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Prefrontal Cortex, Hippocampus, and the Biology of Explicit Memory Storage

EXPLICIT MEMORY—THE CONSCIOUS recall of information about people, places, and objects—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.

The two structures in the mammalian brain that are critical for encoding and storing explicit memories are the prefrontal cortex and the hippocampus. The prefrontal cortex mediates working memory (see Chapter 65). Information stored in working memory can be actively maintained for very short periods and then rapidly forgotten, such as a telephone number that is remembered only until it is dialed, or it can be stored elsewhere in the brain as long-term memory. The hippocampus stores declarative information in a more stable form for periods ranging from days to weeks to years, up to a lifetime. The ultimate storage site for all declarative memories is thought to be in the cerebral cortex. In this chapter we focus on the cellular and molecular mechanisms underlying working memory and long-term storage of explicit memories.

Working Memory Depends on Persistent Neural Activity in the Prefrontal Cortex

In vivo electrophysiological recordings from neurons in the prefrontal cortex of nonhuman primates have provided insights into the neural basis of working memory. Neuronal activity is measured while the animal is engaged in a delayed match-to-sample working memory task. In such tasks the animal is initially shown an image (the sample) and must retain the image in working memory for seconds to minutes after the initial image is extinguished (the delay period). The monkeys are then shown a test image and must press a lever to indicate whether the test image matches the sample image.

Neurons in the prefrontal cortex fire persistently during the delay period, presumably contributing to the neural representation of the image in working memory. Two major mechanisms may contribute to this persistent neural activity: the intrinsic properties of neuronal membranes and recurrent synaptic connectivity.

Intrinsic Membrane Properties Can Generate Persistent Activity

In some cortical neurons a brief electrical stimulation can lead to persistent firing that lasts for seconds or even minutes after the end of the stimulus (Figure 67–1A). Moreover, the rate of firing can be a graded function of the intensity of the stimulation. This persistent firing is not affected by blockers of fast excitatory and inhibitory synaptic transmission, indicating that it depends on the intrinsic membrane properties of the neuron.

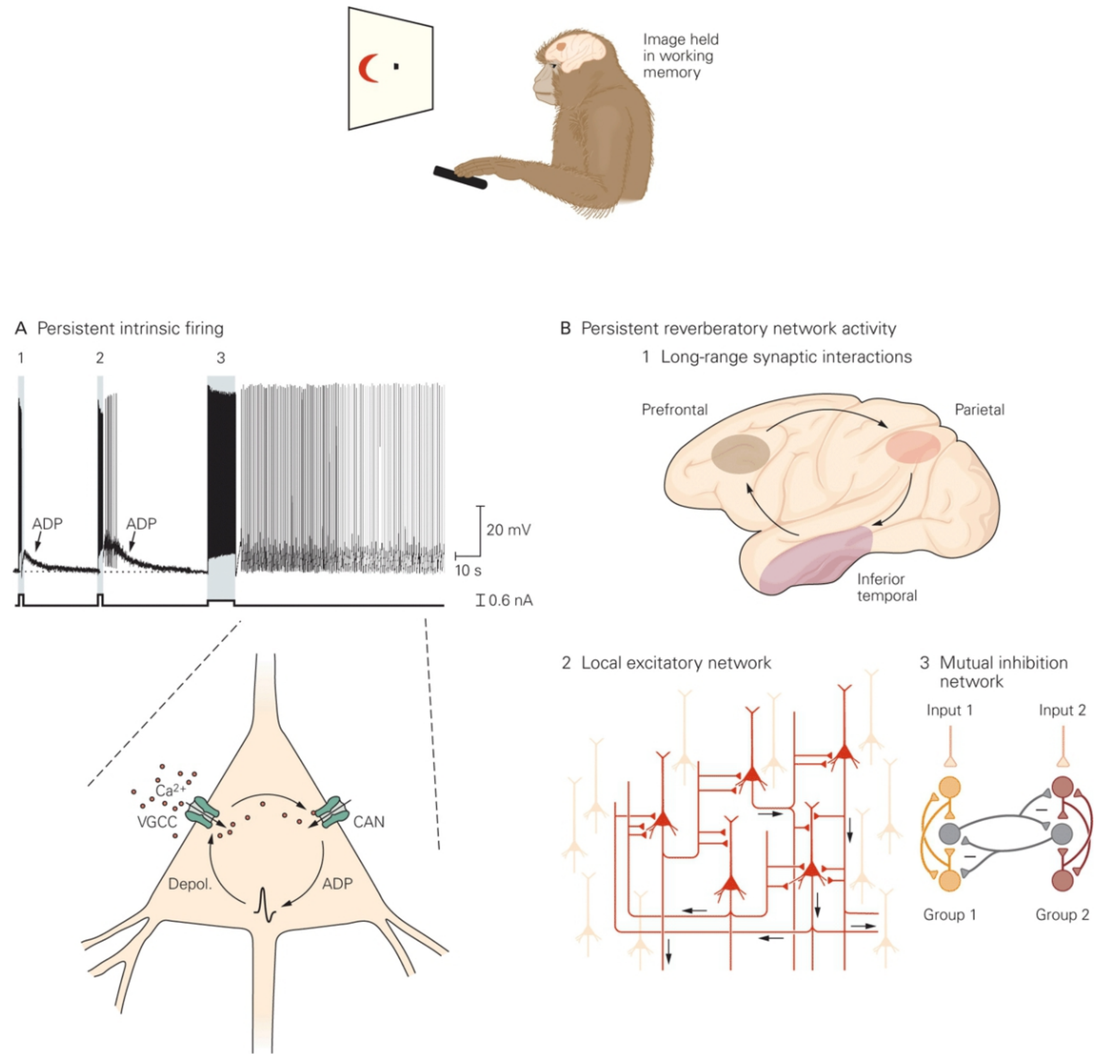


Figure 67–1 Mechanisms of persistent neuronal activity that may contribute to working memory. When a monkey performs a working memory task neurons in prefrontal cortex fire persistently during the delay period of the task.

A. Intrinsic mechanisms of graded persistent activity. A brief depolarizing stimulus to a pyramidal neuron in the entorhinal cortex elicits a short burst of action potentials followed by an afterdepolarization **(ADP)** (**1**). A slightly longer stimulus elicits a longer burst of spikes followed by a larger afterdepolarization (2). When the stimulus is further

lengthened, the afterdepolarization is sufficient to trigger additional action potentials, leading to persistent firing for tens of seconds (3). The diagram illustrates a potential mechanism for the persistent firing. The influx of Ca^{2+} through voltage-gated Ca^{2+} channels **(VGCC)** during an action potential opens Ca^{2+} -activated nonselective cation **(CAN)** channels. The resulting inward current through the CAN channels produces an afterdepolarization that can lead to action potentials. The action potentials further activate VGCCs, perpetuating the cycle. The recordings were obtained in the presence of carbachol, which activates muscarinic acetylcholine (ACh) receptors and a downstream signaling cascade that enables the opening of CAN channels when intracellular Ca^{2+} is also elevated. (Reproduced, with permission, from Egorov et al. 2002.)

B. Recurrent networks of synaptically coupled neurons can lead to persistent reverberatory activity. **1**. Some network interactions occur between two widely separated populations of excitatory neurons in distinct brain regions. **2**. Other excitatory networks are local, illustrated here by reciprocally connected neighboring pyramidal neurons in neocortex. **3**. Persistent activity can be generated through mutual inhibition. This example consists of two populations of neurons, groups 1 and 2. Within each population the neurons are reciprocally connected by excitatory synapses. However, each population mutually inhibits the other. In this manner an excitatory input to group 1 leads to the silencing of group 2. The loss of inhibitory input from group 2 (a process termed *disinhibition*) in turn enhances the firing of group 1 (B1 and B2 reproduced, with permission, from Wang 2001; B3 reproduced, with permission, from Aksay et al. 2007.)

The intrinsic mechanism underlying persistent firing has been best characterized in neurons in the deep layers of the entorhinal cortex. Normally a brief depolarizing current pulse elicits a transient burst of action potentials in these neurons. However, when the entorhinal neurons are exposed to acetylcholine, which activates G-protein coupled muscarinic receptors, a brief depolarizing current elicits a prolonged train of action potentials that persists for tens of seconds, far longer than the current stimulus.

This maintained firing depends on the opening of a type of channel termed the *Ca^{2+} -activated non-selective* (CAN) cation channel. The opening of these channels requires two simultaneous events. First, the muscarinic receptor signaling cascade must be stimulated by extracellular acetylcholine; second, there must be an increase in intracellular Ca^{2+}, normally generated by the opening of voltage-gated Ca^{2+} channels during the firing of a brief burst of action potentials. Ca^{2+} then opens the CAN channel by binding to a site on the channel's cytoplasmic surface. As the cytoplasmic Ca^{2+} level remains elevated for some time after the burst of action potentials, the inward current through the CAN channels leads to a prolonged afterdepolarization following the burst of action potentials.

If the initial stimulation period is sufficiently intense, the Ca^{2+} influx will activate sufficient current through the CAN channels so that the afterdepolarization will trigger a second round of spikes. This in turn leads to more Ca^{2+} influx, which activates more CAN channels, leading to a larger afterdepolarization that can maintain firing that far outlasts the initial stimulus. Thus these CAN channels contribute to persistent firing by participating in a positive feedback loop with voltage-gated Ca^{2+} channels. Recent studies suggest that this mechanism of persistent firing also is observed in prefrontal cortex neurons.

Network Connections Can Sustain Activity

The second type of mechanism for sustained firing depends on recurrent synaptic connections within neural circuits. In the simplest case activity is maintained by recurrent excitatory connections within the active population of neurons. A network can comprise either long-range connections between distinct regions of the brain or local circuits (Figure 67–1B). The firing maintained through such chains is referred to as *reverberatory* activity.

Another circuit that can sustain activity depends on reciprocal inhibitory synapses between two populations of neurons (Figure 67–1B). Neurons in both populations fire spontaneously at a basal level that is normally held in check by the reciprocal inhibitory synapses. However, a brief excitatory input to one population of neurons will transiently enhance their firing rate, which leads to an increase in their inhibitory output onto the second population. As a result, the firing rate of the second population decreases. This decreases the inhibitory input onto the first population of neurons, further enhancing their rate of firing.

This mechanism of positive feedback, termed *disinhibition*, can lead to firing of the first population of neurons that outlasts the initial stimulus. A network of reciprocal inhibitory connections between distinct populations of neurons contributes to the sustained firing of oculomotor neurons such as those of the goldfish, which are responsible for remembering eye position. It is likely that persistent activity during working memory involves a combination of network and intrinsic mechanisms.

Working Memory Depends on the Modulatory Transmitter Dopamine

Although the relative importance of intrinsic activity versus network activity in working memory remains uncertain, it is clear that the efficiency of working memory and persistent activity in prefrontal cortex neurons depends on the state of activation of the D_1 type of dopamine receptors. These receptors are coupled to the G protein G_s and the production of cyclic adenosine monophosphate (cAMP).

Patricia Goldman-Rakic and colleagues have found that there is an inverted U-shaped relation between the extent of D_1 receptor activation and working memory: Working memory is most efficacious at intermediate levels of D_1 receptor activation. Defects in the dopaminergic regulation of working memory in prefrontal cortex are thought to contribute to the cognitive deficits associated with schizophrenia.

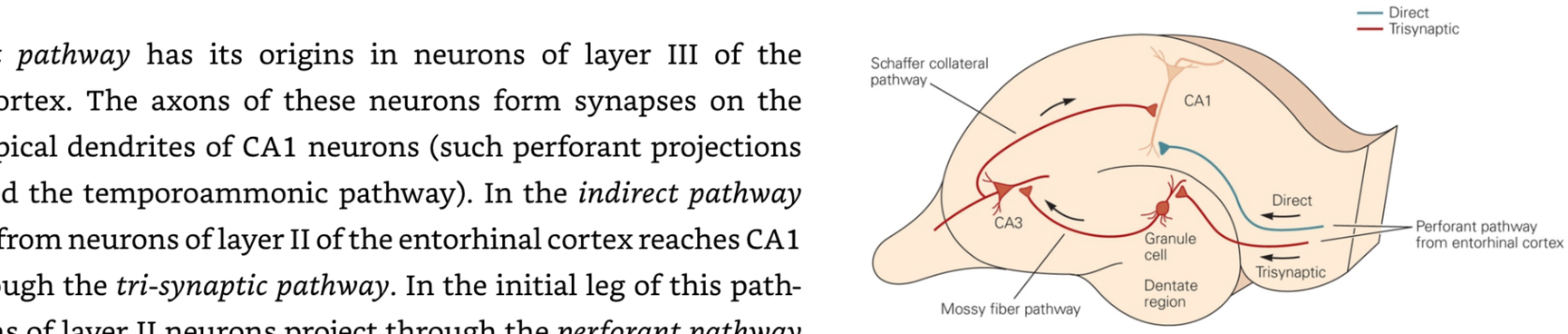
Explicit Memory in Mammals Involves Different Forms of Long-Term Potentiation in the Hippocampus

What neural mechanisms are responsible for long-term explicit memory mediated by the hippocampus and its associated structures in the medial temporal lobe of the mammalian brain? Unlike working memory, long-term storage of information by the hippocampus is not thought to depend on persistent neural firing but rather to involve long-lasting changes in the strength of synaptic connections.

The hippocampus receives multimodal sensory and spatial information from the nearby entorhinal cortex. The major output of the hippocampus is through the pyramidal neurons in the CA1 region, which project back to the entorhinal cortex and to the subiculum, another medial temporal lobe structure. 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, which has been complemented by numerous studies in animal models. Information from the entorhinal cortex reaches CA1 neurons along two excitatory pathways, one direct

pathway and one indirect. Together these inputs are termed the *perforant pathways*.

The *direct pathway* has its origins in neurons of layer III of the entorhinal cortex. The axons of these neurons form synapses on the very distal apical dendrites of CA1 neurons (such perforant projections are also called the temporoammonic pathway). In the *indirect pathway* information from neurons of layer II of the entorhinal cortex reaches CA1 neurons through the *tri-synaptic pathway*. In the initial leg of this pathway the axons of layer II neurons project through the *perforant pathway* to the granule cells of the dentate gyrus (an area considered part of the hippocampus). The granule cell axons project in the *mossy fiber pathway* to excite the pyramidal cells in the CA3 region of the hippocampus. Finally, the CA3 axons project through the *Schaffer collateral pathway* to make excitatory synapses on more proximal regions of CA1 pyramidal cell dendrites (Figure 67–2).



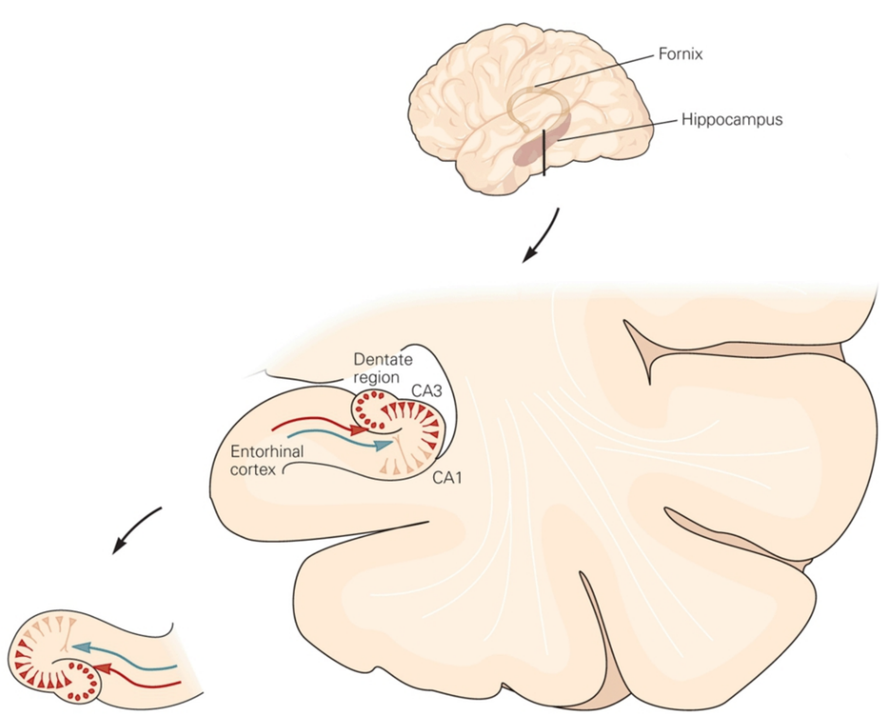
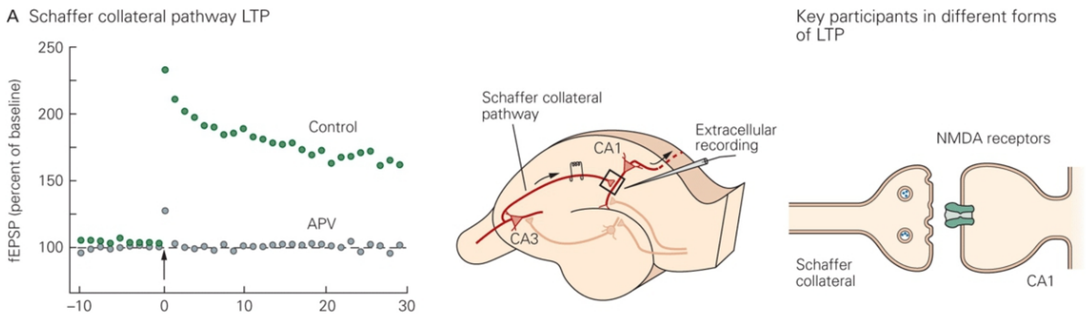


Figure 67–2 The hippocampal synaptic circuit is important for declarative memory. Information arrives in the hippocampus from entorhinal cortex through the *perforant pathways*, which provide both direct and indirect input to CA1 pyramidal neurons, the major output neurons of the hippocampus. (Arrows denote the direction of impulse flow.) In the indirect *trisynaptic pathway* neurons in layer II of 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 entorhinal cortex project through the perforant path to make excitatory synapses on the distal dendrites of CA1 pyramidal neurons without intervening synapses.

The fact that CA1 pyramidal neurons receive cortical information through two pathways has led to the view that CA1 neurons compare information in the indirect circuit with sensory input from the direct pathway. Lesion studies indicate that both direct and indirect inputs to CA1 may be necessary for normal learning and memory. Lesions of the indirect Schaffer collateral pathway limit the ability of mice to perform a complex spatial learning and memory task, although some form of spatial learning remains intact. Lesions of the direct pathway to CA1 do not

appear to alter initial formation of memory, but inhibit the ability of an animal to store those initial memories as long-term memory, a process termed *consolidation*. Genetic inactivation of the direct path also interferes with episodic memory, in which an animal must learn about the temporal relation between two or more events.

In 1973 Timothy Bliss and Terje LomØ discovered that the initial stage of the trisynaptic pathway—the perforant pathway from layer II of the entorhinal cortex to the dentate granule neurons—is remarkably sensitive to previous activity. A brief high-frequency train of stimuli (a tetanus) gives rise to *long-term potentiation* (LTP), a long-lasting increase in the amplitude of the excitatory postsynaptic potentials (EPSPs) in the dentate granule neurons. (In Chapter 66 we saw how a similar form of synaptic potentiation at synapses in the amygdala contributes to fear conditioning.) Subsequent studies showed that brief high-frequency trains of stimulation can induce forms of LTP at all three synapses of the trisynaptic pathway as well as at the direct perforant path synapses with CA1 neurons (Figure 67–3). Long-term potentiation can last for days or even weeks when induced in the intact animal using implanted electrodes. LTP can also be examined in slices of hippocampus and in cell culture, where it can last several hours.



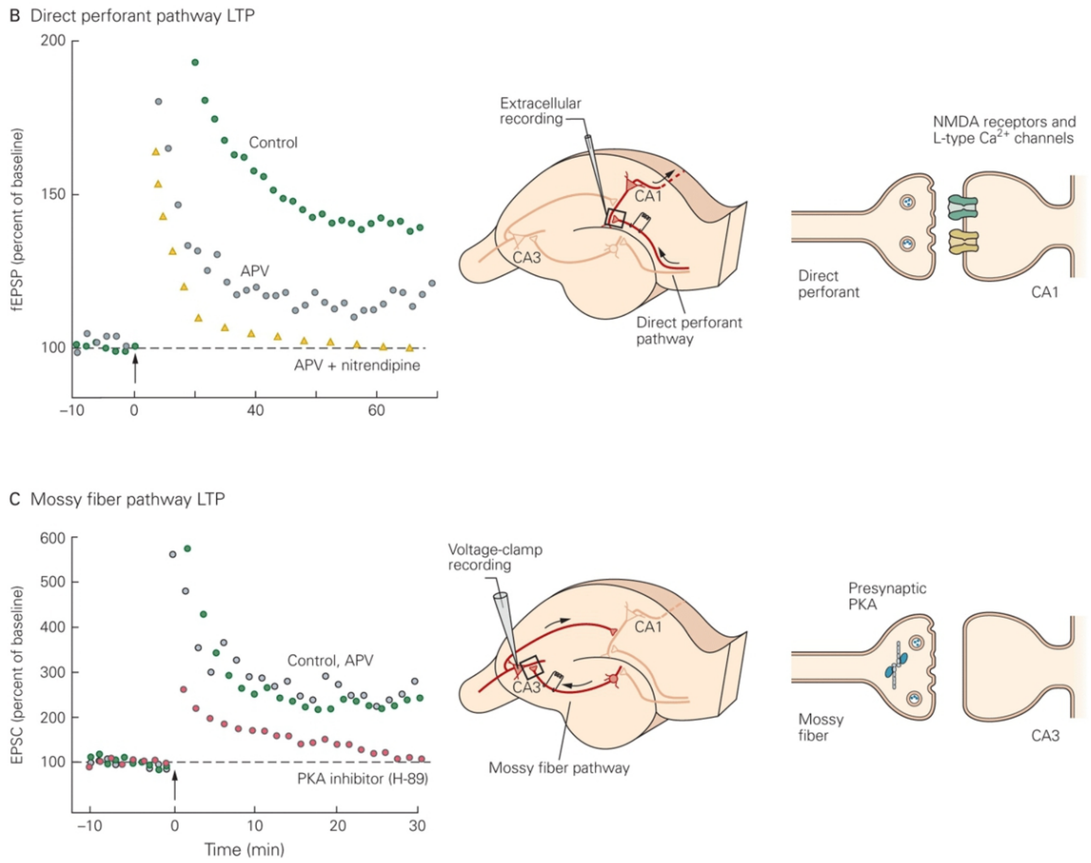


Figure 67–3 Different neural mechanisms underlie long-term potentiation at each of the three synapses in the trisynaptic pathway in the hippocampus. Long-term potentiation (LTP) is present at synapses throughout the hippocampus but depends to differing degrees on activation of NMDA-type glutamate receptors.

A. Tetanic stimulation of the Schaffer collateral pathway (at arrow) induces LTP at the synapses between presynaptic terminals of CA3 pyramidal neurons and their postsynaptic CA1 pyramidal neurons. The graph plots the size of the extracellular field EPSP (fEPSP) expressed as a percent of the initial baseline fEPSP prior to induction of LTP. At these synapses LTP requires activation of the NMDA receptors in the CA1 neurons as it is completely blocked when the tetanus is delivered in the

presence of the NMDA receptor antagonist APV. (Reproduced, with permission, 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 partially on activation of the NMDA receptors and partially on activation of L-type voltage-gated Ca^{2+} channels. It is therefore only partially blocked by APV. Addition of APV and nitrendipine, a dihydropyridine that blocks L-type channels, is needed to fully inhibit LTP. (Reproduced, with permission, from Remondes and Schuman 2003.)

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 was measured under voltage-clamp conditions. This LTP does not require activation of the NMDA receptors and so is not blocked by APV. It does require activation of protein kinase A and so is blocked by the kinase inhibitor H-89. (Reproduced, with permission, from Zalutsky and Nicoll 1990.)

Studies in these different pathways have shown that LTP is not a single form of synaptic plasticity. 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. However, these distinct processes also 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. However, the various forms of LTP differ in the relative importance of different receptors and ion channels. In addition, different forms of LTP may recruit different second-messenger signaling pathways either in the presynaptic cell, altering transmitter release, or in the postsynaptic cell, altering its sensitivity to the neurotransmitter glutamate.

The similarities and differences in the mechanisms of LTP at the Schaffer collateral, mossy fiber, and entorhinal inputs to CA1 can be seen by examining the role of the postsynaptic NMDA type of glutamate receptor in the induction of LTP in the three pathways. In all three pathways synaptic transmission is persistently enhanced in response to a brief tetanic stimulation. However, the contribution of the NMDA receptor to the induction of LTP differs in the three pathways.

At the Schaffer collateral synapses with CA1 pyramidal neurons, 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). However, 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 67–3). In the next two sections we consider the mechanisms of LTP in more detail, first in the mossy fiber pathway and then in the Schaffer collateral pathway.

Long-Term Potentiation in the Mossy Fiber Pathway Is Nonassociative

Glutamate released at the mossy fiber synapses binds to both the NMDA and AMPA type of glutamate receptors in the postsynaptic membrane of the CA3 neurons. However, under most conditions the NMDA receptors have only a minor role in synaptic transmission in this pathway. Moreover, as noted above, blocking these receptors has no effect on LTP (Figure 67–3C). Rather, LTP in the mossy fiber pathway is triggered by the large Ca^{2+} influx into the presynaptic terminals during a tetanus. In the presynaptic cell the Ca^{2+} influx activates a calcium/calmodulin–dependent adenylyl cyclase complex, thereby increasing the production of cAMP and activating protein kinase A. This leads to an increase in the release

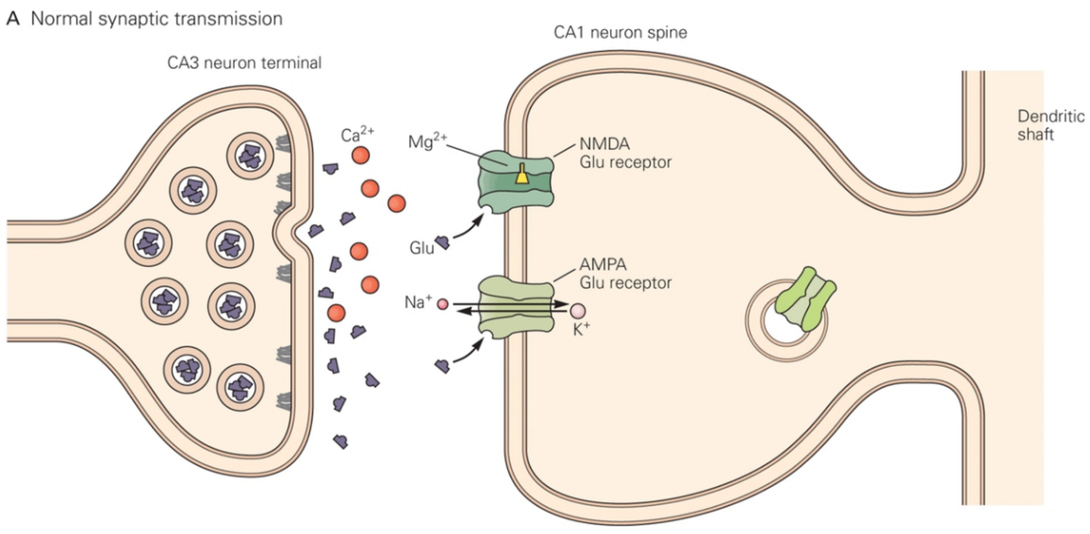
of glutamate from the mossy fiber terminals, resulting in LTP. Activity in the postsynaptic cell is not required for this form of LTP. Thus, mossy fiber LTP is nonassociative.

The increase in transmitter release is thought to depend on the ability of protein kinase A to phosphorylate RIM1α, a synaptic vesicle protein that interacts with several other presynaptic proteins important for exocytosis (see Chapter 12). Thus mossy fiber LTP is abolished in mice in which the gene for RIMα, has been deleted through genetic engineering. The importance of presynaptic protein kinase A in mossy fiber LTP resembles aspects of the synaptic changes responsible for associative learning in the gill-withdrawal reflex of *Aplysia* and amygdala-based learned fear in rodents (see Chapter 66). Another similarity with the synaptic changes in *Aplysia* is that induction of mossy fiber LTP is under the control of a system of modulatory inputs. Just as the activation of adenylyl cyclase by serotonin is important for long-term facilitation in *Aplysia*, mossy fiber LTP is facilitated by the binding of norepinephrine to β-adrenergic receptors, enhancing the activation of adenylyl cyclase.

Long-Term Potentiation in the Schaffer Collateral Pathway Is Associative

Like the mossy fiber terminals in the CA3 region, glutamate released from the Schaffer collateral terminals activates both AMPA and NMDA receptors in the postsynaptic membrane of CA1 pyramidal neurons. However, unlike the mossy fiber system, LTP in the Schaffer collateral pathway requires activation of the NMDA receptors in the postsynaptic cell, which triggers a complex postsynaptic signaling cascade.

The opening of the NMDA receptors, unlike the AMPA receptors, requires that two events occur simultaneously. First, like any ionotropic receptor, glutamate must bind to the NMDA receptor to open the channel. However, when the membrane is at the resting potential or only modestly depolarized by a weak synaptic input, glutamate binding by itself is not sufficient for the NMDA receptors to conduct ions because the pore of the receptor-channel is blocked by extracellular Mg^{2+} (Figure 67–4A; see Chapter 10). For the receptor to function efficiently, the postsynaptic membrane must undergo a significant depolarization to expel the bound Mg^{2+} by electrostatic repulsion. In this manner the receptor acts as a coincidence detector: It is functional only when action potentials in the presynaptic neuron release glutamate that binds to the receptor *and* the membrane potential of the postsynaptic cell is sufficiently depolarized.



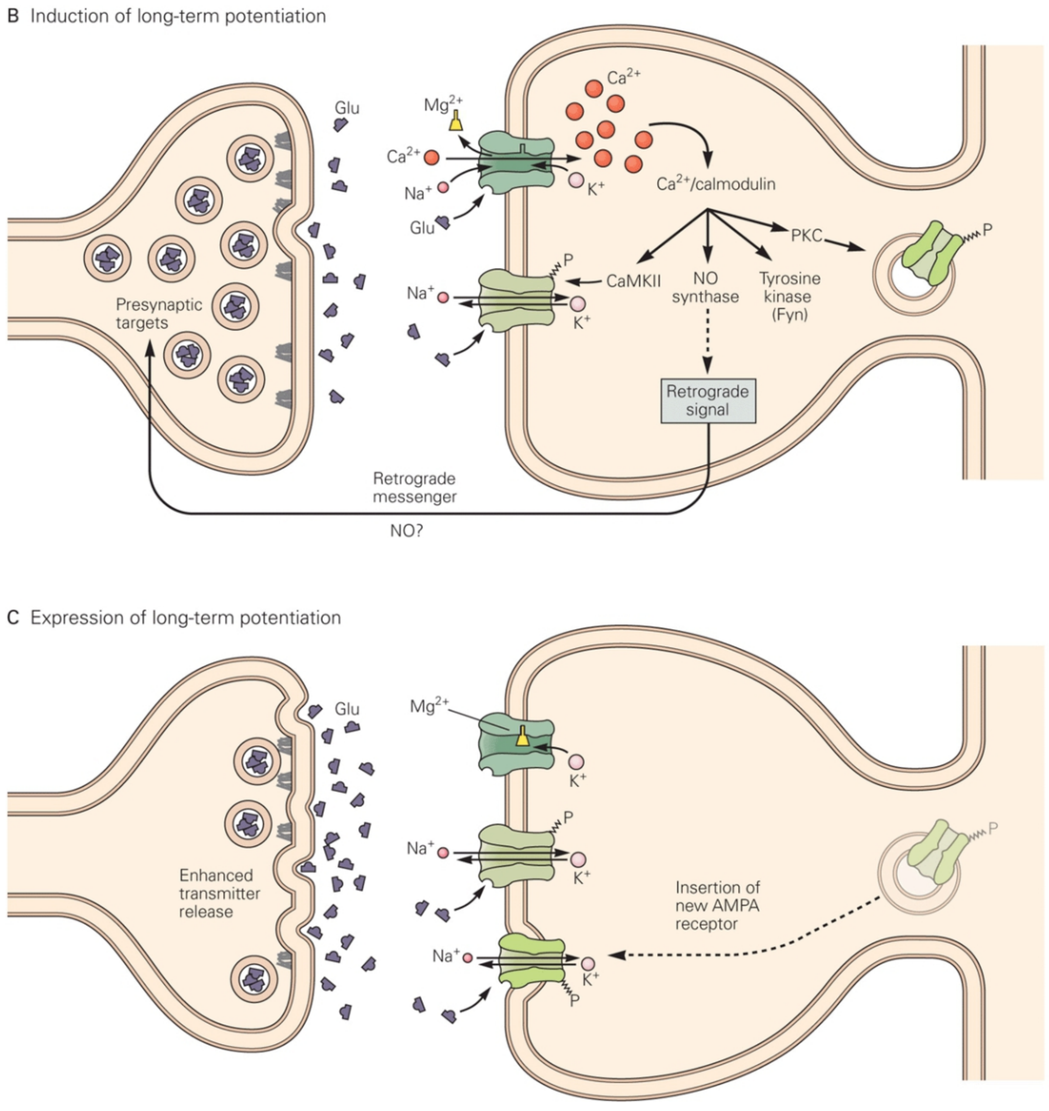


Figure 67–4 (Opposite) A model for the induction of long-term potentiation at Schaffer collateral synapses.

A. During normal, low-frequency synaptic transmission glutamate released from the terminals of CA3 Schaffer collateral axons acts on both NMDA and AMPA receptors in the postsynaptic membrane of dendritic spines (the site of excitatory input) of CA1 neurons. Sodium and K^+ flow through the AMPA receptors but not through the NMDA receptors because their pore is blocked by Mg^{2+} at negative membrane potentials.

B. During a high-frequency tetanus the large depolarization of the postsynaptic membrane (caused by strong activation of the AMPA receptors) relieves the Mg^{2+} blockade of the NMDA receptors, allowing Ca^{2+}, Na^+, and K^+ to flow through these channels. The resulting increase of Ca^{2+} in the dendritic spine triggers calcium-dependent kinases—calcium/calmodulin– dependent kinase (CaMKII) and protein kinase C (PKC)—as well as the tyrosine kinase Fyn, leading to induction of LTP.

C. 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 receptors, in part by causing insertion of new receptors into the spine synapses. In addition, the postsynaptic cell releases (in ways that are still not understood) retrograde messengers that activate protein kinases in the presynaptic terminal to enhance subsequent transmitter release. One such retrograde messenger may be nitric oxide (NO), produced by the enzyme NO synthase (shown in part B).

Because of the Mg^{2+} blockade of the NMDA receptors, at negative voltages near the resting potential EPSPs are largely generated by the opening of AMPA receptors. The burst of strong synaptic activity during induction of LTP opens a large number of AMPA receptors, generating an EPSP that is sufficient to trigger a postsynaptic action potential. The action potential generates a large depolarization that is able to expel Mg^{2+} from the pore of the NMDA receptor, permitting the receptor to conduct cations and contribute to the postsynaptic depolarization.

Why are the NMDA receptors required to induce LTP if the AMPA receptors are sufficient to produce a large postsynaptic depolarization? The answer lies in the fact that in addition to conducting monovalent Na^+, and K^+ ions, similar to the conductance properties of the AMPA receptors,

the NMDA receptors also have a high permeability to Ca^{2+}. Thus activation of these receptors leads to a significant increase in the intracellular Ca^{2+} concentration in the postsynaptic cell. The Ca^{2+} elevation is vital to the induction of LTP; injection of a chemical chelator of Ca^{2+} into the postsynaptic CA1 cell blocks the induction of LTP. The increase in Ca^{2+} activates several downstream signaling pathways, including calcium/calmodulin–dependent protein kinase II (CaMKII), protein kinase C (PKC), and tyrosine kinases. These signaling pathways lead to changes that both enhance the response of the postsynaptic cell to glutamate and increase the amount of glutamate released from the presynaptic Schaffer collateral terminals (Figure 67–4B).

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

Recent studies suggest that the cellular mechanisms underlying the expression of LTP vary depending on the precise pattern of activity that induces LTP. In many cases LTP that is induced solely by Ca^{2+} influx through NMDA receptors appears to be largely caused by an increase in the response of the postsynaptic membrane of the CA1 neuron to glutamate. But other patterns of stimulation elicit other forms of LTP at the same synapse and these also have presynaptic effects 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” (Figure 67–5). In some recordings from pairs of hippocampal pyramidal neurons, stimulation of an action potential in one neuron fails to elicit a synaptic response in a second (postsynaptic) neuron when that neuron is at its resting potential (approximately –70 mV).

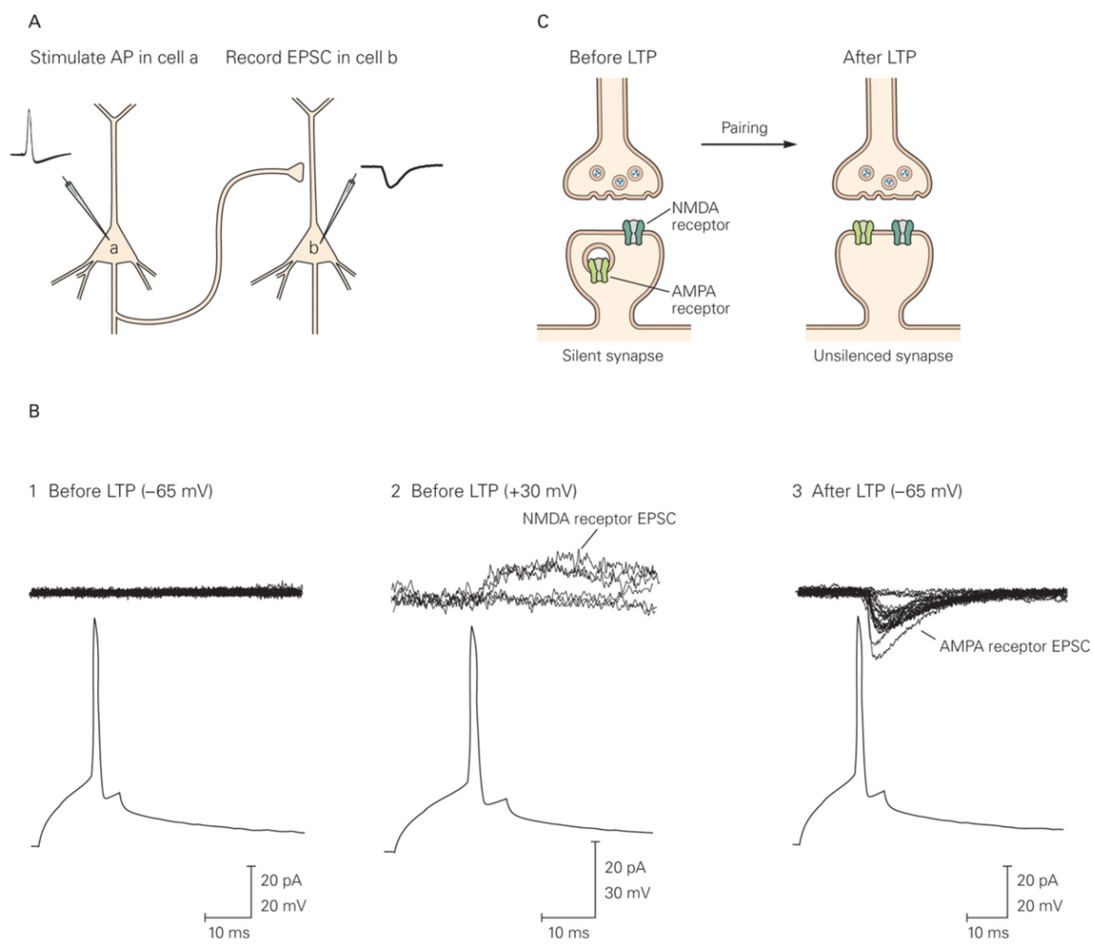


Figure 67–5 Unsilencing of silent synapses during long-term potentiation.

A. Intracellular recordings are obtained from a pair of hippocampal pyramidal neurons. An action potential 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. Effect of induction of LTP on silent synapses. Before induction of LTP there is no EPSC in cell *b* in response to an action potential in cell *a* when the membrane potential of neuron *b* is at its resting value of −65 mV (1). However, slow NMDA receptor-mediated EPSCs are observed when neuron *b* is depolarized by the voltage clamp to +30 mV (2). LTP is then induced by pairing action potentials in neuron *a* with postsynaptic depolarization in neuron *b* to relieve Mg^{2+} block of the NMDA receptors. After this pairing fast AMPA receptor-mediated EPSCs are seen at −65 mV (3).

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 new receptors on the spine.

This result is not surprising as any given hippocampal presynaptic neuron is connected to only a small fraction of other neurons. However, what is surprising is that, in some neuronal pairs, when the second neuron is depolarized under voltage clamp to +30 mV, which removes the Mg^{2+} block from the NMDA receptors, stimulation of the presynaptic neuron elicits a large excitatory postsynaptic current (EPSC) in the postsynaptic neuron, mediated by the NMDA receptors. This result indicates that the two neurons were synaptically connected all along but the postsynaptic neuron contained only NMDA receptors at its synaptic contact with the presynaptic neuron. These connections are called silent synapses because they do not generate an EPSP at the normal resting potential of the cell as a result of the Mg^{2+} block of the NMDA receptors. Synapses from other presynaptic neurons on the same postsynaptic cell may have AMPA receptors in addition to NMDA receptors (nonsilent synapses).

The key finding from these experiments is seen following the induction of LTP. Pairs of neurons initially connected solely by silent synapses now often exhibit large EPSPs at the resting potential mediated by AMPA receptors. These results indicate that LTP must involve an increase in the response of AMPA receptors to glutamate at the previously silent synapses, a process Roberto Malinow refers to as "AMPAfication."

How does the induction of LTP increase the response of AMPA receptors at previously silent synapses? The strong synaptic stimulation used to induce LTP will trigger glutamate release at both silent and nonsilent synapses on the same postsynaptic neuron. This leads to the opening of a large number of AMPA receptors at the nonsilent synapses, which in turn produces a large postsynaptic depolarization. The depolarization will propagate throughout the neuron to relieve Mg^{2+} block of the NMDA receptors at both the nonsilent and silent synapses. At the silent synapses the Ca^{2+} influx through the NMDA receptors activates a biochemical cascade that ultimately leads to the insertion of clusters of AMPA receptors in the postsynaptic membrane from a pool of intracellular receptors stored in recycling endosomal vesicles. The fusion of these vesicles with the plasma membrane is triggered by the phosphorylation by protein kinase C of the cytoplasmic tail of the endosomal AMPA receptors (Figure 67–4B,C).

As discussed earlier, LTP is not a unitary process even at a single synapse. At Schaffer collateral synapses LTP generated by a brief 100 Hz tetanus depends solely on Ca^{2+} influx through NMDA receptors, whereas LTP induced by a 200 Hz tetanus depends on Ca^{2+} influx through both NMDA receptors and L-type voltage-gated Ca^{2+} channels. (A similar mechanism contributes to LTP in the direct entorhinal pathway to CA1 neurons.) This high-frequency form of LTP is expressed both through presynaptic mechanisms that enhance glutamate release and through

postsynaptic mechanisms that increase the membrane response to glutamate. Thus both the induction and expression of LTP depend on a family of presynaptic and postsynaptic processes.

Because induction of LTP requires Ca^{2+} influx into the postsynaptic cell, the increase in transmitter release during LTP implies that the presynaptic cell must receive information from the postsynaptic cell that LTP has been induced. There is now evidence that Ca^{2+}-activated second messengers in the postsynaptic cell, or perhaps Ca^{2+} itself, cause the postsynaptic cell to release one or more chemical messengers that diffuse to the presynaptic terminals to enhance release (see Figure 67–4B,C and Chapter 11). 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 in the Schaffer Collateral Pathway Follows Hebbian Learning Rules

The NMDA receptors endow LTP in the Schaffer collateral pathway with several interesting properties that have direct relevance to learning and memory. First, LTP in this pathway requires the near simultaneous activation of a large number of afferent axons, a feature called *cooperativity* (Figure 67–6). This requirement stems from the fact that relief of Mg^{2+} block of the NMDA receptor requires a large depolarization.

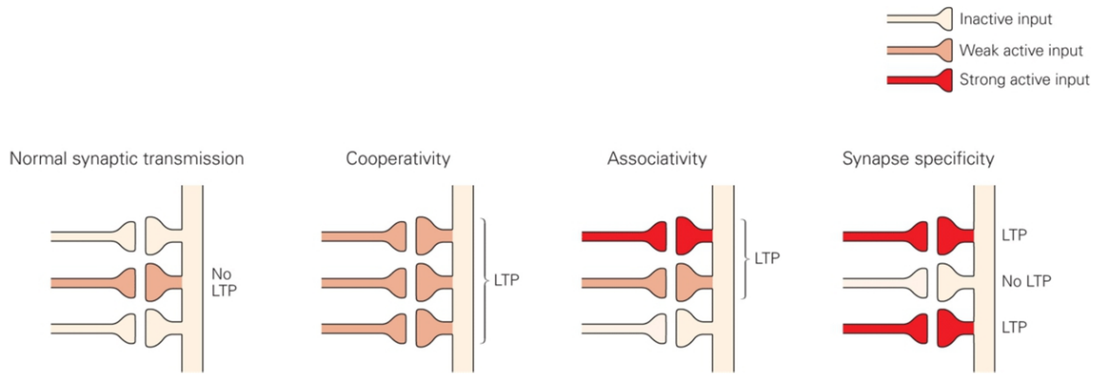


Figure 67–6 Long-term potentiation in CA1 pyramidal neurons of the hippocampus shows cooperativity, associativity, and synapse specificity. With normal synaptic transmission a single action potential in one or a few axons (weak input) leads to a small EPSP that is insufficient to expel Mg^{2+} from the NMDA glutamate 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 action potential firing and results 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 in spite of the strong stimulation of neighboring synapses. This ensures that memories are selectively formed at active synapses (synapse specificity).

The second important property of LTP in the Schaffer collateral pathway is that it is *associative*. A weak synaptic input normally does not produce enough postsynaptic depolarization to induce LTP. However, if that weak input is coactivated or paired with a strong synaptic input that does produce supra-threshold depolarization, then the large depolarization will be able to propagate to the synapse with weak input, leading to relief of the Mg^{2+} blockade of the NMDA receptors in the postsynaptic membrane at that site and the induction of LTP.

The third key property of LTP is that it 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 of cooperativity, associativity, and synapse specificity underlies key components 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). Finally, synapse specificity ensures that inputs that convey information not related to a particular event will not be strengthened to participate in a given memory.

The finding that the induction of LTP in the Schaffer collateral pathway requires that presynaptic activity be strong enough to elicit firing in the postsynaptic neuron provides evidence for *Hebb's rule*, proposed in 1949 by the psychologist Donald Hebb as a theoretical mechanism for how neuronal circuits are modified by experience: "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." A similar principle is involved in fine-tuning synaptic connections during the late stages of development (see Chapter 56).

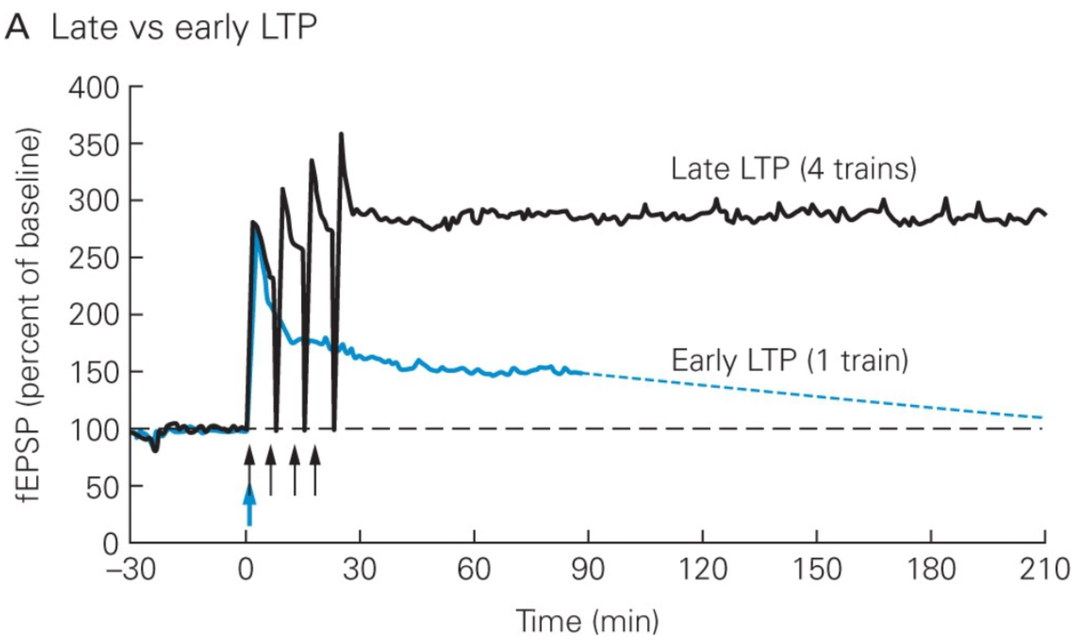
The Hebbian nature of LTP is best illustrated by the phenomenon of spike timing-dependent plasticity. Under most circumstances hippocampal neurons do not produce the high-frequency trains of action potentials typically used to induce LTP. However, a form of LTP can be induced by pairing a single presynaptic stimulus with the firing of a single action potential in the postsynaptic cell. In fact, this coincidence detection is very strict. In agreement with Hebb's postulate, the pairing protocol produces LTP only if the postsynaptic cell fires a few milliseconds *after* the EPSP. That is, the presynaptic cell must fire before the postsynaptic cell. If the postsynaptic cell fires just before the EPSP, a long-lasting decrease in the size of the EPSP occurs (this long-term depression is described more fully below.) If the action potential occurs more than a hundred milliseconds before or after the EPSP, the synaptic strength will not change.

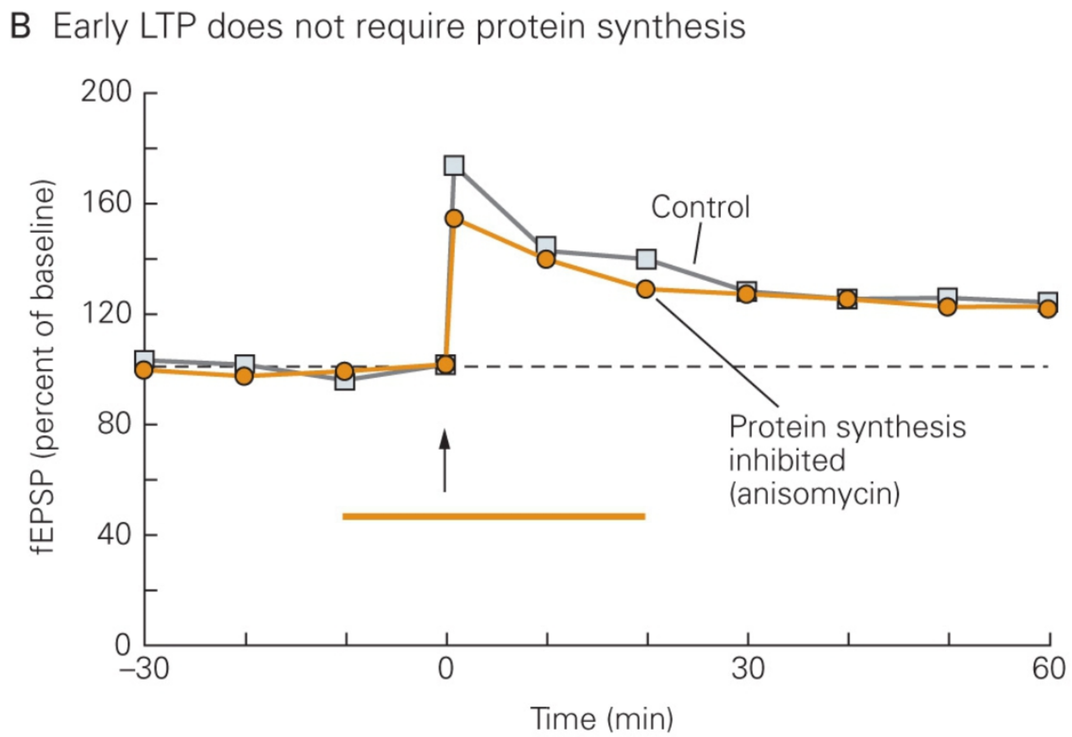
The pairing rules of spike timing-dependent plasticity result in large part from the cooperative properties of the NMDA receptor. If the postsynaptic spike occurs during the EPSP, the spike is able to relieve the Mg^{2+} blockade of the receptor at a time when the NMDA receptor-channel has been activated by the binding of glutamate. This leads to a large influx of Ca^{2+} through the receptor and the induction of LTP. However, if the postsynaptic action potential occurs prior to glutamate release, 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 little influx of Ca^{2+} through the receptor to induce LTP.

These studies of the Schaffer collateral pathway indicate that two sequential associative mechanisms ensure that the induction of LTP is restricted to those synapses at which there is both presynaptic and postsynaptic activity, in accord with Hebb's learning rule. The first mechanism is the associative property of the NMDA glutamate receptor. The second is the selective action of retrograde messengers released from the postsynaptic cell at only those presynaptic sites that are active. As we saw in Chapter 66, these two associative mechanisms in series also contribute to associative classical conditioning in *Aplysia* and in the amygdala. Thus mechanisms of synaptic plasticity important for learning and memory have been conserved throughout evolution of the species at broad classes of synapses and for distinct forms of learning.

Long-Term Potentiation Has Early and Late Phases

Long-term potentiation has two phases. One train of action potentials produces a phase of LTP lasting 1 to 3 hours called *early LTP*. This component, which is the phase we have been considering up to now, does not require new protein synthesis, cAMP, or PKA activation. However, four or more trains of synaptic stimulation induce a late LTP that lasts up to 24 hours; this late LTP does require cAMP and PKA, as well as changes in gene transcription and the synthesis of new proteins (Figure 67–7).





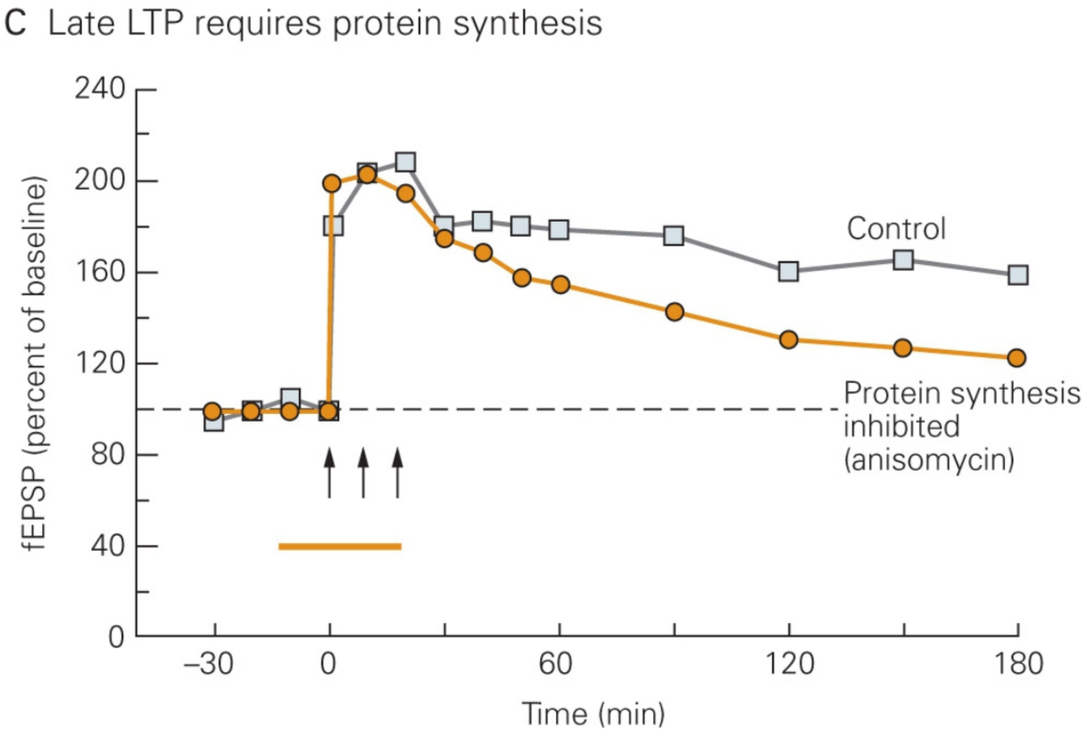


Figure 67–7 Early and late phases of long-term potentiation in the CA1 region of the hippocampus.

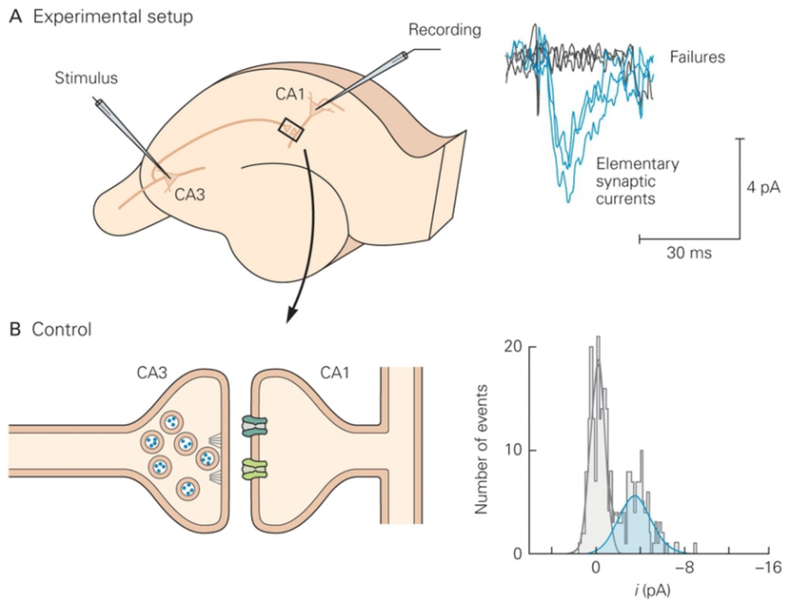
A. Early LTP is induced by a single tetanus lasting 1 second at 100 Hz. Late LTP is induced by four tetani given 10 minutes apart. Early LTP of the fEPSP lasts only 1 to 2 hours, whereas the late LTP lasts more than 8 hours (only the first 3.5 hours are shown). (Reproduced, with permission, from Kandel 2001.)

B. Early LTP induced by one tetanus is not blocked by anisomycin, an inhibitor of protein synthesis. Bar indicates application of anisomycin during the LTP induction protocol. (Reproduced, with permission, from Huang and Kandel 1994.)

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

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. In both pathways late LTP recruits the cAMP and PKA signaling pathway, which recruits the cAMP response element binding protein (CREB) transcription factor, leading to the synthesis of new mRNAs and proteins.

How do the synaptic mechanisms for the expression of early and late LTP differ? Quantal analysis (see Chapter 12) was used to examine synaptic transmission between a single presynaptic CA3 neuron and a single postsynaptic CA1 cell (Figure 67–8).



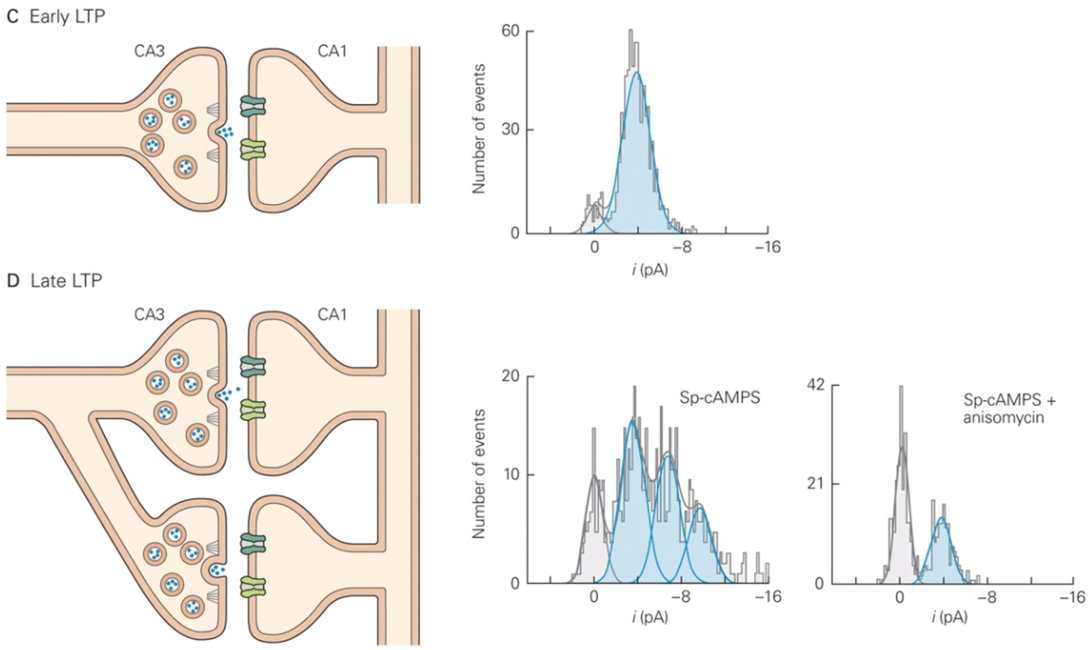


Figure 67–8 (Opposite) Quantal analysis of early and late phases of long-term potentiation. (Reproduced, with permission, from Bolshakov et al. 1997.)

A. When a single presynaptic CA3 cell is stimulated to fire an action potential, it produces a small excitatory postsynaptic current (EPSC) in a postsynaptic CA1 cell recorded under voltage clamp conditions. When the CA3 cell is stimulated successively at a frequency too low to induce LTP or LTD, the EPSC varies from stimulus to stimulus. The stimulus either evokes an EPSC (a success) or does not evoke any measurable response (a failure). The amplitude of the successes is equal to that of the miniature EPSC, the elementary or quantal response.

B. Before LTP, stimulation of the presynaptic cell results in many failures; the synapse has a low probability of releasing a vesicle. The distribution of the EPSC amplitudes can be approximated by two Gaussian curves, one centered on zero current (the failures) and the other centered on −4 pA (the successful responses). These histograms are consistent with the type of synapse illustrated here, in which a single CA3 cell makes a single synaptic connection with a CA1 cell. This connection has a single active zone from which a single vesicle is released in an all-or-none manner (failures or successes) in response to successive stimuli. The postsynaptic membrane contains both NMDA and AMPA receptors, the latter of which are responsible for the rapid EPSC at negative potentials.

C. Once early LTP has been induced, the probability of release increases significantly, leading to a decrease in the fraction of failures and an increase in the fraction of successes. The EPSC histogram is again fitted by two Gaussian curves, consistent with the view that there is still only a single release site that releases at most a single vesicle but now with a high probability of release. (This study examined LTP at nonsilent synapses under conditions where insertion of new AMPA receptors was not observed.)

D. When late LTP is induced by prolonged application of a membrane-permeable analog of cAMP (Sp-cAMPS), the distribution of successful responses is no longer fitted by a single Gaussian curve. Instead, three or four Gaussian curves are fitted, suggesting that a single presynaptic action potential releases multiple quanta (synaptic vesicles) of transmitter. These effects are blocked by anisomycin, an inhibitor of protein synthesis. The increase in number of quanta is consistent with the growth of new sites of synaptic transmission between the presynaptic and postsynaptic neurons. (Sp-cAMPS, Sp-diastereomer of adenosine cyclic 3′,5′-phosphorothioate.)

Prior to LTP a CA3 neuron typically forms only one functional synapse with a CA1 neuron. At this synapse a presynaptic action potential releases with low probability a single vesicle of transmitter. This weak connection between a single CA3 and single CA1 neuron means that a large number of CA3 neurons must be co-activated to trigger a spike in

the postsynaptic CA1 cell. Following induction of early LTP, the probability that a presynaptic action potential will release a vesicle is increased (Figure 67–8C).

Induction of the late phase of LTP by direct application of a chemical analog of cAMP dramatically changes the response to synaptic stimulation. Under these conditions a presynaptic action potential elicits a very large EPSP through the release of multiple quanta of transmitter (Figure 67–8D). Because each release site (active zone) in the presynaptic terminal is thought to release at most one vesicle in an all-or-none fashion, the increase in the number of quanta indicates that late LTP recruits new presynaptic release sites apposed to new clusters of AMPA receptors in the postsynaptic membrane. Moreover, the formation of new synapses requires new protein synthesis, consistent with the idea that late LTP involves a growth process. Light microscopic imaging studies of live neurons in hippocampal slices provide direct evidence that LTP induces the formation of new dendritic spines, the sites of new excitatory synaptic input.

Like sensitization of the gill-withdrawal reflex in *Aplysia*, 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 at the second set of nonstimulated synapses is not altered. However, Uwe Frey and Richard Morris found that if a single tetanus is applied to the second set of synapses soon after the four tetani are applied to the first set, the single train is able to induce late LTP at the synapses it activates. This phenomenon is similar to the synapse-specific capture of long-term facilitation at the sensory-motor neuron synapses in *Aplysia* (see Chapter 66). At the Schaffer collateral synapses the single tetanus somehow marks the activated synapses allowing them to respond to, or capture, the new proteins synthesized in response to signals from the synapses that received the four tetani.

How can a few brief trains of synaptic stimulation produce such long-lasting increases in synaptic transmission? Studies from Todd Sacktor have shown that the maintenance of late LTP depends on a novel isoform of protein kinase C termed PKMζ (PKM zeta). Most isoforms of PKC contain both a regulatory domain and a catalytic domain (see Chapter 11). Binding of diacylglycerol, phospholipids, and Ca^{2+} to the regulatory domain of PKC relieves its inhibitory binding to the catalytic domain, which is then free 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. This mRNA is present in the CA1 neuron dendrites, enabling its local translation to rapidly alter synaptic strength. Blockade of PKMζ with a specific inhibitor does not block early LTP but does block late LTP. Moreover, application of the PKMζ blocker several hours after the LTP induction protocol can reverse late LTP after it has been established. This result indicates that the maintenance of late LTP requires the persistent and ongoing activity of PKMζ, which leads to the persistent increase in insertion of AMPA receptors in the postsynaptic membrane (Figure 67–9).

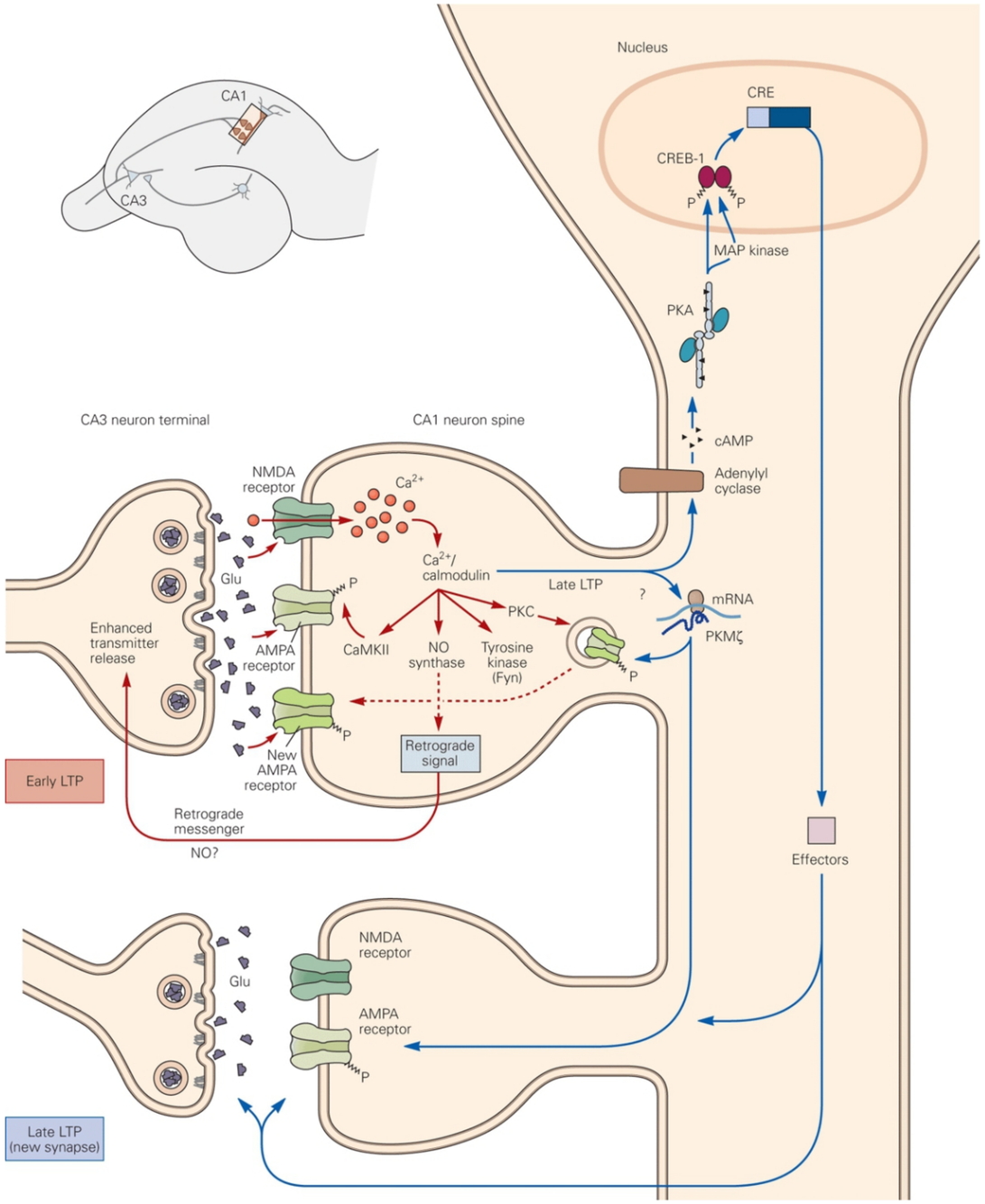


Figure 67–9 A model for the molecular mechanisms of early and late phases of long-term potentiation. A single tetanus induces early LTP by activating NMDA receptors, triggering Ca^{2+} influx into the postsynaptic cell and the activation of a set of second messengers. With repeated tetani the Ca^{2+} influx also recruits an adenylyl cyclase, which generates cAMP that activates PKA. This leads to the activation of MAP kinase, which translocates to the nucleus where it phosphor-ylates 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. Repeated stimulation also activates translation in the dendrites of mRNA encoding PKMζ, a constitutively active isoform of PKC. This leads to a long-lasting increase in the number of AMPA receptors in the postsynaptic membrane. A retrograde signal, perhaps NO, is thought to diffuse from the postsynaptic cell to the presynaptic terminal to enhance transmitter release.

Spatial Memory Depends on Long-Term Potentiation in the Hippocampus

Long-term potentiation is an experimentally induced change in synaptic strength produced by strong direct stimulation of neural pathways. Does this form of synaptic change occur physiologically for explicit memory storage? If so, how does it affect the normal processing of information for memory storage in the hippocampus?

To date a large number of experimental approaches have shown that inhibiting LTP interferes with spatial memory. One spatial memory test uses a pool filled with an opaque fluid (the Morris water maze). To escape from the liquid a mouse must find a platform submerged below the surface of 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 *contextual cues*—markings on the walls of the room in which the pool is

located. This task requires the hippocampus. In a *noncontextual* 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 the platform using brain pathways that do not require an intact hippocampus.

When NMDA receptors are blocked by injection of a pharmacological antagonist into the hippocampus, the animal can find the visible platform in the non-contextual version of the task but cannot remember the location of the hidden platform in the contextual version. These experiments thus suggest that some mechanism involving NMDA receptors in the hippocampus, perhaps LTP, is involved in spatial learning. As we saw above, NMDA receptors are required for the induction but not for the persistence or maintenance of LTP. Similarly, injection of an NMDA receptor blocker into the hippocampus *after* an animal has learned a spatial memory task does not inhibit subsequent memory recall for that task.

As we also saw above, PKMζ is required for the maintenance of LTP but is not involved in its initial induction. Todd Sacktor and his colleagues have found a corresponding requirement for PKMζ in the persistence of memory. Thus injection of a pharmacological inhibitor of PKMζ into the hippocampus 1 day after an animal has been trained on a spatial task disrupts the memory for that task.

More direct evidence for the correlation of memory 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 mutant mice in which the NR1 deletion is restricted to CA1 pyramidal neurons and occurs only 1 or 2 weeks after birth (Box 67–1). These mice survive into adulthood and show a loss of LTP in the Schaffer collateral pathway. This disruption is highly localized; nevertheless the mutant mice have a serious deficit in spatial memory (Figure 67–12).

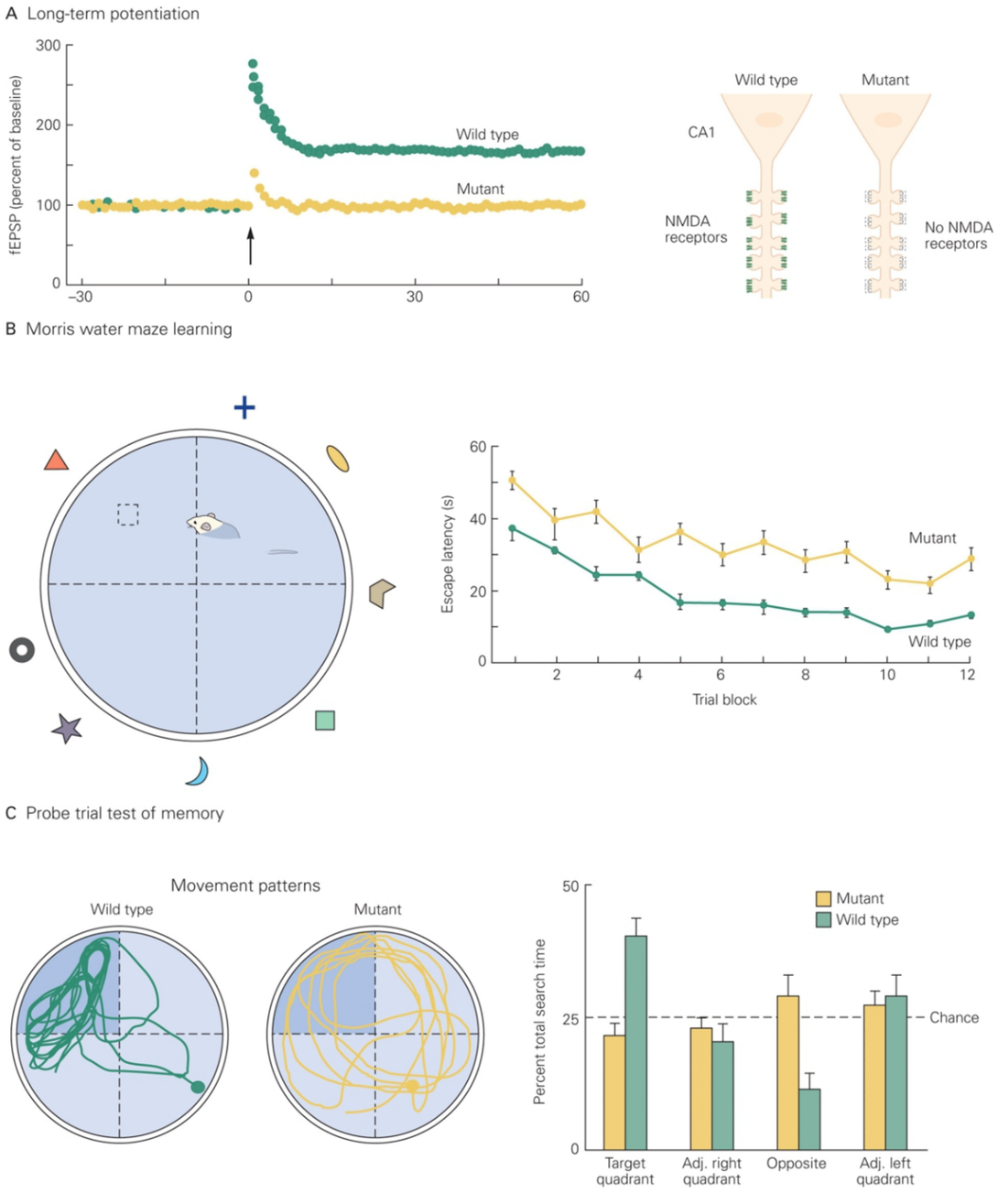


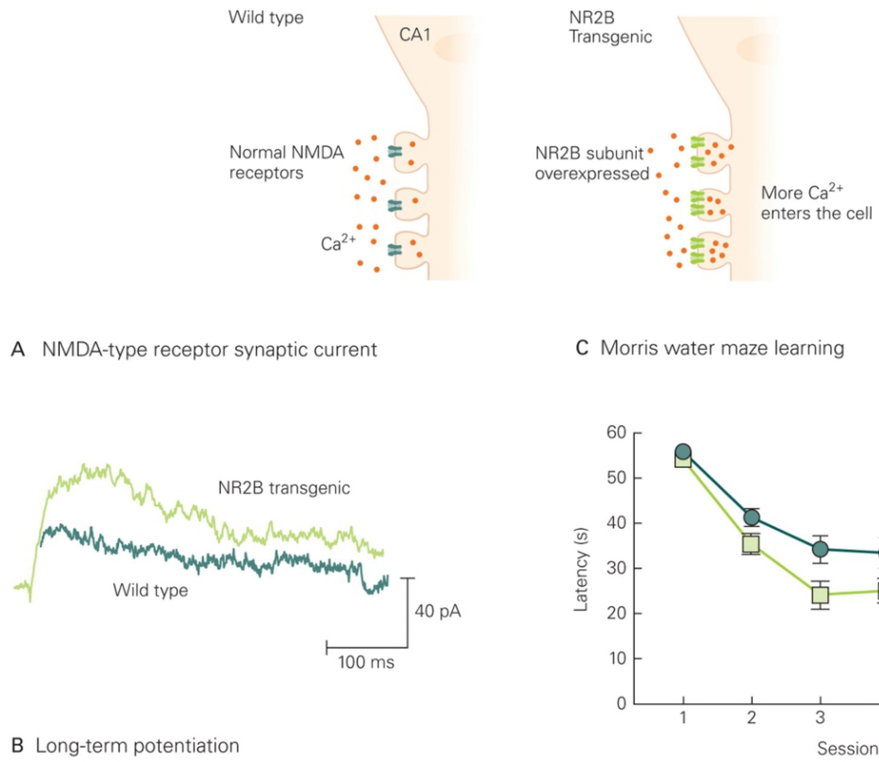
Figure 67–12 Long-term potentiation, and spatial learning and memory are impaired in mice that lack the NMDA receptor in the CA1 region of the hippocampus. (Reproduced, with permission, from Tsien, Huerta, and Tonegawa 1996.)

A. LTP is abolished in mice in which the NMDA receptor is selectively deleted in CA1 pyramidal neurons by knocking out the NR1 subunit gene. Field EPSPs were recorded in response to Schaffer collateral stimulation. Tetanic stimulation at 100 Hz for 1 s (arrow) caused a large potentiation in wild-type mice but failed to induce LTP in the NMDA receptor knockout (mutant) mice.

B. 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 (the 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.

C. 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, ie, they perform at chance level, indicating deficient memory.

Although it is perhaps not surprising that genetic manipulations can impair neuronal function, 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 mouse mutant that overexpresses the NR2B subunit of the NMDA receptor. This subunit is normally present at early stages of development but is downregulated at adult hippocampal synapses. Receptors that incorporate this subunit allow more Ca^{2+} influx than those that do not. 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 67–13).



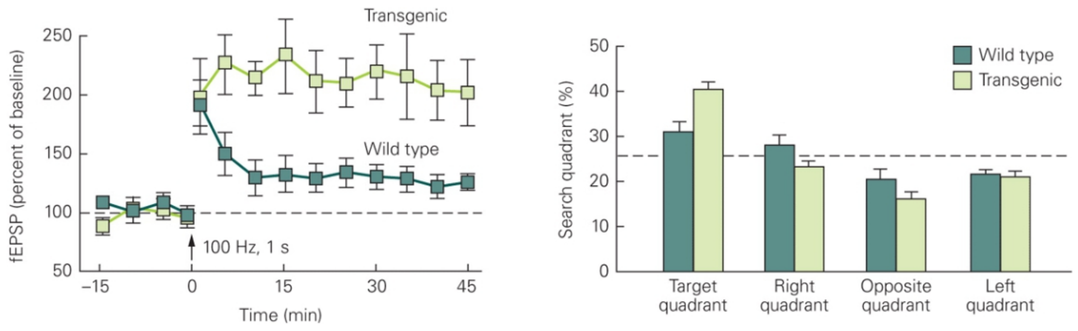


Figure 67–13 Learning and memory are enhanced in mice that overexpress the NR2B subunit of the NMDA glutamate receptor. (Reproduced, with permission, from Tang et al. 1999.)

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 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.

C. Spatial learning is enhanced in the transgenic mice as demonstrated in the 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. Spatial memory is also enhanced in the transgenic mice as demonstrated in the probe trial (lower plot). Transgenic mice spend more time in the target quadrant, which previously contained the hidden platform, than do wild-type mice (see Figure 67–12C).

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 (see Box 67–1).

Reversible gene expression has been used to explore the role of the enzyme CaMKII, whose function in LTP was discussed above. After a brief exposure to Ca^{2+}, CaMKII can be converted to a Ca^{2+} independent state through its autophosphorylation at threonine-286 (Thr286). This ability to become persistently active in response to a transient Ca^{2+} stimulus led to the suggestion that CaMKII may act as a simple molecular switch to maintain memory. Mutation of Thr286 to the negatively charged amino acid aspartate mimics the effect of autophosphorylation at Thr286 and converts the CaMKII to a Ca^{2+} -independent form.

Transgenic expression of this dominant mutation of CaMKII (CaMKII–Asp286) results in a systematic shift in the relation between stimulus frequency during a tetanus and the resultant change in synaptic strength during long-term plasticity. In the transgenic mice intermediate-frequency tetanic stimulation at 10 Hz, which normally induces a small amount of LTP, induces long-term depression of synaptic transmission in the Schaffer collateral pathway (Figure 67–14A). In contrast, Schaffer collateral LTP in response to a 100 Hz tetanus is not altered. The defect in synaptic plasticity with intermediate frequency stimulation is associated with an inability of the mutant mice to remember spatial tasks (Figure 67–14C). However, the defects in LTP and in spatial memory can be fully rescued when the mutant gene is switched off in the adult, thereby showing that the memory defect is not due to a developmental abnormality (Figure 67–14).

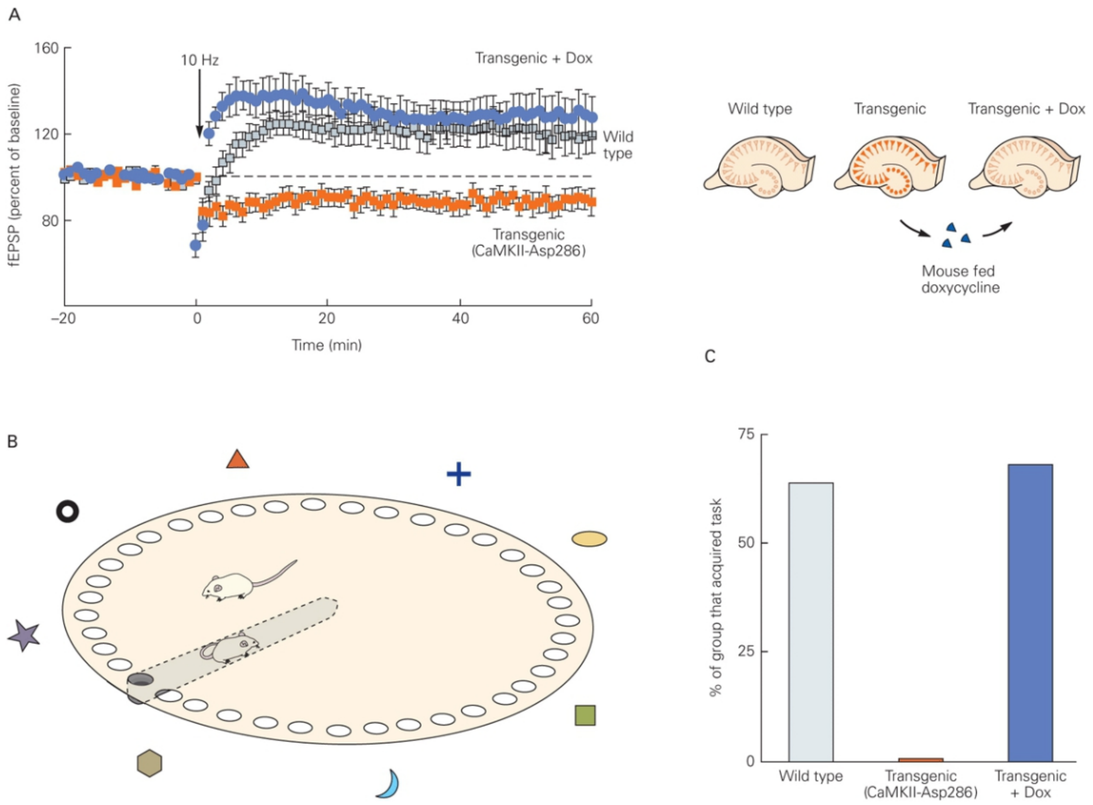


Figure 67–14 Deficits in long-term potentiation and spatial memory due to a transgene are reversible. (Reproduced, with permission, from Mayford et al. 1996.)

A. An LTP deficit is seen in hippocampal slices from transgenic mice that overexpress a constitutively active form of CaMKII, CaMKII-Asp286. Expression of this kinase is under control of *tetO* and the tTA transcription factor (see Box 67–1). Four groups of mice were tested: transgenic mice that are fed doxycycline (Dox), 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 are fed doxycycline the deficit in LTP is reversed.

B. The effect of the kinase on spatial memory was tested in the Barnes maze. This 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 by using distinctive markings on the four walls as cues for hippocampal-dependent spatial memory.

C. Transgenic mice that express the CaMKII-Asp286 kinase and 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 (in which the kinase is highly expressed) 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 in the Schaffer collateral pathway are also required for spatial memory. However, in addition to receiving input through the Schaffer collaterals, CA1 neurons also receive excitatory input from the entorhinal cortex. Synaptic plasticity at the entorhinal inputs in CA1 may also contribute to spatial learning and memory. The HCN1 hyperpolarization-activated cation channel is strongly expressed in the very distal dendrites of CA1 neurons, the site of the entorhinal inputs. These channels are partially open at the resting potential, which decreases the membrane resistance (R_m) of the dendrite. This reduces the size of the EPSP in response to a given excitatory synaptic current ($\Delta V_{EPSP} = I_{EPSP} \times R_m$) and decreases the mem-

brane time constant ($\tau_m = R_m \times C_m$). The net effect is a reduction in the spatial and temporal integration of EPSPs in the perforant path.

Box 67–1 Restricting Gene Knockout and Regulating Transgenic Expression

Biological analysis of learning requires the establishment of a causal relation between specific molecules and learning. In the past this relationship was difficult to demonstrate in mammals but now can be studied successfully in mice either by the use of transgenes or gene knockout.

With gene knockout, deletion of a specific gene is induced in embryonic stem cells through homologous recombination (see Figure 3–8). Experiments using transgenes and gene knockout have made it possible to examine the relationship of NMDA receptors and different second-messenger-dependent protein kinases to long-term potentiation in the hippocampus and to spatial learning.

Conventional gene knockout is unrestricted; animals inherit the genetic deletion in all of their cell types. Global genetic deletion may cause developmental defects that interfere with the later functioning of neural circuits important for memory storage. As a result, interpretation of the results from experiments using conventional gene knockout run into two types of problems.

First, it is often difficult to exclude the possibility that the abnormal phenotype observed in mature animals results directly or indirectly from a developmental defect rather than because that gene plays a specific, active role in learning and memory. Second, global gene knockout makes it difficult to attribute abnormal phenotypes to a particular type of cell or specific region within the brain.

Regional Control of Gene Expression

To improve the utility of gene knockout technology, methods have been developed that restrict deletions to cells in a specific tissue or at specific points in an animal's development. One method of regional restriction exploits the *Cre/loxP* system. The *Cre/loxP* system is a site-specific recombination system, derived from the P1 phage, in which the phage enzyme Cre recombinase catalyzes recombination between 34 bp *loxP* recognition sequences, which are normally not present in animal genomes.

The *loxP* sequences can be inserted into the genome of embryonic stem cells by homologous recombination such that they flank one or more exons of a gene of interest (called a *floxed* gene). When the stem cells are injected into an embryo, a mouse can be eventually bred in which the gene of interest is floxed and still functional in all cells of the animal.

A second line of transgenic mice can then be generated that expresses Cre recombinase under the control of a neural promoter sequence that is normally expressed in a restricted brain region. By crossing the Cre transgenic line of mice with the line of mice with the floxed gene of interest, the gene will only be deleted in those cells that express the Cre transgene (Figure 67–10).

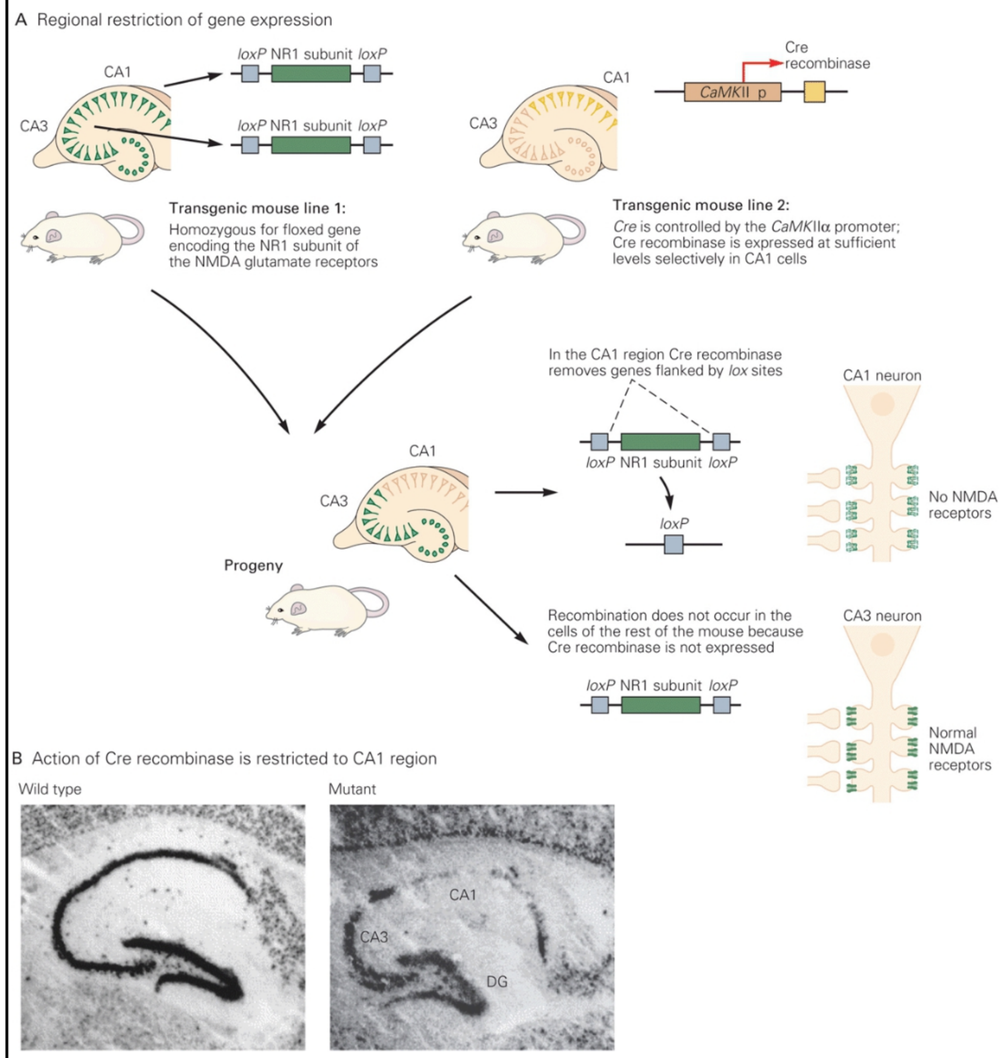


Figure 67–10 The *Cre/loxP* system for gene knockout.

A. A line of mice is bred in which the gene encoding the NR1 subunit of the NMDA receptor has been flanked by *loxP* genetic elements (transgenic mouse line 1). These so-called "floxed NR1" mice are then crossed with a second line of mice in which a transgene coding for Cre recombinase is placed under the control of a transcriptional promoter specific to a cell type or a tissue type (transgenic mouse line 2). In this example the promoter from the *CaMKIIa* gene is used to drive expression of the *Cre* gene. In progeny that are homozygous for the floxed gene and that carry the Cre recombinase transgene, the floxed gene will be deleted by *Cre*-mediated *loxP* recombination only in cell type(s) in which the promoter driving *Cre* expression is active.

B. 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 *CaMKIIa* promoter. Note that NR1 mRNA expression (dark staining) is greatly reduced in the CA1 region of the hippocampus but remains normal in CA3 and the dentate gyrus (DG). (Reproduced, with permission, from Tsien, Huerta, and Tonegawa 1996.)

In the example shown in Figure 67–10 the gene encoding the NR1 (or GluN1) subunit of the NMDA glutamate receptor has been flanked with *loxP* elements and then crossed with a mouse line expressing Cre recombinase under control of the *CaMKII* promoter, which normally is expressed in forebrain neurons. In this particular line expression was fortuitously limited to the CA1 region of the hippocampus, resulting in selective deletion of the NR1 subunit in this brain region. Because the *CaMKII* promoter only activates gene transcription postnatally, early developmental changes are minimized by this strategy.

Temporal Control of Gene Expression

In addition to regional restriction of gene expression, effective use of genetically modified mice requires control over the timing of gene expression. The ability to turn a transgene on and off gives the investigator an additional degree of flexibility and can exclude the possibility that any abnormality observed in the phenotype of the mature animal is the result of a developmental defect produced by

the transgene. This can be done in mice by constructing a gene that can be turned on or off with a drug.

One starts by creating two lines of mice. Line 1 carries a particular transgene, for example *CaMKIIα Asp286*, a mutated form of the gene *CaMKIIα* coding for a constitutively active kinase. Instead of being attached to its normal promoter, the transgene is attached to the promoter *tetO* that is ordinarily found only in bacteria (Figure 67–11).

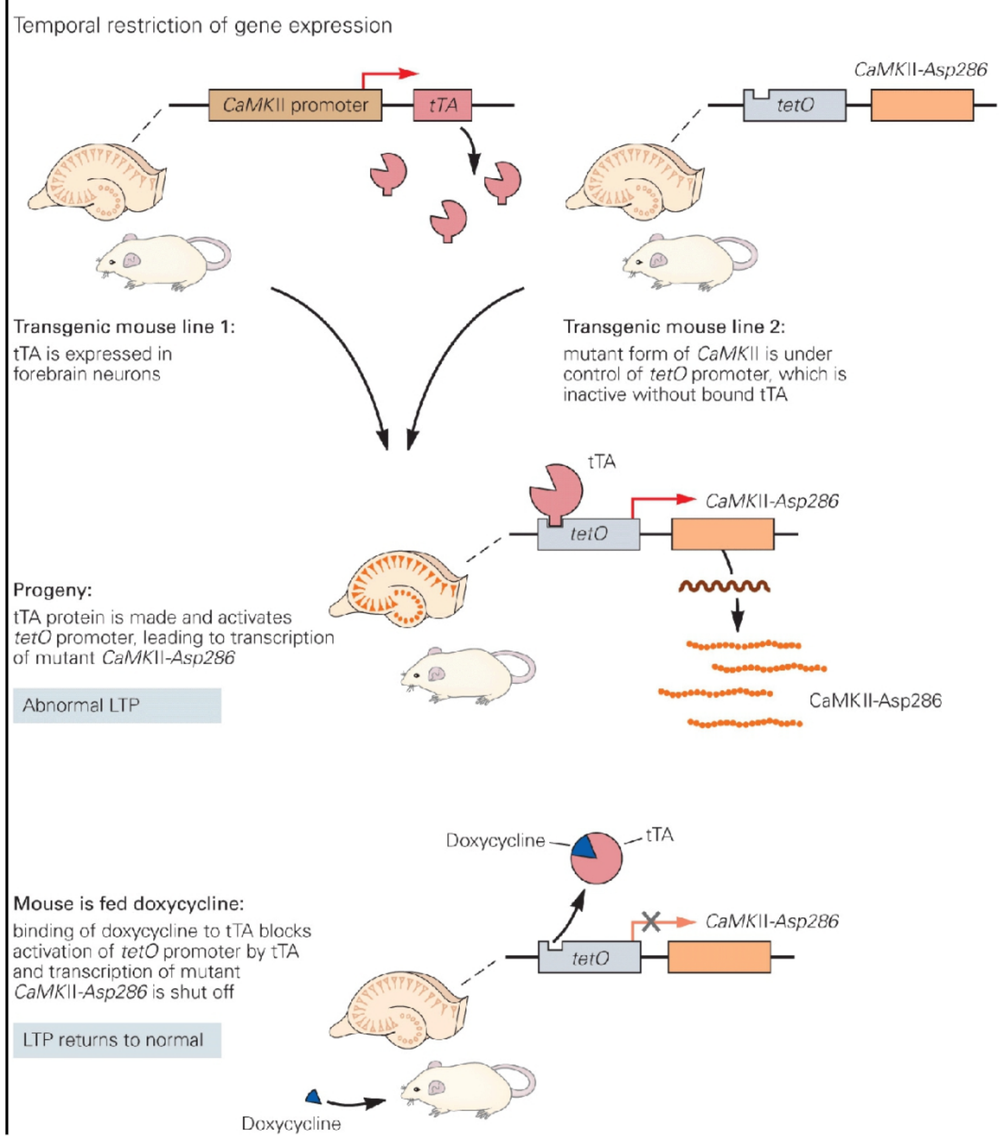


Figure 67–11 The tetracycline system for temporal and spatial regulation of transgene expression. Two independent lines of transgenic mice are bred. One line expresses, under the control of the *CaMKIIα* promoter, the tetracycline transactivator (tTA), an engineered protein incorporating a bacterial transcription factor that recognizes the bacterial *tetO* operon. The second line contains a transgene of interest—here encoding a constitutively active form of CaMKII (*CaMKIIα–Asp286*) that makes the kinase persistently active in the absence of Ca^{2+} —whose expression is under control of *tetO*. When these two lines are mated the offspring express the tTA protein in a pattern restricted to the forebrain. When the tTA protein binds to *tetO* it will activate transcription of the downstream gene of interest. Tetracycline (or doxycycline) given to the offspring binds to the tTA protein and causes a conformational change that leads to the unbinding of the protein from *tetO*, blocking transgene expression. In this manner mice will express CaMKII–Asp286 in the forebrain, and this expression can be turned off by administering doxycycline to the mice. (Reproduced, with permission, from Mayford et al. 1996.)

This promoter cannot by itself turn on the gene; it needs to be activated by a specific transcriptional regulator. Thus the second line of mice expresses a second transgene that encodes a hybrid transcription factor, the tetracycline transactivator (tTA), which recognizes and binds to the *tetO* promoter. Expression of tTA is placed under the control of a region-specific promoter, such as the promoter for *CaMKIIα*

When the two lines of mice are mated, some of the offspring will carry both transgenes. In these mice the tTA binds to the *tetO* promoter and activates the mutated *CaMKIIα* gene. This mutant causes abnormalities in long-term potentiation (see Figure 67–14).

But when the antibiotic doxycycline (similar to tetracycline) is administered, the drug binds to the transcription factor tTA, causing it to undergo a change in shape that makes it come off the promoter. In the presence of the antibiotic, cells stop expressing CaMKIIα-Asp286 and long-term potentiation returns to normal, demonstrating that the transgene exerts its effect by perturbing signaling in the adult brain rather than by interfering with neural development.

One can also generate mice that express a mutant form of tTA called reverse tTA (rtTA). This transactivator will not bind to *tetO* unless the animal is fed doxycycline. In this case the transgene is always turned off unless the drug is given.

Mice lacking the HCN1 subunit show markedly enhanced temporal summation of EPSPs in the distal dendrites in response to a tetanus because of the increased membrane time constant. This contributes to a large increase in the magnitude of LTP at the perforant path synapses. In contrast, the same mice show relatively little change in EPSPs or LTP at the Schaffer collateral synapses, which are formed on more proximal regions of CA1 dendrites where expression of HCN1 is relatively modest. Importantly, the mutant mice get smarter, exhibiting a significantly faster rate at which they learn to find the hidden platform in the Morris maze relative to littermates that express normal levels of HCN1. Such experiments support the view that LTP at the most distal perforant path inputs to CA1 neurons also contributes to spatial learning and memory.

The preceding experiments demonstrate that a wide range of pharmacological and genetic manipulations that alter LTP are correlated with changes in spatial learning and 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 colleagues addressed this question by monitoring the strength of synaptic transmission at the Schaffer collateral CA1 synapses in vivo in rats using an array of extracellular recording electrodes.

Recordings were made of synaptic strength as rats were trained to avoid one side of a box through administration of a foot shock. These experiments show that after training there is a small but significant increase in the amplitude of synaptic transmission at a subset of the recording electrodes. Importantly, at electrode sites where the enhancement is greatest, LTP in response to tetanic stimulation through an independent electrode is diminished. This result implies that the enhanced synaptic transmission following spatial training is actually caused by the induction of LTP; because the amount of LTP at a given synapse is finite, the prior induction of LTP during learning occludes the subsequent induction of LTP by electrical stimulation. This effect is similar to the occlusion of LTP in the amygdala during fear learning, as discussed in Chapter 66.

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

In 1971 John O'Keefe and John Dostrovsky made the remarkable discovery in rats that the hippocampus contains a cognitive map of an animal's spatial environment. An animal's familiarity with a particular environment is represented in the hippocampus by the firing pattern of populations of pyramidal cells, termed *place cells*, in the CA3 and CA1 regions. A place cell fires when an animal enters a certain location in a specific environment, the cell's *place field* (Figure 67–15). The population of place fields specify the environment. When the animal enters a new environment new place fields are formed within minutes and are stable for weeks to months. Thus, if one records the electrical activity of a number of place cells it is possible to predict where the animal is in its

environment. In this manner the hippocampus is thought to constitute a cognitive map of the animal's surroundings.

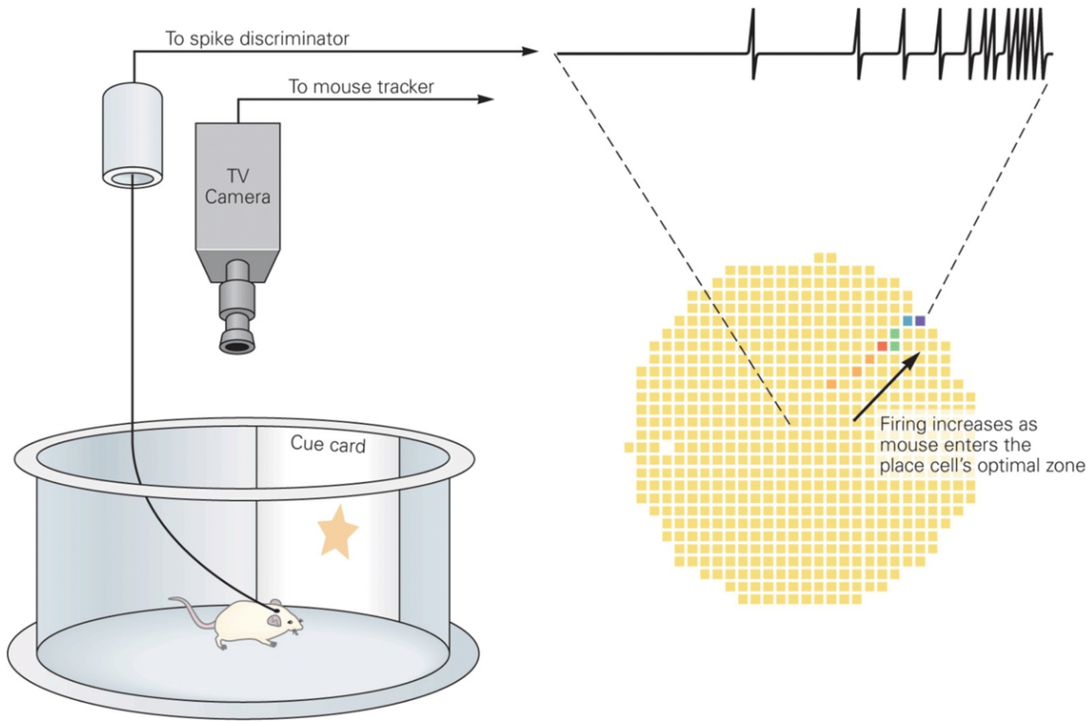


Figure 67–15 The firing patterns of pyramidal cells in the hippocampus create an internal representation of the animal's location in its surroundings. Electrodes implanted in the hippocampus of a mouse are attached to a recording cable, which is connected to an amplifier attached to a computer-based spike-discrimination program. The mouse is placed in a cylinder with an overhead TV camera that transmits to a device that detects the position of the mouse. The cylinder also contains a visual cue to orient the animal. Spikes in individual hippocampal pyramidal neurons (place cells) are detected by the 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 map of color-coded firing rates for the cell, from which the cell's place field can be determined. Yellow, orange, red, green, blue, and purple pixels show regions with progressively increasing rates of firing. The place field is the location in space that elicits optimal firing in the cell. (Adapted, with permission, from Muller, Kubie, and Ranck 1987.)

O'Keefe's demonstration of place cells provided the first evidence for a neural representation of the environment that allows an animal to move deliberately around the world. The idea of a cognitive map was predicted earlier by the great cognitive psychologist Edward Tolman. He proposed that somewhere in the brain there must be a representation of the environment. This cognitive map is not topographic or egocentric in its organization, like the maps for touch or vision on the surface of the cerebral cortex. Rather the map is allocentric (or geocentric); it is fixed with respect to a point in the outside world.

How is the spatial map formed? What type of spatial information is carried by the afferent connections to the hippocampal place cells? In 2005 Edvard and May-Britt Moser and their colleagues in Norway discovered that neurons in the medial entorhinal cortex, whose axons form the perforant pathway to the hippocampus, map space in a very different manner from the hippocampal place cells. Instead of firing when the animal is in a unique location, like the place cells, the entorhinal neurons, termed *grid cells*, fire whenever the animal is at any of several, regularly spaced positions forming a triangular grid-like array. This grid allows the animal to locate its body within a Cartesian-like external coordinate system that is independent of context, landmarks, or specific markings. The gridded spatial information conveyed by the entorhinal inputs is transformed within the hippocampus into unique spatial locations represented by the firing of place cells.

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 experi-

mental LTP, a natural question is whether LTP is important. This question was addressed in experiments in mice in which LTP was disrupted.

Surprisingly, 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, place fields of hippocampal neurons in 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 protein kinase A. In these mice place fields also form but the firing patterns of individual cells are stable only for an hour or so (Figure 67–16). Thus late LTP is required not for the formation but for long-term stabilization of place fields.

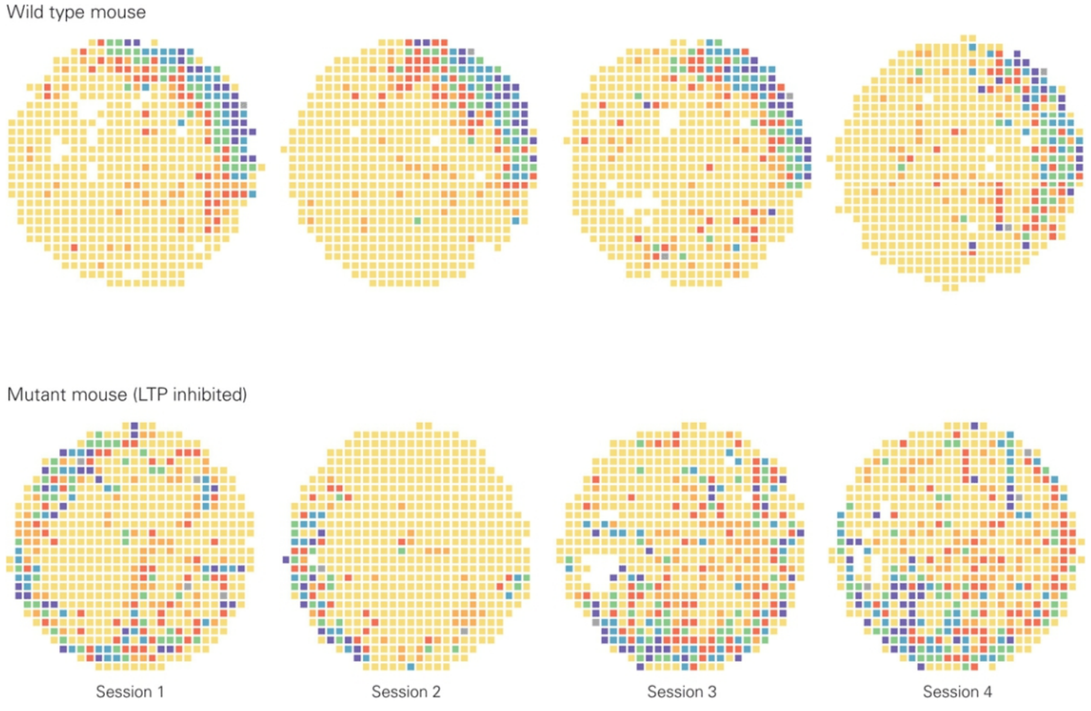


Figure 67–16 Disruption of long-term potentiation alters the stability of place field formation in hippocampal cells. Color-coded firing-rate maps (see Figure 67–15) show the place fields recorded in four successive sessions from a single hippocampal pyramidal neuron in a wild-type mouse and from a neuron 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 cylinder 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. In this example the place cell fires whenever the animal is in the upper right region of the enclosure. By contrast, the place field of the cell in the mutant mouse is unstable across the different sessions. (Reproduced, with permission, from Rotenberg et al. 1996.)

These experiments raise a final question: 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 a neuron's place field 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 as it forages for food, 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 action of dopamine on the D_1/D_5 type of receptor, which stimulates adenylyl cyclase, leading to production of cAMP and activation of PKA. This demonstrates that, rather than being a form

of implicit memory that is stored and recalled without conscious effort, long-term memory of a stably formed place field requires the animal to attend to its environment, as is the case for explicit memory in humans.

Different Subregions of the Hippocampus Are Required for Pattern Separation and for Pattern Completion

Explicit memory is used to store facts (semantic memory) and episodes (episodic memory). Successful storage and recall of explicit memory requires the ability to distinguish between two closely related images, episodes, or spatial configurations—an ability called *pattern separation*. Explicit memory can also use partial cues to retrieve previously stored memories by filling in an incomplete pattern based on preexisting knowledge—an ability called *pattern completion*. Both capabilities are essential for optimal memory performance. We need to distinguish similar memories when the differences between them are important, and we need to recall memories when only partial clues for recall are available.

A variety of cell-physiological and computation studies, beginning with the theoretical work of David Marr in the 1970s, suggests that pattern completion depends on the recurrent connections between the CA3 pyramidal cells and that pattern separation depends on the direct projection from the entorhinal cortex to the dentate gyrus. The dentate's role in pattern separation was suggested because the number of granule cells in the dentate far exceeds the number of CA3 or CA1 pyramidal neurons. These ideas have now received support from genetic experiments by Susumu Tonegawa and his colleagues.

The importance of LTP between CA3 neurons is seen in studies performed on mice in which the NMDA glutamate receptor is selectively deleted in 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 mossy fiber synapses onto the CA3 neurons or at the Schaffer collateral synapses between CA3 and CA1 neurons. Despite this deficit, the mice show normal learning and memory in the water maze. However, when the mice are asked to find a hidden platform with fewer than the normal number of spatial cues, their performance is impaired. This indicates that LTP at the recurrent synapses between CA3 neurons is important for pattern completion.

Similarly, the mechanism of pattern separation has been examined in mice in which a critical subunit of the NMDA receptor is selectively deleted from granule neurons in the dentate gyrus, or in which the neural activity of the granule neurons is blocked. Mutant mice lacking a functional NMDA receptor in the dentate gyrus have difficulty distinguishing between two similar contexts—that is, the mice cannot perform pattern separation.

One of the most unexpected findings in neuroscience has been the realization that neurogenesis is not limited to early stages of development. New neurons continue to be born throughout adulthood and become incorporated into neural circuits. However, adult neurogenesis is limited to two types of neurons in two brain regions: inhibitory granule cells in the olfactory bulb and the excitatory granule neurons of the dentate gyrus. When neurogenesis in the dentate is blocked, either by X-ray irradiation of specific brain regions or administration of a chemical that interferes with DNA synthesis, there is a variable effect on learning and memory; some forms of hippocampal-dependent memory are impaired whereas others appear intact. However, recent exciting results show that pattern separation specifically requires the participation of adult-born granule neurons. Moreover, procedures that stimulate neurogenesis enhance the ability of a mouse to perform pattern separation during a contextual memory task, which requires the animal to discriminate between

closely related environments. Methods to enhance neurogenesis are now being explored as a means of treating different types of age-related memory loss.

Memory 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 connection might reach a point beyond which further enhancement is not possible. Yet individuals are able to learn and store new memories throughout a lifetime. This paradox led to the suggestion that neurons must have mechanisms to downregulate synaptic function to counteract LTP.

In fact 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, the induction of LTD requires prolonged periods of low-frequency synaptic stimulation such as stimulation at 1 Hz for 15 minutes. LTD can also be induced by a pairing protocol, in which an EPSP is evoked after a postsynaptic cell fires an action potential. This suggests an anti-Hebbian learning rule: Synapses that do not contribute to the firing of a cell are weakened. Like LTP, a number of molecular and synaptic mechanisms are available to produce LTD.

Surprisingly many of the forms of LTD also require activation of the same receptors involved in LTP, namely the NMDA receptors (Figure 67–17A). Moreover, like LTP, LTD is thought to require Ca^{2+} influx through the NMDA receptors into the postsynaptic neuron. How can activation of a single type of receptor leading to elevated levels of a single second messenger, Ca^{2+}, produce both potentiation and depression?

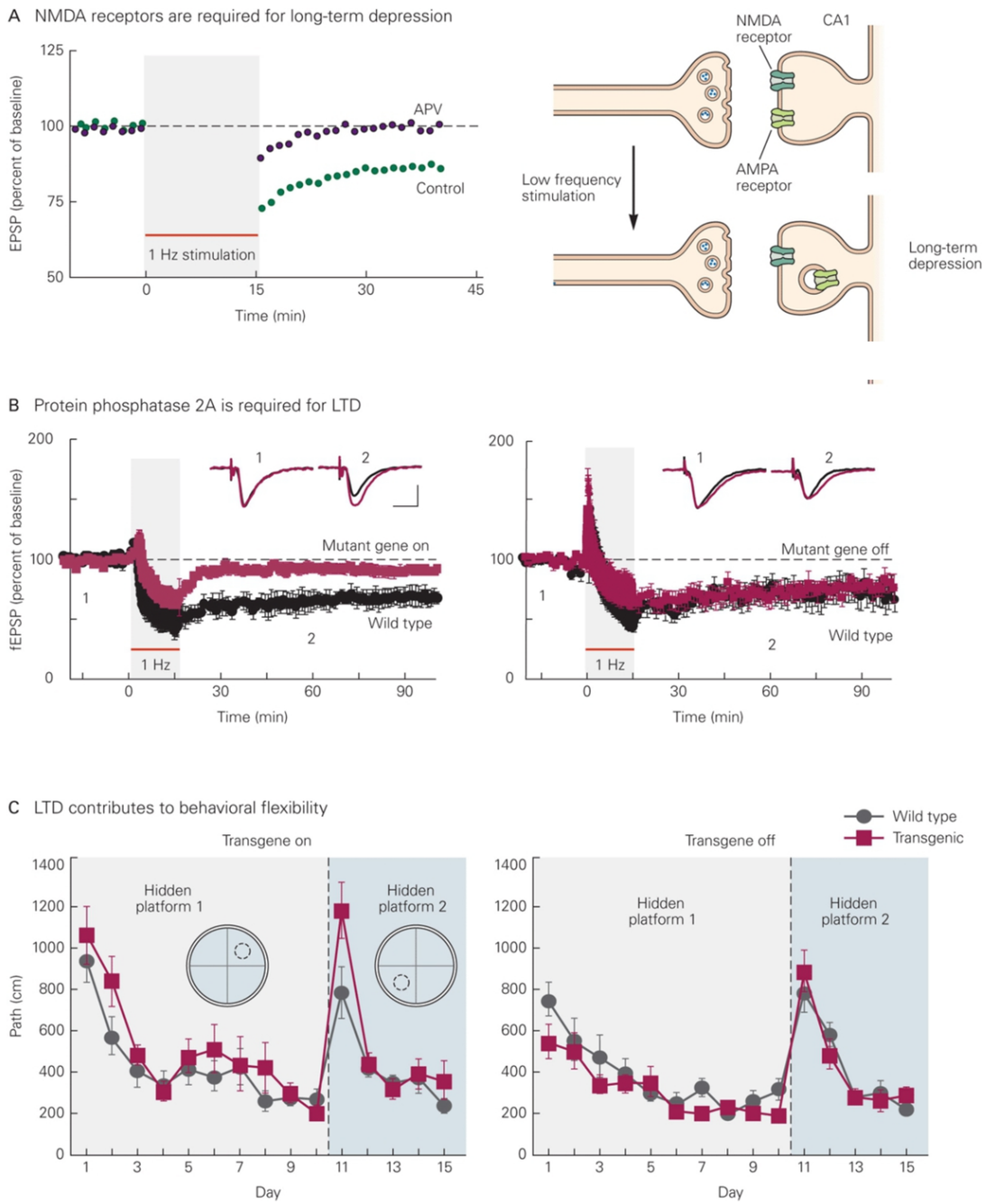


Figure 67–17 Long-term depression of synaptic transmission requires NMDA receptors and phosphatase activity.

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 EPSP in the hippocampal CA1 region, a decrease that outlasts the period of stimulation (control). The diagram shows that long-term depression (LTD) results from removal of AMPA receptors from the postsynaptic membrane by endocytosis. This LTD is blocked when the 1 Hz stimulation is given in the presence of APV to block NMDA receptors. (Reproduced, with permission, from Dudek and Bear 1992.)

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

C. Inhibition of phosphatase 2A alters behavioral flexibility. Transgenic mice expressing the phosphatase inhibitor learn the location of the submerged platform in the Morris maze at the same rate as wild-type mice (days 1 through 10). Learning is measured by the daily decrease in the path length the mice traverse as they search for the platform during training. 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 require significantly longer path lengths to find the platform on the first day of retesting (day 11). 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.)

A key difference lies in the 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 post-synaptic depolarization and thus is much less effective at relieving the Mg^{2+} block of the NMDA receptors. As a result, the increase in the postsynaptic Ca^{2+} concentration is much smaller than observed during induction of LTP. This low concentration of Ca^{2+} is thought to be insufficient to activate CaMKII, the enzyme implicated in LTP. Rather, LTD is thought to result from the activation of the calcium-dependent phosphatase calcineurin, an enzyme complex that has a higher affinity for Ca^{2+} compared to that of CaMKII (see Chapter 11).

The activated calcineurin triggers a signaling cascade that activates still other phosphatases that dephosphorylate a number of proteins, including the GluA1 (also known as GluR1) subunit of the AMPA receptor. In addition to activating phosphatases, the LTD induction protocol also increases the phosphorylation of the GluA2 (or GluR2) subunit of the AMPA receptor by protein kinase C. The combined effects of dephosphorylation of GluA1 and phosphorylation of GluA2 trigger the endocytosis of the AMPA receptors, reducing the number of receptors in the postsynaptic membrane and thus reducing the size of the EPSP.

Distinct forms of LTD can be induced through the activation of metabotropic glutamate receptors. Such forms do not require activation of phosphatases but depend on activation of mitogen-activated protein (MAP) kinase signaling pathways (see Chapter 11). These types of LTD lead to a reduction in synaptic transmission through a decrease in glutamate release from the Schaffer collateral 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 recent studies using a transgenic mouse that expresses an inhibitor of protein phosphatase under regulated control. This mouse shows a deficit in NMDA receptor-dependent LTD when the transgene is expressed but shows normal LTD when trans-

gene expression is suppressed (Figure 67–17B). In contrast, transgene expression does not affect LTP or forms of LTD that involve metabotropic glutamate receptors. The transgene-expressing mice also show normal learning the first time they are tested in the Morris maze. However, when the mutant 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 67–17C). This result suggests that LTD is needed for behavioral flexibility. Thus LTD may be necessary not only to prevent LTP saturation, but also as an active participant in memory storage.

Epigenetic Changes in Chromatin Structure Are Important for Long-Term Synaptic Plasticity and Learning and Memory

As is the case with long-term implicit memory, long-term explicit memory storage also requires covalent alterations in chromatin structure. Such changes are termed epigenetic. One form of epigenetic regulation involves acetylation of histone proteins, the protein component of the nucleosome repeat unit of chromatin. CREB binds to the cAMP-recognition element (CRE) promoter and recruits a transcriptional coregulator, the CREB-binding protein (CBP), which functions as a histone acetylase. Acetylation of the N-terminal tails of histones by CBP disrupts repressive chromatin structure and opens up the promoter region of target genes, allowing the binding of RNA polymerase II to initiate transcription (Figure 67–18A).

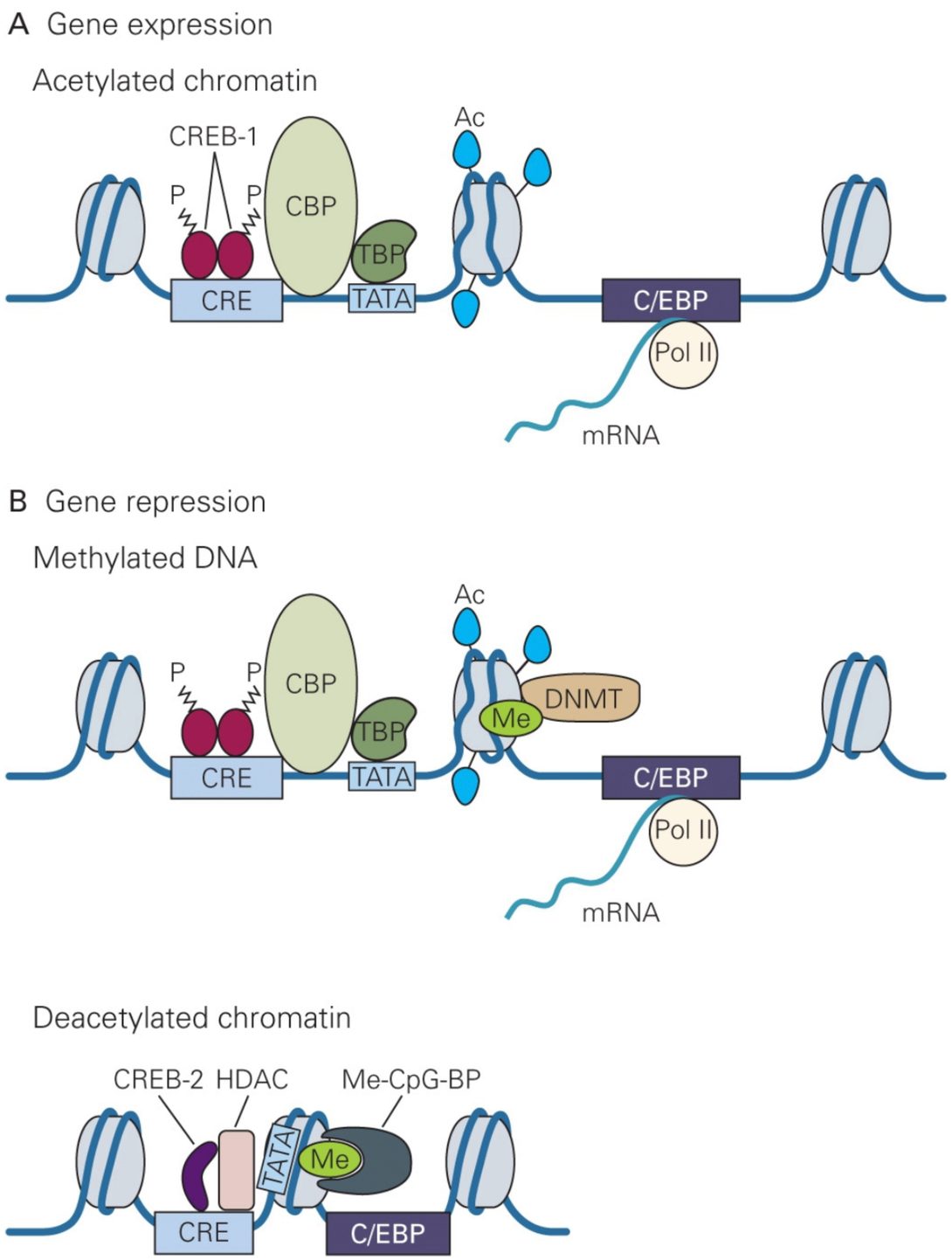


Figure 67–18 Long-term memory requires epigenetic alterations in chromatin structure.

A. Phosphorylation of CREB-1 following induction of the late phase of LTP in the hippocampus recruits the binding of CBP to the promoter region of target genes. In turn CBP acetylates certain positively charged lysine residues on the N-terminal tails of histone proteins, which form the nucleosome unit of chromatin. Acetylation loosens the binding of negatively charged DNA to the histones, allowing transcription to proceed and resulting in the late phase of LTP.

B. The induction of LTP also leads to changes in methylation of certain cytosine bases that precede guanine nucleotides (CpG sites) in DNA through the action of DNA methyltransferases (DNMT). This recruits methyl-CpG-binding proteins (Me-CpG-BP), which in turn recruit histone deacetylases (HDAC), leading to a decrease in histone acetylation, which, together with CREB-2, represses transcription.

Mutations in CBP underlie Rubinstein-Taybi syndrome, a rare condition characterized by mental retardation and skeletal abnormalities. Rubinstein-Taybi syndrome can be modeled in mice by insertion of a truncated nonfunctional form of CBP into the mouse genome. These mice show a reduction in histone acetylation and impairment in the late phase of LTP that is associated with a deficit in certain forms of hippocampal-dependent long-term memory.

Whereas defects in histone acetylation result in memory impairment, enhancement of histone acetylation using inhibitors of histone deacetylase (HDAC) can enhance memory. Remarkably, in a mutant mouse line that undergoes significant hippocampal degeneration, memory capabilities that have been lost following neuronal death can be recovered by treatment of the mice with HDAC inhibitors, raising the possible clinical usefulness of these compounds, which were first developed as anticancer agents.

A second form of chromatin modulation that has been implicated in learning and memory involves DNA methylation (Figure 67–18B). Certain cytosine bases that precede guanine bases in DNA (CpG sites) can be methylated by DNA methyltransferases. DNA methylation recruits methyl-CpG-binding proteins that in turn bind transcriptional corepressor complexes that inhibit gene expression, in part because these complexes contain HDACs. DNA methylation is thought to be of particular importance during early development, when it helps initially to determine cell fate and maintain cell identity. Evidence that DNA methylation is important for proper development of the human nervous system is illustrated by the fact that mutations in the methyl-CpG-binding protein 2 underlie Rett syndrome, a pervasive neurodevelopmental disorder discussed in Chapter 64.

Recent experiments suggest that DNA methylation may also play an acute role in learning and memory in the adult. Thus contextual fear conditioning in mice causes an increase in methylation of the gene encoding the neurotrophin BDNF in the CA1 region of the hippocampus, which leads to changes in BDNF expression. Injection of pharmacological inhibitors of DNA methylation into the CA1 region both inhibits the change in BDNF expression and impairs fear memory 24 hours after conditioning. Consistent with findings for other forms of hippocampal-dependent learning and memory, the changes in DNA methylation require activation of the NMDA receptor. At present the mechanism linking NMDA receptor activation to changes in DNA methylation is unknown.

Are There Molecular Building Blocks for Learning?

Three key findings have emerged from cellular studies of implicit and explicit memory storage. First, the molecular mechanisms of some associative forms of synaptic plasticity are based on those of nonassociative forms in the same cell. This suggests that there may be molecular building blocks for synaptic plasticity. Simpler forms of plasticity might serve

as components of more complex mechanisms, such as the joint recruitment of presynaptic and postsynaptic mechanisms of plasticity.

Second, the molecular mechanisms of elementary forms of associative learning, both implicit and explicit, are similar. The two synaptic mechanisms for memory storage we have considered—activity-dependent presynaptic facilitation for storing implicit memory and associative long-term potentiation for storing explicit memory—seem to derive from the associative properties of specific proteins (eg, the responsiveness of adenylyl cyclase or the NMDA receptor when two independent signals are simultaneously present).

Finally, despite their clear differences, implicit and explicit memory storage seem to rely on elements of a common multi-component genetic switch involving protein kinase A, CREB, and epigenetic changes in chromatin structure that convert labile short-term memory into long-term memory. Moreover, these mechanisms of synaptic plasticity do not operate in isolation. Rather, they are embedded in distributed neural circuits that have considerable computational power and thus can add substantial complexity to the actions of individual cells.

An Overall View

The demonstration that changes in the effectiveness of neural connections underlie memory, and thus learning, has revised our view of the relationship between social and biological processes in the shaping of an individual's behavior, both in health and disease. Until recently the majority view in medicine and psychiatry was that biological and social determinants of behavior act on separate components of the mind. For example, psychiatric illnesses were traditionally classified as either organic or functional. Organic mental illnesses included the dementias such as Alzheimer disease and the toxic psychoses such as those that follow the chronic use of alcohol. Functional mental illnesses included the various depressive syndromes, the schizophrenias, and the neuroses.

This distinction dates to the 19th century when neuropathologists examined the brains of patients coming to autopsy and found gross and readily demonstrable distortions in the architecture of the brain in some psychiatric diseases but not in others. Diseases that produce anatomical evidence of brain lesions were called *organic*; those lacking these features were called *functional*.

This distinction is no longer tenable, as the last two chapters of this book make clear. Everyday events—sensory stimulation, deprivation, and learning—can effectively weaken synaptic connections in some circumstances and strengthen them in others. We no longer think that only certain diseases ("organic diseases") affect mentation through biological changes in the brain whereas others ("functional diseases") do not. The basis of contemporary neural science is that all mental processes are biological and therefore any alteration in those processes is necessarily organic.

The question of the relative roles of nature and nurture in human behavior has shifted. We now ask, how do specific biological processes of the brain give rise to specific mental events and how in turn do social factors modulate the brain's biological structure? In the attempt to understand a particular mental illness it is more appropriate to ask, to what degree is this biological process determined by genetic and developmental factors? To what degree is it determined by a toxic or infectious agent, or by a developmental abnormality? To what degree is it socially determined? Even those mental disturbances that are considered most heavily determined by social factors must have a biological aspect, as it is the activity of the brain that is being modified by experience.

Insofar as social intervention works—whether through psychotherapy, counseling, or the support of family or friends—it must work by

acting on the brain and quite likely on the strength of connections between nerve cells. Moreover, the absence of detectable structural changes does not rule out the possibility that important biological changes are nevertheless occurring. They may simply occur at a subcellular or even molecular level that is below the level of detection with the techniques available to us.

Demonstrating the biological nature of mental functioning requires more sophisticated anatomical methodologies than the light-microscopic histology of 19th-century pathologists. To clarify these issues it will be necessary to develop a neuropathology of mental illness that is based on anatomical structure *and* function. Imaging techniques—positron emission tomography and functional magnetic resonance imaging among others—have allowed the noninvasive exploration of the human brain on a cell-biological level, the level of resolution that is required to understand the physical mechanisms of mentation and therefore of mental disorders. This approach is being pursued in the study of schizophrenia and depression.

In studying the specific cellular changes that underlie memory storage, we should look for altered gene expression in abnormal as well as normal mental states. There is now substantial evidence that the susceptibility to major psychotic illnesses—schizophrenia and bipolar disorder—is heritable. Nevertheless, a sibling whose identical twin develops schizophrenia has only a 50% chance of also developing a psychosis. Thus environmental factors must also be important. The cell-biological data on learning and long-term memory reviewed in this chapter suggest that neurotic illnesses acquired by learning are likely to involve alterations in the *regulation* of gene expression (Figure 67–19).

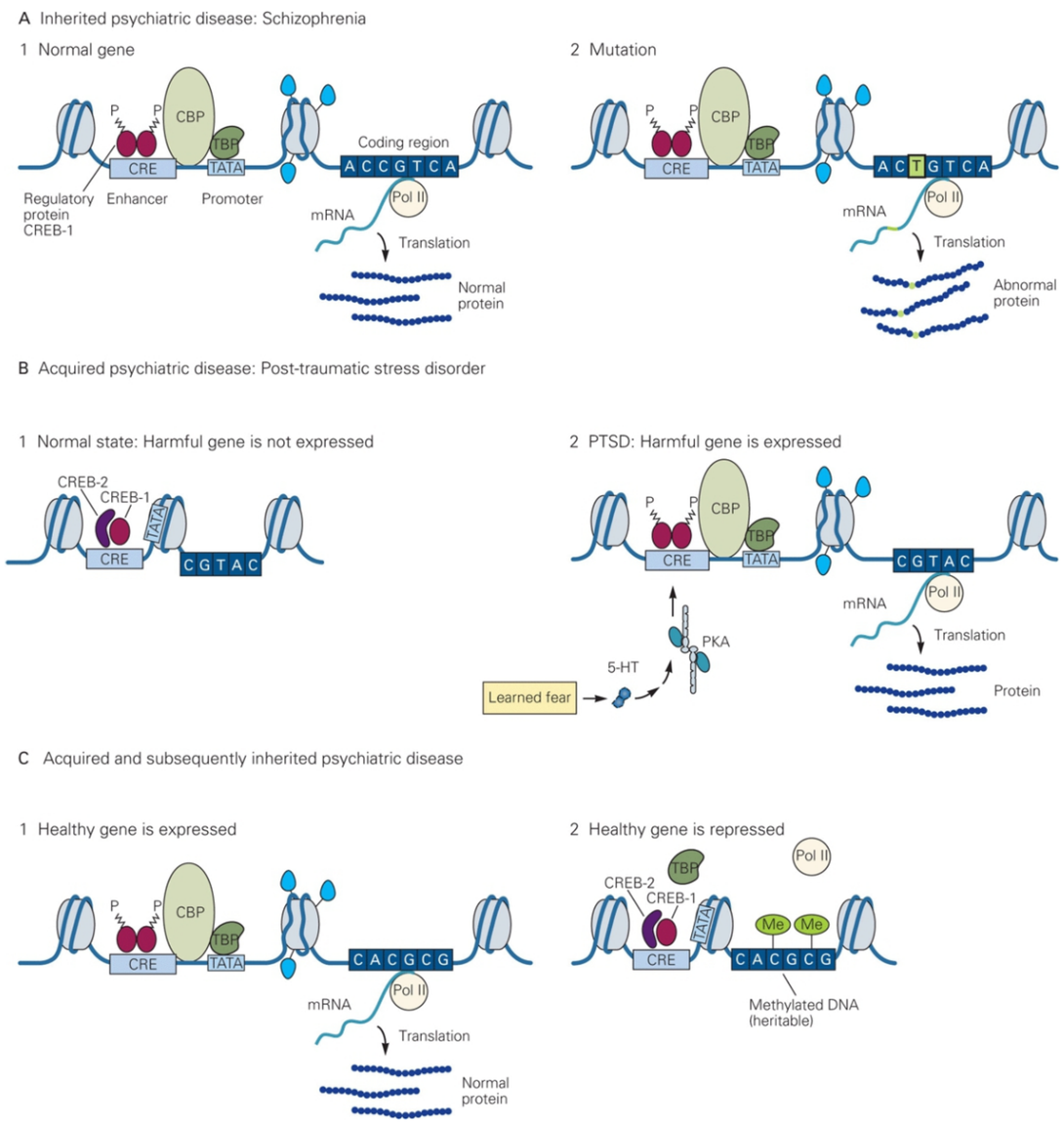


Figure 67–19 (Opposite) Inherited and acquired illnesses both involve genetic changes. Inherited illnesses result from the expression of altered genes, whereas acquired illnesses (neuroses) involve the modulation of normal gene expression by environmental stimuli. The gene illustrated here has two segments. A coding region is transcribed into mRNA by an RNA polymerase and the mRNA in turn is translated into a specific protein. A regulatory segment consists of enhancer and promoter regions (see Box 11–1). In this example the RNA polymerase can transcribe the

gene when the regulatory proteins CREB-1 and CBP bind to the enhancer region. For binding to occur, CREB-1 must be phosphorylated (P).

A. Inherited psychiatric disease. **1**. Under normal conditions the phosphorylated CREB regulatory protein binds the enhancer segment, thereby activating transcription of the structural gene, leading to the production of the protein. **2**. A mutant form of the coding region of the structural gene, for example, in which a thymidine (T) has been substituted for cytosine (C), leads to transcription of an altered messenger RNA. This in turn produces an abnormal protein, giving rise to the disease state. This alteration in gene structure may become established in the germline and therefore heritable.

B. Acquired disease. **1**. If the regulatory protein for a normal structural gene is not phosphorylated, it cannot bind the promoter site and thus gene transcription cannot be initiated. **2**. A specific frightening experience can lead to the activation of a modulatory transmitter such as serotonin **(5-HT)**, which leads to elevation of cAMP and activation of protein kinase A. The catalytic subunit phosphorylates the regulatory protein, which then can bind to the enhancer segment and thus initiate gene transcription. By this means an abnormal learning experience could lead to the altered expression of a protein that gives rise to symptoms of a psychiatric disorder such as post-traumatic stress disorder.

C. Heritable epigenetic changes. Certain experiences, for example extreme stress during early childhood, can lead to changes in DNA methylation, which alters gene expression. Such changes in methylation can be maintained even during DNA replication as a result of the activity of maintenance DNA methyltransferases. In some instances changes in DNA methylation can be passed from a mother to her offspring.

Development, hormones, stress, and learning are all factors that alter gene expression by modifying the binding of transcriptional regulatory proteins to each other and to the regulatory regions of genes. It is likely that at least some neurotic illnesses (or components of them) result from effects of gene regulation that are reversible. It is intriguing to think, then, that insofar as psychotherapy is successful in changing behavior it does so by producing alterations in gene expression. If so, treatment of neurosis or character disorders by psychotherapeutic intervention would, if successful, also produce structural changes in the nervous system. Thus we face the attractive possibility, for which there is now preliminary evidence, that improved brain imaging techniques might ultimately be useful not only for diagnosing various psychiatric illnesses but also for monitoring the progress of psychotherapy.

When we consider together what we know about synapse formation and synaptic plasticity, it is clear there are two overlapping stages in the development and maintenance of synapses. The first stage, the initial steps of synapse formation, occurs primarily early in development and is under the control of genetic and developmental processes, commonly diffusible signals, cell matrix interactions, and cell-cell interactions. The second stage, the fine tuning of synapses by experience, occurs daily throughout later life. The activity-dependent mechanisms at work during critical periods of development are thought to be closely related to the activity-dependent cellular mechanisms involved in associative learning. Recent evidence indicates that age-related memory loss, including that which occurs in the early stages of Alzheimer disease, may primarily result from a defect in synaptic plasticity. Drugs that enhance the ability to induce synaptic plasticity, and in particular LTP, offer a promising approach for the treatment of memory loss.

The finding that epigenetic changes play important roles in learning, memory and behavioral modifications adds a new dimension to our understanding of how environmental and social interactions can produce long-lasting changes in the nervous system. What is particularly striking is that these epigenetic changes allow environmental influences to be passed from a mother onto her offspring. Infant neglect and child

abuse are extremely stressful for the developing child and are thought to contribute to cognitive and social deficits that lead to the later development of psychiatric illnesses such as anxiety states and depression.

We now know that early prenatal and postnatal environmental influences, including maternal behavior, can lead to persistent chromatin modifications in the form of direct methylation of the genes that encode hormones, growth factors, and receptors that are important for learning, memory, and emotional states. Moreover, females that experienced maltreatment during infancy later produce offspring that inherit this DNA methylation for at least one generation. Thus, not only our own experience, but that of our mothers, can have a direct and lasting influence on our genetic landscape.

The convergence of neurobiology, cognitive psychology, neurology, and psychiatry that we have emphasized throughout this book is filled with promise. Modern cognitive psychology has shown that the brain stores an internal representation of the world while neurobiology has shown that this representation can be understood in terms of the activity of individual nerve cells and their interconnections. This synthesis has given us a deeper understanding of perception, learning, and memory as well as profound new biological insight into the nature of mental illnesses.

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