Whereas the early phase of LTP is mediated by changes at existing synapses, late LTP is thought to result from the growth of new synaptic connections between pairs of co-activated neurons.

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

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

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

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

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

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

Constitutively active forms of protein kinases may not be the only mechanism for maintaining long-lasting synaptic changes in the hippocampus. Repeated stimulation may lead to the formation of new synaptic connections, just as long-term facilitation leads to the formation of new synapses during learning in *Aplysia*. In addition, long-lasting synaptic changes likely involve epigenetic changes in chromatin structure. During late LTP, phosphorylated CREB activates gene expression by recruiting the CREB binding protein (CBP), which acts as a histone acetylase, transferring an acetyl group to specific lysine residues on histone proteins, and thereby producing

long-lasting changes in gene expression. Mutations in CBP impair late LTP and learning and memory in mice. In humans, de novo mutations in the CBP gene underlie Rubinstein-Taybi syndrome, a developmental disorder associated with intellectual impairment. Other studies implicate a second epigenetic mechanism, DNA methylation, in long-lasting synaptic plasticity and learning and memory.

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

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

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

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

NMDA receptor–dependent LTP at the Schaffer collateral pathway and other hippocampal pathways has three properties with direct relevance to learning and memory (Figure 54–6). First, LTP in such pathways requires the near-simultaneous activation of a large number of afferent inputs, a feature called *cooperativity* (Figure 54–6). This requirement stems from the fact

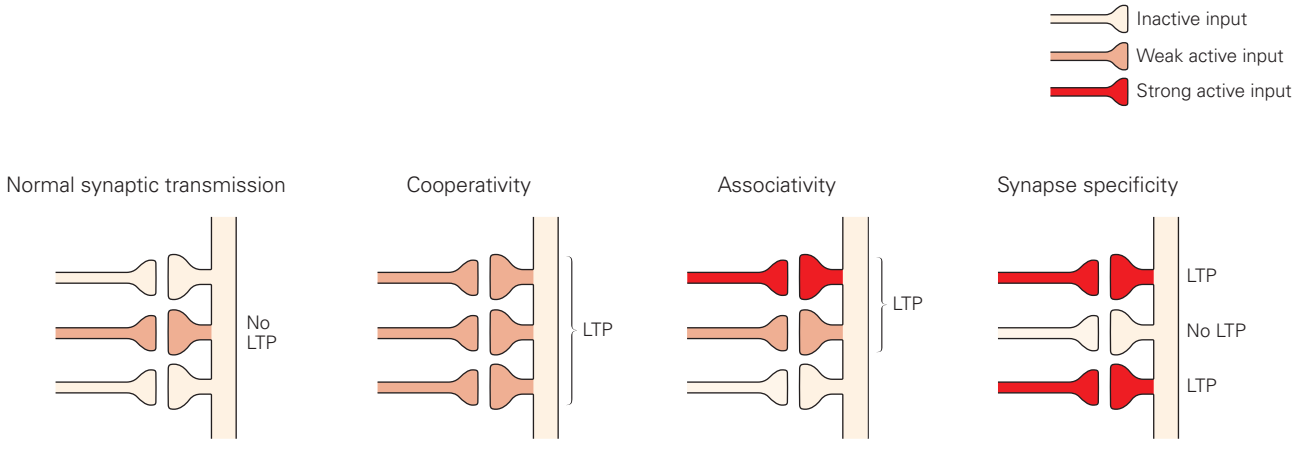


Figure 54–6 Long-term potentiation (LTP) in CA1 pyramidal neurons of the hippocampus shows cooperativity, associativity, and synapse specificity. With normal synaptic transmission, a single action potential in one or a few axons (weak input) leads to a small excitatory postsynaptic potential (EPSP) that is insufficient to expel Mg^{2+} from the *N*-methyl-D-aspartate (NMDA) receptor-channels and thus cannot induce LTP. This ensures that irrelevant stimuli are not remembered. The near-simultaneous activation of several weak inputs during strong activation (cooperativity) produces a suprathreshold EPSP that triggers an action potential, resulting in LTP in all pathways. Stimulation of strong and weak inputs together (associativity) causes LTP in both pathways. In this way, a weak input becomes significant when paired with a powerful one. An unstimulated synapse does not undergo LTP despite the strong stimulation of neighboring synapses. This ensures that memory is selectively stored at active synapses (synapse specificity).

that relief of Mg^{2+} block of the NMDA receptor-channel requires a large depolarization, which is achieved only when the postsynaptic cell receives input from a large number of presynaptic cells.

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

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

Each of these three properties—cooperativity, associativity, and synapse specificity—underlies a key requirement of memory storage. Cooperativity ensures that only events of a high degree of significance, those that activate sufficient inputs, will result in memory storage. Associativity, like associative Pavlovian conditioning, allows an event (or conditioned stimulus) that has little significance in and of itself to be endowed with a higher degree of meaning if that event occurs just before or simultaneously with another more significant event (an unconditioned stimulus). In a network with strong recurrent connections, such as CA3, associative LTP enables a pattern of activity in one group of cells to become linked to a distinct pattern of activity in a separate, but partially overlapping, group of synaptically coupled cells. Such linkages of cell assemblies are thought to enable related events to become associated with one another and to be important for storing and recalling large varieties of experiences, as occurs with explicit memory. Finally, synapse specificity ensures that inputs that convey information not related to a particular event will not be strengthened. Synapse specificity is critical when large amounts of information must be stored in one network, because much more information can be stored in a cell through functional alterations at individual synapses than through blanket changes in a property of the cell, such as its excitability.

Spatial Memory Depends on Long-Term Potentiation

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

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

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

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

localized, the mutant mice have a serious deficit in spatial memory (Figure 54–7).

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

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

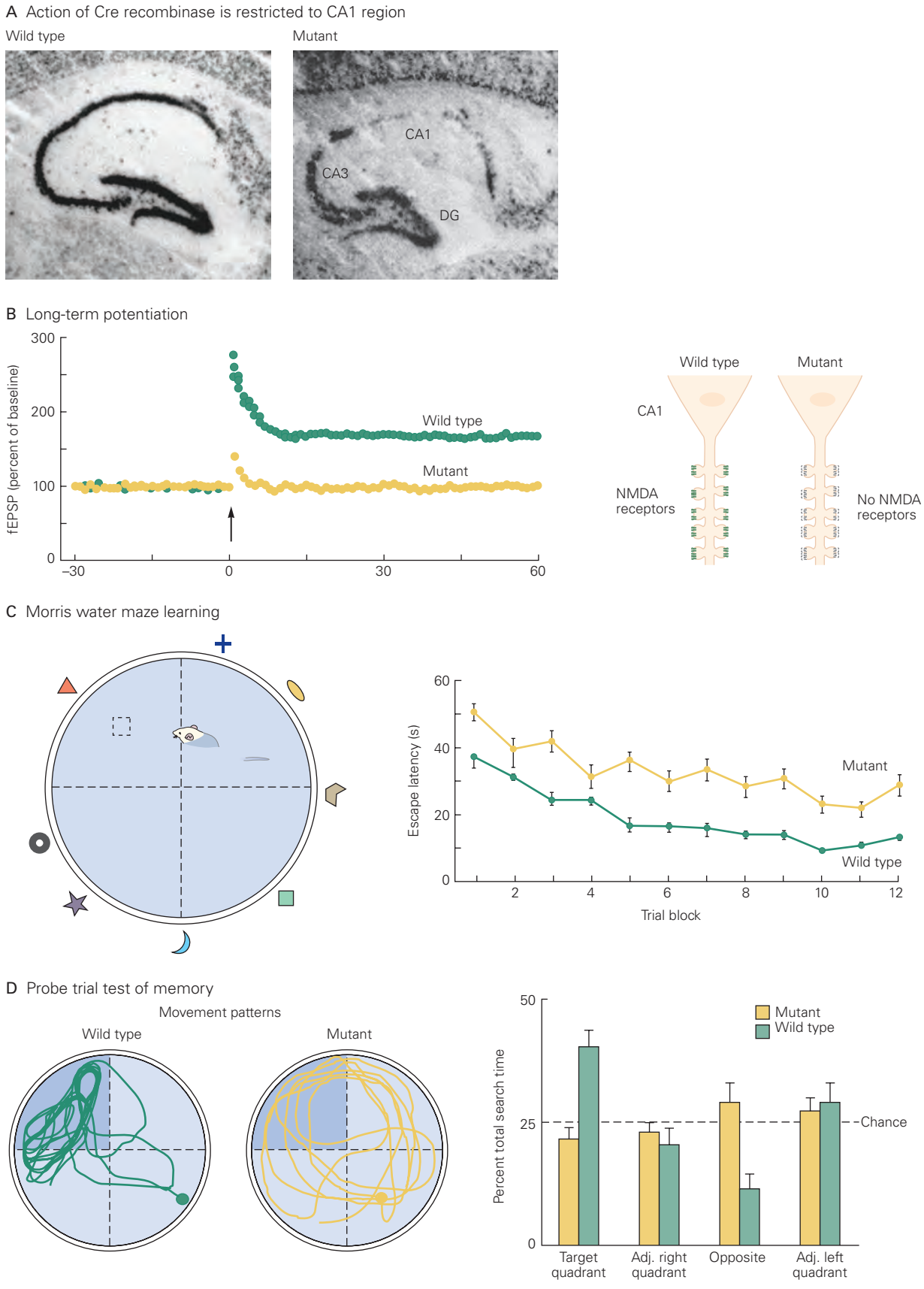
Reversible gene expression has been used to explore the role of CaMKII, whose autophosphorylation properties and function in LTP were discussed earlier in this chapter (see also Chapter 2, Figure 2–9, for a description of the methodology). Mutation of the autophosphorylation Thr286 site to the negatively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

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

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

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

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


A Action of Cre recombinase is restricted to CA1 region
Wild type
Mutant
CA1
CA3
DG
B Long-term potentiation
fEPSP (percent of baseline)
300
200
100
0
–30
0
30
60
Wild type
Mutant
Wild type
Mutant
CA1
NMDA receptors
No NMDA receptors
C Morris water maze learning
Escape latency (s)
60
40
20
0
2
4
6
8
10
12
Trial block
Mutant
Wild type
D Probe trial test of memory
Movement patterns
Wild type
Mutant
Percent total search time
50
25
0
Mutant
Wild type
Chance
Target quadrant
Adj. right quadrant
Opposite
Adj. left quadrant

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

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

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

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

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

Figure 54–7 (Opposite) Long-term potentiation (LTP) and spatial learning and memory are impaired in mice that lack the *N*-methyl-D-aspartate (NMDA) receptor in the CA1 region of the hippocampus. (Reproduced, with permission, from Tsien, Huerta, and Tonegawa 1996.)

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

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

C. Mice that lack the NMDA receptor in CA1 pyramidal neurons have impaired spatial memory. A platform (**dashed square**) is submerged in an opaque fluid in a circular tank (a Morris water maze). To avoid remaining in the water, the mice have to find the platform using spatial (contextual) cues on the walls surrounding the tank and then climb onto the platform. The graph shows escape latency or the time required by mice to find the hidden platform in successive trials. The mutant mice display a longer escape latency in every block of trials (four trials per day) than do the wild type mice. Also, mutant mice do not reach the optimal performance attained by the control mice after 12 training days, even though they show some improvement with training.

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

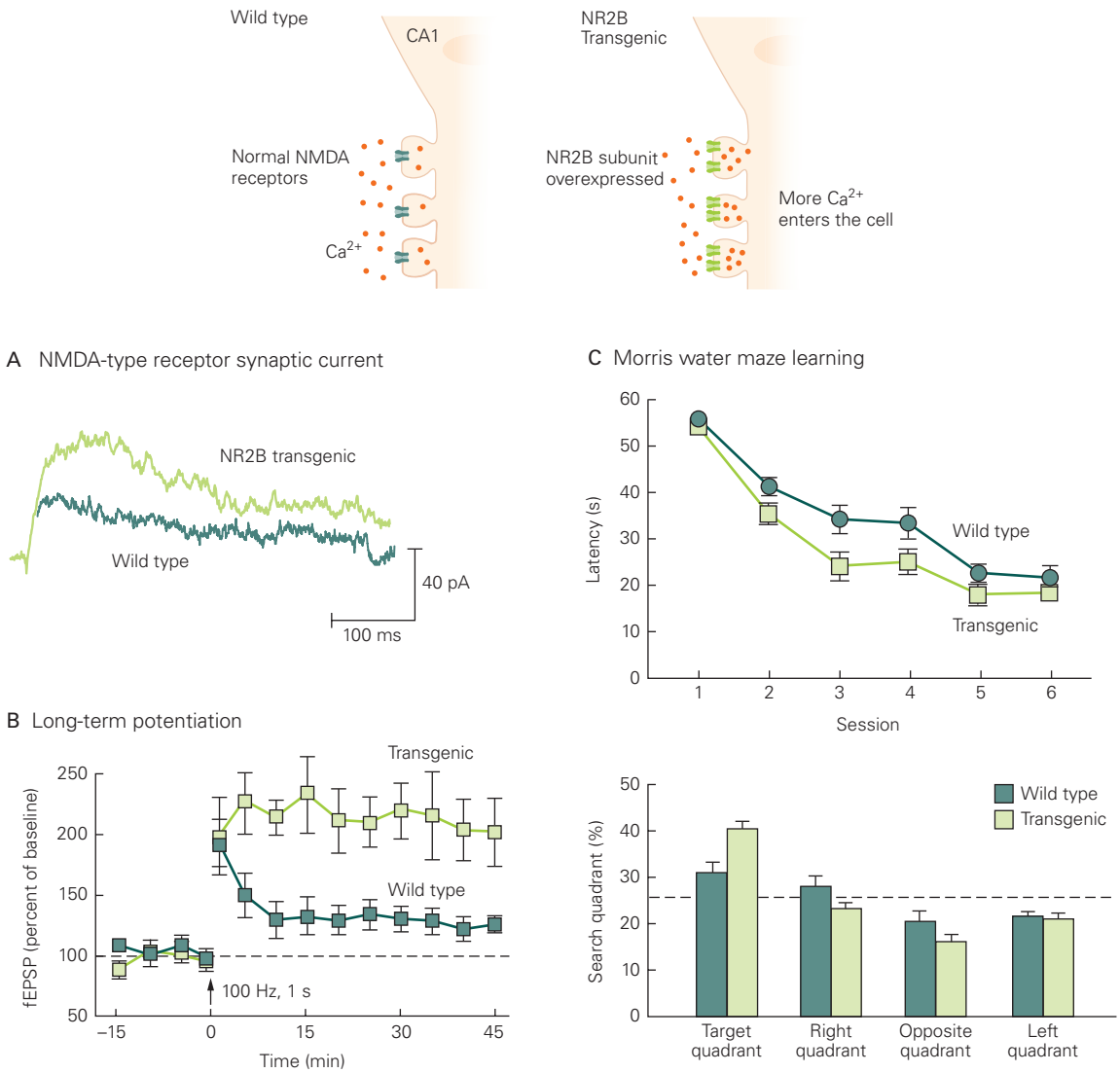


Figure 54–8 Learning and memory are enhanced in mice that overexpress a subunit of the *N*-methyl-D-aspartate (NMDA) glutamate receptor. (Reproduced, with permission, from Tang et al. 1999. Copyright © 1999 Springer Nature.)

A. The amplitude of the current generated by the NMDA receptors in response to a brief pulse of glutamate is enhanced and its time course prolonged in hippocampal neurons obtained from mice that contain a transgene that expresses higher levels of the receptor's NR2B subunit compared to wild type mice.

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

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

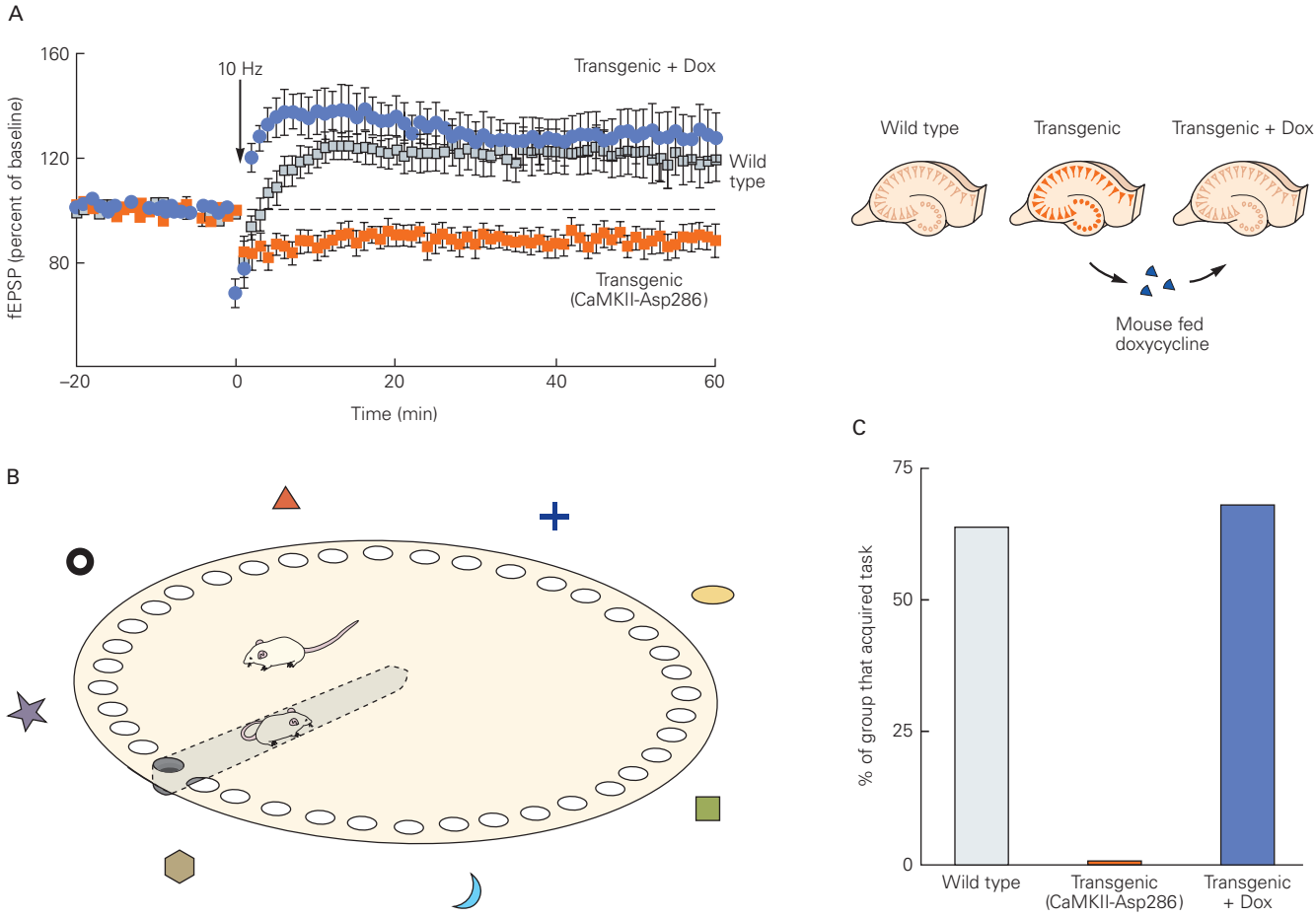


Figure 54–9 **Deficits in long-term potentiation (LTP) and spatial memory due to a transgene are reversible.** (Reproduced, with permission, from Mayford et al. 1996.)

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

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

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

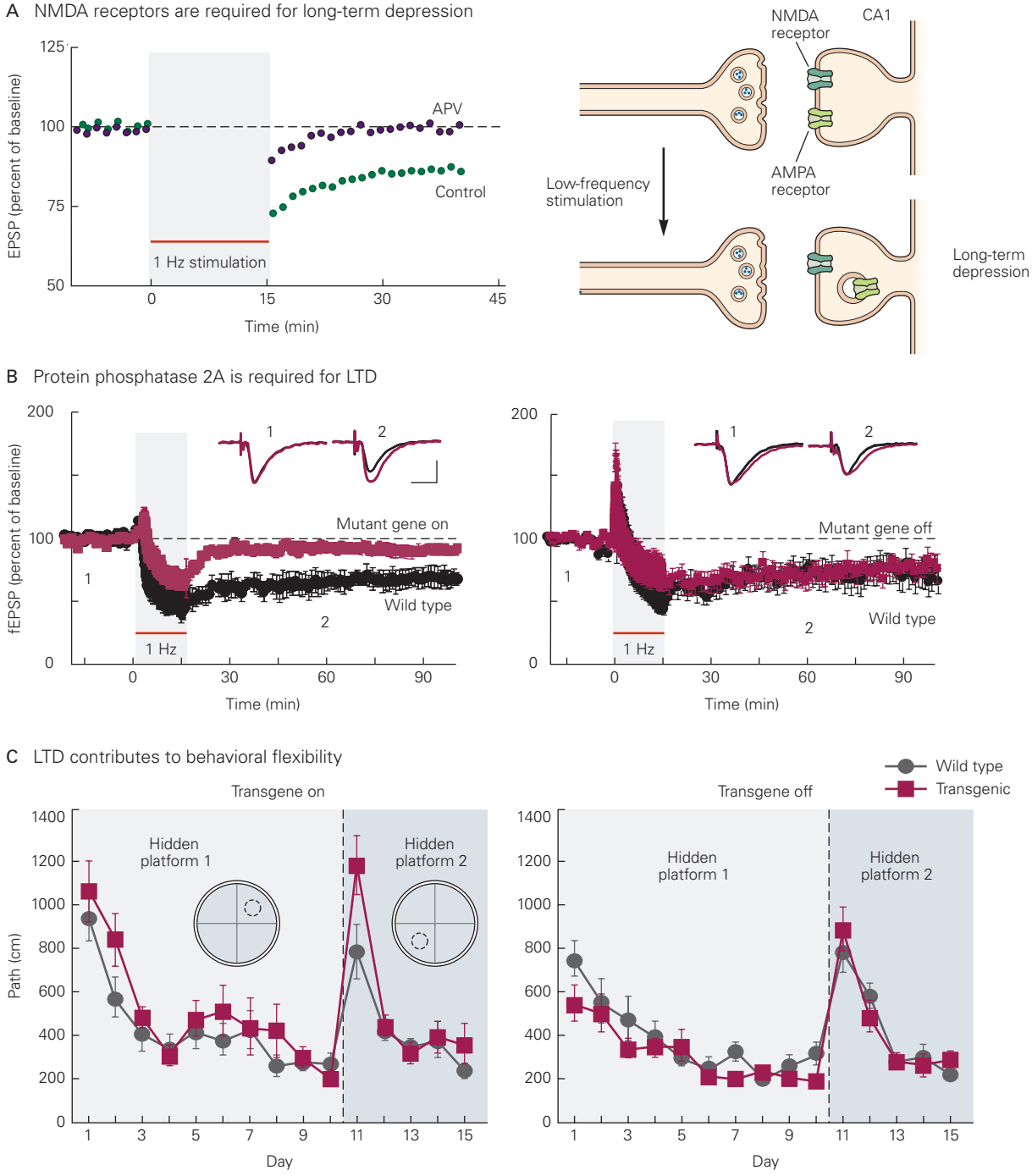


Figure 54–10 Long-term depression of synaptic transmission requires *N*-methyl-D-aspartate (NMDA) receptors and phosphatase activity.

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

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

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