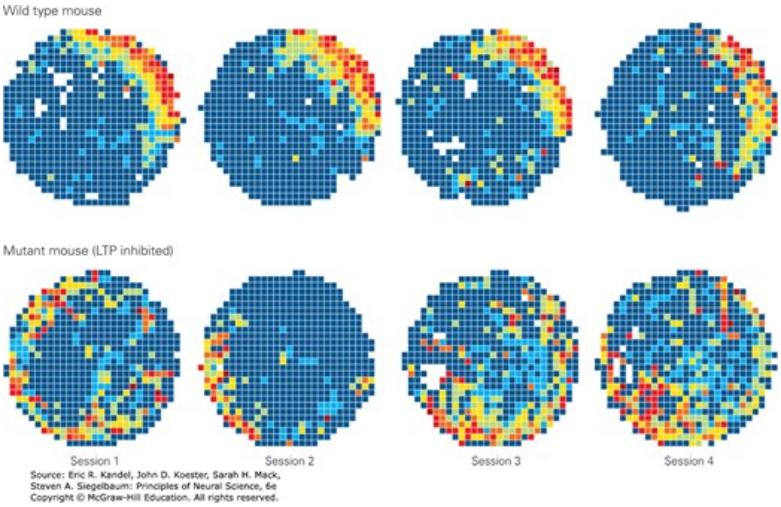
Once the firing pattern of a population of hippocampal neurons is formed for a given environment, how is it maintained? Because the place cells are the same hippocampal pyramidal neurons that undergo experimental LTP, a natural question is whether LTP is important. This question was addressed in experiments in mice in which LTP was disrupted.

In mice lacking the NR1 subunit of the NMDA receptor, hippocampal pyramidal neurons still fire in place fields despite the fact that LTP is blocked. Thus, this form of LTP is not required for the transformation of spatial sensory information into place fields. However, the place fields of the mutant mice are larger and fuzzier in outline than those in normal animals. In a second experiment with mutant mice, late LTP and long-term spatial memory were selectively disrupted by expression of a transgene that encodes a protein inhibitor of PKA. In these mice, place fields also form, but the firing patterns of individual cells are stable only for an hour or so (Figure 54–16). Thus, late LTP is required for long-term stabilization of place fields but not their formation.

Figure 54–16

Disruption of long-term potentiation (LTP) degrades the stability of place field formation in the hippocampus. Color-coded firing rate

maps (see Figure 54–12) show place fields recorded in four successive sessions from a single hippocampal pyramidal neuron in a wild type mouse and from a neuron in a mutant mouse that expresses the persistently active CaMKII (which inhibits the induction of LTP). Before each recording session, the animal is taken out of the enclosure and sometime later reintroduced into it. In each of the four sessions, the place field for the cell in the wild type animal is stable; the cell fires whenever the animal is in the upper right region of the enclosure. By contrast, the place field for the cell in the mutant mouse is unstable across the four sessions. (Reproduced, with permission, from Rotenberg et al. 1996.)



To what degree do these maps of an animal's surroundings mediate explicit memory? In humans, explicit memory is defined as the conscious recall of facts about people, places, and objects. Although consciousness cannot be studied empirically in the mouse, selective attention, which is required for conscious recall, can be examined.

When mice are presented with different behavioral tasks, the long-term stability of place fields correlates strongly with the degree of attention required to perform the task. When a mouse does not attend to the space it walks through, place fields form but are unstable after 3 to 6 hours. Animals with unstable place fields are unable to learn a spatial task. However, when a mouse is forced to attend to the space, for example, when trained to run to a specific location, the place fields are stable for days.

How does this attentional mechanism work? Studies in primates have shown the importance of the prefrontal cortex and the modulatory dopaminergic system during attention. Indeed, the formation of stable place fields in mice requires the activation of the dopamine D_1/D_5 type of receptor, which has been shown to enhance the formation of late LTP through production of cAMP and activation of PKA. These results suggest that long-term memory of a place field, rather than being a form of implicit memory that is stored and recalled without conscious effort, requires the animal to attend to its environment, as is the case for explicit memory in humans.

Disorders of Autobiographical Memory Result From Functional Perturbations in the Hippocampus

Our sense of identity is greatly dependent on our store of explicit autobiographical memories and our ability to recognize and navigate through familiar spatial environments. Neurological and psychiatric disorders that disrupt these abilities often occur as a result of changes in neural circuitry and plasticity mechanisms within the hippocampus and related regions in the temporal lobe.

There is now substantial evidence that the devastating memory loss associated with Alzheimer disease is associated with an accumulation of extracellular plaques of the protein fragment β-amyloid (Aβ) and intracellular neurofibrillary tangles of tau, a microtubule associated protein (Chapter 64). However, even before plaques and tangles are apparent, elevated levels of soluble Aβ and tau are thought to disrupt a number of cellular processes, particularly by reducing the magnitude of both early and late LTP at certain synapses. Mouse models of Alzheimer disease also show alterations in hippocampal place cell stability and population-level synchrony, which may contribute to memory loss and spatial disorientation. Changes in grid-cell function have also been observed in electrophysiological recordings in mouse disease models and in humans through functional magnetic resonance imaging studies. Although a number of preclinical studies have shown that agents that decrease levels of Aβ can rescue synaptic function and memory in rodents, so far these treatments have been less successful in treating patients with Alzheimer disease, perhaps because

treatment must be initiated at early stages prior to irreversible synaptic changes.

Altered hippocampal function may also contribute to cognitive problems experienced by individuals with schizophrenia, including disturbances in working memory (Chapter 60). Recent studies using a genetic mouse model of schizophrenia report reduced synchrony between the hippocampus and prefrontal cortex associated with working memory. Furthermore, the place fields of place cells in the hippocampus CA1 region may be overly rigid in this mouse, suggesting that the ability of the hippocampus to distinguish different contexts may be impaired. Finally, a deficit in social memory in these mice has been linked to a reduction of parvalbumin-positive inhibitory neurons in the CA2 region; a similar loss of inhibitory neurons has been observed in postmortem brain tissue from individuals with schizophrenia and bipolar disorder.

Thus, studies of the hippocampus and related temporal lobe structures offer the great promise of providing fundamental insight into how explicit memories are stored and recalled and how functional alterations in these structures may contribute to neuropsychiatric disease. In turn, such insight may aid in the discovery of new treatments for these devastating disorders.

Highlights

1. Explicit memory has both a short-term component, termed working memory, and a long-term component. Both forms depend on the prefrontal cortex and hippocampus.
2. Long-term memory is thought to depend on activity-dependent long-term synaptic plasticity at synapses within the cortico-hippocampal circuit. A brief high-frequency train of tetanic stimulation leads to long-term potentiation (LTP) of excitatory synaptic transmission at each stage of the cortico-hippocampal circuit.
3. LTP at many synapses depends on calcium influx into the postsynaptic cell mediated by the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor. This receptor acts as a coincidence detector: It requires both glutamate release and strong postsynaptic depolarization to conduct calcium.
4. The expression of LTP depends on either the insertion of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type of glutamate receptors in the postsynaptic membrane or an increase in presynaptic glutamate release, depending on the type of synapse and intensity of tetanic stimulation.
5. LTP has both early and late phases. Early LTP depends on covalent modifications, whereas late LTP depends on new protein synthesis, gene transcription, and growth of new synaptic connections.
6. Pharmacological and genetic manipulations that disrupt LTP often lead to an impairment of long-term memory, indicating that LTP may provide an important cellular mechanism for memory storage.
7. Memories are stored by cell assemblies. LTP may be required for forming event-specific assemblies. Recall of memory may reflect reactivation of the same assemblies that were active during the original event.
8. The hippocampus encodes both spatial and nonspatial signals. Many hippocampal neurons act as place cells, firing action potentials when an animal visits a particular location in its environment.
9. The entorhinal cortex, the area of the cortex that provides most of the input to hippocampus, also encodes both nonspatial and spatial information. The medial portion of entorhinal cortex contains neurons, called grid cells, that fire when an animal crosses the vertices of a hexagonal grid-like lattice of spatial locales. Grid cells are organized into semi-independent semi-topographically organized modules with distinct grid frequencies. The entorhinal map also contains border cells, object-vector cells, head direction cells, and speed cells.
10. Within a grid-cell module, pairs of grid cells maintain firing relationships rigidly across environments and experiences, suggesting that grid cells form a universal map that is expressed similarly in all environments. In contrast, place cells in the hippocampus form maps that are plastic as they are completely uncorrelated between environments.
11. Neuropsychiatric disorders such as Alzheimer disease and schizophrenia have been associated with deficits in hippocampal and entorhinal synaptic function, place-cell properties, and learning and memory. Treatments aimed at restoring such function may yield new therapeutic

approaches to disease.

12. Despite their clear differences, implicit (Chapter 53) and explicit memory storage rely on a common logic. Both activity-dependent presynaptic facilitation for storing implicit memory and associative long-term potentiation for storing explicit memory rely on the associative properties of specific proteins: Adenylyl cyclase activation in implicit memory requires neurotransmitter plus intracellular Ca^{2+}, whereas NMDA receptor activation in explicit memory requires glutamate plus postsynaptic depolarization. Such similarities indicate the fundamental importance of associative learning rules for memory storage.

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