

The initial problem in this work was to develop behavioral tests that could identify atypical learning or memory defects in large populations of flies. This challenge was met by Seymour Benzer and his colleagues Chip Quinn and Bill Harris at the California Institute of Technology, who developed the olfactory and visual learning tests that have become the basis for most subsequent analyses of learning and memory in the fruit fly (see figure). Behavioral paradigms pairing odors or light with an aversive stimulus allowed Benzer and his colleagues to assess associative learning in flies. The design of an ingenious testing apparatus controlled for non-learning-related sensory cues that had previously complicated such behavioral testing. Moreover, the appa-

ratus allowed large numbers of flies to be screened relatively easily, expediting the analysis of mutagenized populations.

These studies led to the identification of an ever-increasing number of single gene mutations that disrupt learning or memory in flies. The behavioral and molecular studies of the mutants (given whimsical but descriptive names such as *dunce*, *rutabaga*, and *amnesiac*) suggested that a central pathway for learning and memory in the fly is signal transduction mediated by the cyclic nucleotide cAMP. Thus, the gene products of the *dunce*, *rutabaga*, and *amnesiac* loci are, respectively, a phosphodiesterase (which degrades cAMP), an adenylyl cyclase (which converts ATP to cAMP), and a peptide transmitter that stimulates

adenylyl cyclase. This conclusion about the importance of cAMP has been confirmed by the finding that genetic manipulation of the CREB transcription factor also interferes with learning and memory in typical flies.

These observations in *Drosophila* accord with conclusions reached in studies of *Aplysia* and mammals (see text) and have emphasized the importance of cAMP-mediated learning and memory in a wide range of additional species. More generally, the genetic accessibility of *Drosophila* continues to make it a powerful experimental system for understanding the genetic underpinnings of learning and memory.

Second, these plastic changes in synaptic function can be either short-term effects that rely on post-translational modification of existing synaptic proteins, or they can be long-term changes that require changes in gene expression, new protein synthesis, and growth of new synapses (as well as enlarging or eliminating existing synapses). Thus, it appears that short- and long-term changes in synaptic function have different mechanistic underpinnings. As the following sections show, these generalizations apply to synaptic plasticity in the mammalian brain and have helped guide our understanding of these forms of synaptic plasticity.

Long-Term Potentiation at a Hippocampal Synapse

Long-term synaptic plasticity has also been identified in the mammalian brain. Here, some patterns of synaptic activity produce a long-lasting increase in synaptic strength known as **long-term potentiation (LTP)**, whereas other patterns of activity produce a long-lasting decrease in synaptic strength, known as **long-term depression (LTD)**. LTP and LTD are broad terms that describe only the direction of change in synaptic efficacy; in fact, different cellular and molecular mechanisms can be involved in producing LTP or LTD at different synapses throughout the brain. In general, LTP and LTD are produced by different histories of activity and are mediated by different complements of intracellular signal transduction pathways in the nerve cells involved.

Long-term synaptic plasticity has been most thoroughly studied at excitatory synapses in the mammalian hippocampus. The hippocampus is especially important in the formation and retrieval of some forms of memory (see Chapter 30). In humans, functional imaging shows that the hippocampus is activated during certain kinds of memory tasks and that damage to this brain region results in an inability to form certain types of new memories. Although many other brain areas are involved in the complex process of memory formation, storage, and retrieval, these

observations from imaging and lesion (damage) studies have led many investigators to study long-term synaptic plasticity of hippocampal synapses.

Work on LTP began in the late 1960s, when Terje Lomo and Timothy Bliss, working in the laboratory of Per Andersen in Oslo, Norway, discovered that a few seconds of high-frequency electrical stimulation can enhance synaptic transmission in the rabbit hippocampus for hours or longer. More recently, however, progress in understanding the mechanism of LTP has relied heavily on in vitro studies of slices of living hippocampus. The arrangement of neurons

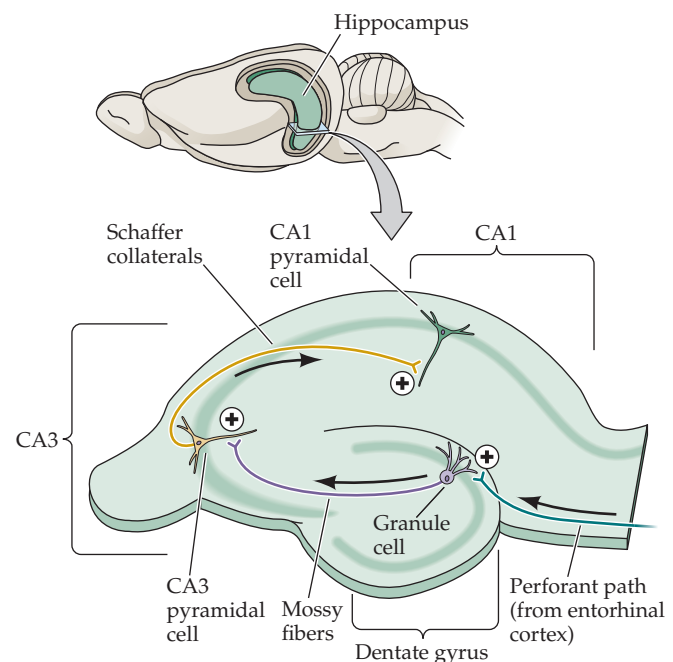


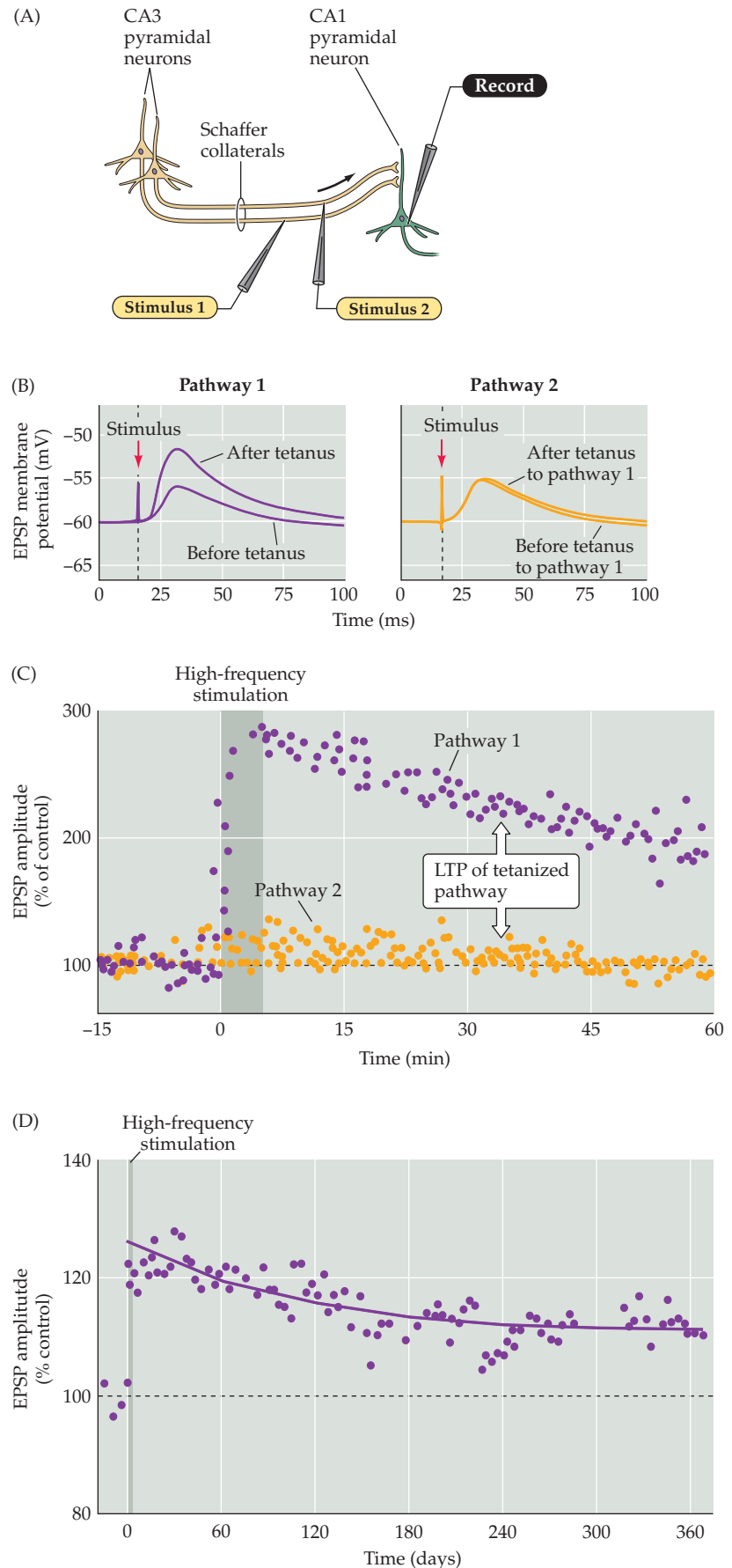
FIGURE 8.6 The trisynaptic circuit of the hippocampus. A section through the rodent hippocampus diagrams excitatory pathways and synaptic connections. Long-term potentiation (plus signs) has been observed at each of the three synaptic connections shown here.

allows the hippocampus to be sectioned such that most of the relevant circuitry is left intact. In such preparations, the cell bodies of the pyramidal neurons lie in a single densely packed layer that is readily apparent (Figure 8.6). This layer is divided into several distinct regions, the major ones being CA1 and CA3. “CA” refers to *cornu Ammonis*, Latin for Ammon’s horn—the ram’s horn that resembles the shape of the hippocampus. The dendrites of pyramidal cells in the CA1 region form a thick band (the stratum radiatum), where they receive synapses from Schaffer collaterals, the axons of pyramidal cells in the CA3 region.

Much of the work on LTP has focused on the synaptic connections between the Schaffer collaterals and CA1 pyramidal cells. Electrical stimulation of Schaffer collaterals generates EPSPs in the postsynaptic CA1 cells (Figure 8.7A,B). If the Schaffer collaterals are stimulated only two or three times per minute, the size of the EPSP elicited in the CA1 neurons remains constant. However, a brief, high-frequency train of stimuli to the same axons causes LTP, which is evident as a long-lasting increase in EPSP amplitude (Figure 8.7B,C). While the maximum duration of LTP is not known, in some cases LTP can last for more than a year (Figure 8.7D). The long duration of LTP shows that this form of synaptic plasticity is capable of serving as a mechanism for long-lasting storage of information. LTP occurs at each of the three excitatory synapses of the hippocampus

FIGURE 8.7 LTP of Schaffer collateral-CA1 synapses.

(A) Arrangement for recording synaptic transmission. Two stimulating electrodes (1 and 2) activate separate populations of Schaffer collaterals, providing test and control synaptic pathways. (B) Left: Synaptic responses recorded in a CA1 neuron in response to single stimuli of synaptic pathway 1, minutes before and 1 hour after a high-frequency train of stimuli. The high-frequency stimulus train increases the size of the EPSP evoked by a single stimulus. Right: The size of the EPSP produced by stimulating synaptic pathway 2, which did not receive high-frequency stimulation, is unchanged. (C) Time course of changes in the amplitude of EPSPs evoked by stimulation of pathways 1 and 2. High-frequency stimulation of pathway 1 (darker bar) enhances EPSPs in this pathway (purple dots). This LTP of synaptic transmission in pathway 1 persists for more than an hour, while the amplitude of EPSPs produced by pathway 2 (orange dots) remains constant. (D) Recordings of EPSPs from the hippocampus *in vivo* reveal that high-frequency stimulation can produce LTP that lasts for more than 1 year. (A–C after Malinow et al., 1989; D from Abraham et al., 2002.)



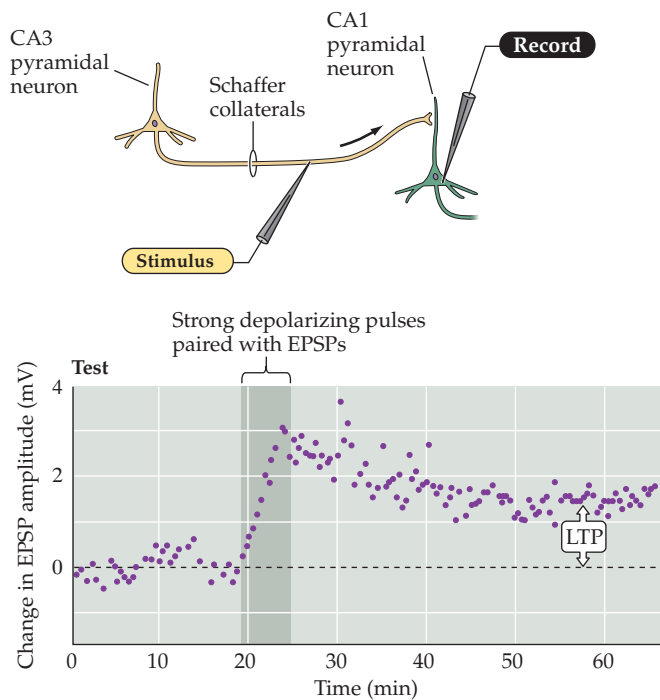


FIGURE 8.8 Pairing presynaptic and postsynaptic activity causes LTP. Single stimuli applied to a Schaffer collateral synaptic input evoke EPSPs in the postsynaptic CA1 neuron. These stimuli alone do not elicit any change in synaptic strength. However, brief polarization of the CA1 neuron's membrane potential (by applying current pulses through the recording electrode), in conjunction with the Schaffer collateral stimuli, results in a persistent increase in the EPSPs. (After Gustafsson et al., 1987.)

shown in Figure 8.6. LTP also is found at excitatory synapses in a variety of brain regions—including the cortex, amygdala, and cerebellum—and at some inhibitory synapses as well.

LTP of the Schaffer collateral synapse exhibits several properties that make it an attractive neural mechanism for

information storage. First, LTP requires strong activity in both presynaptic and postsynaptic neurons. If action potentials in a small number of presynaptic Schaffer collaterals—which evoke transmitter release that produces subthreshold EPSPs that would not normally yield LTP—are paired with strong depolarization of the postsynaptic CA1 cell, the activated Schaffer collateral synapses undergo LTP (Figure 8.8). This increase in synaptic transmission occurs only if the paired activities of the presynaptic and postsynaptic cells are tightly linked in time, such that the strong postsynaptic depolarization occurs within about 100 milliseconds of transmitter release from the Schaffer collaterals. Such a requirement for coincident presynaptic and postsynaptic activity is the central postulate of a theory of learning devised by Donald Hebb in 1949. Hebb proposed that coordinated activity of a presynaptic terminal and a postsynaptic neuron would strengthen the synaptic connection between them, precisely as is observed for LTP. This indicates the involvement of a **coincidence detector** that allows LTP to occur only when both presynaptic and postsynaptic neurons are active. Hebb's postulate has also been useful in thinking about the role of neuronal activity in other brain functions, most notably development of neural circuits (see Chapter 25).

A second property that makes LTP a particularly attractive neural mechanism for information storage is that LTP is input specific: When LTP is induced by activation of one synapse, it does not occur in other, inactive synapses that contact the same neuron (see Figure 8.7C). Thus, LTP is restricted to activated synapses rather than to all of the synapses on a given cell (Figure 8.9A). Such specificity of LTP is consistent with its involvement in memory formation (or at least the selective storage of information at synapses). If activation of one set of synapses led to all other synapses—even inactive ones—being potentiated, it would be difficult to selectively enhance particular sets of inputs, as is presumably required to store specific information. The synapse specificity of LTP means that each of the tens of thousands of synapses on a hippocampal neuron can store information, thereby making it possible for the millions of neurons in the hippocampus to store a vast amount of information.

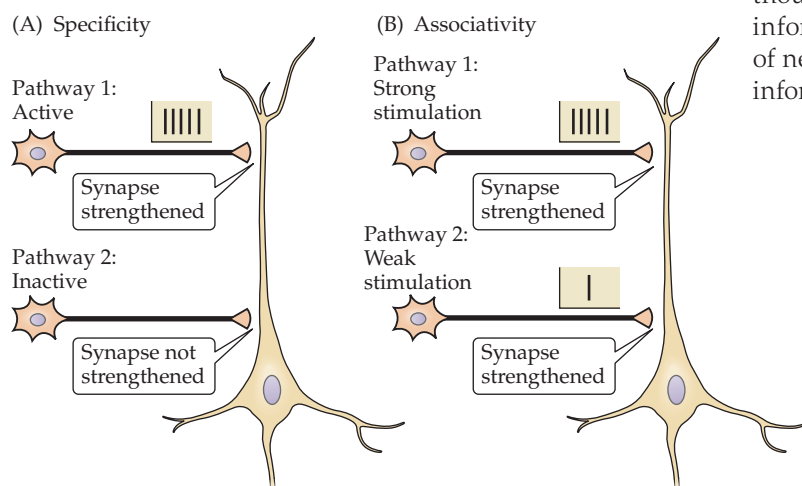


FIGURE 8.9 Properties of LTP at a CA1 pyramidal neuron receiving synaptic inputs from two independent sets of Schaffer collateral axons.

(A) Strong activity initiates LTP at active synapses (pathway 1) without initiating LTP at nearby inactive synapses (pathway 2). (B) Weak stimulation of pathway 2 alone does not trigger LTP. However, when the same weak stimulus to pathway 2 is activated together with strong stimulation of pathway 1, both sets of synapses are strengthened.

Another important property of LTP is **associativity** (Figure 8.9B). As noted, weak stimulation of a pathway will not by itself trigger LTP. However, if one pathway is weakly activated at the same time that a neighboring pathway onto the same cell is strongly activated, both synaptic pathways undergo LTP. Associativity is another consequence of the coincidence detection feature of LTP, specifically pairing of the activity of the weak synapse with the coincident generation of action potentials by the strong synapse. The selective enhancement of conjointly activated sets of synaptic inputs is often considered a cellular analog of associative learning, where two stimuli are required for learning to take place. The best-known type of associative learning is classical (Pavlovian) conditioning. More generally, associativity is expected in any network of neurons that links one set of information with another.

Although there is clearly a gap between understanding LTP of hippocampal synapses and understanding learning, memory, or other aspects of behavioral plasticity in mammals, this form of long-term synaptic plasticity provides a plausible mechanism for producing enduring neural changes within a part of the brain that is known to be involved in certain kinds of memories.

Mechanisms Underlying Long-Term Potentiation

The molecular underpinnings of LTP now are well understood. A key advance was the discovery that antagonists of the NMDA-type glutamate receptor prevent LTP during high-frequency stimulation of the Schaffer collaterals, but have no effect on the synaptic response evoked by low-frequency stimulation. At about the same time, the unique biophysical properties of the NMDA receptor were first appreciated and provided a critical insight into how LTP

is selectively induced by high-frequency activity. As described in Chapter 6, the NMDA receptor channel is permeable to Ca^{2+} but is blocked by Mg^{2+} at the normal resting membrane potential. The NMDA receptor is a molecular coincidence detector: The channel of this receptor opens (to induce LTP) only when glutamate is bound to the receptor, and the postsynaptic cell is depolarized to relieve the Mg^{2+} block of the channel pore. During low-frequency synaptic transmission, glutamate released by the Schaffer collaterals binds to both NMDA-type and AMPA-type glutamate receptors; however, Mg^{2+} blockade prevents current flow through the NMDA receptors, so that the EPSP is mediated entirely by the AMPA receptors (Figure 8.10, left). Because blockade of the NMDA receptor by Mg^{2+} is voltage-dependent, summation of EPSPs during high-frequency stimulation (as in Figure 8.7) leads to a prolonged depolarization that expels Mg^{2+} from the NMDA channel pore (see Figure 8.10, right). Removal of Mg^{2+} then allows Ca^{2+} to enter the dendritic spines of postsynaptic CA1 neurons.

These properties of the NMDA receptor can account for many of the characteristics of LTP. First, the requirement for strong coincident presynaptic and postsynaptic activity to induce LTP (see Figure 8.8) arises because presynaptic activity releases glutamate, while the coincident postsynaptic depolarization relieves the Mg^{2+} block of the NMDA receptor. The specificity of LTP (see Figure 8.9A) can be explained by the fact that NMDA channels will be opened only at synaptic inputs that are active and releasing glutamate, thereby confining LTP to these sites even though EPSPs generated at active synapses depolarize the postsynaptic neuron. With respect to associativity (see Figure 8.9B), a weakly stimulated input releases glutamate but cannot sufficiently depolarize the postsynaptic cell to relieve the Mg^{2+} block. If a large number of other inputs are strongly stimulated, however, they provide the “associative” depolarization

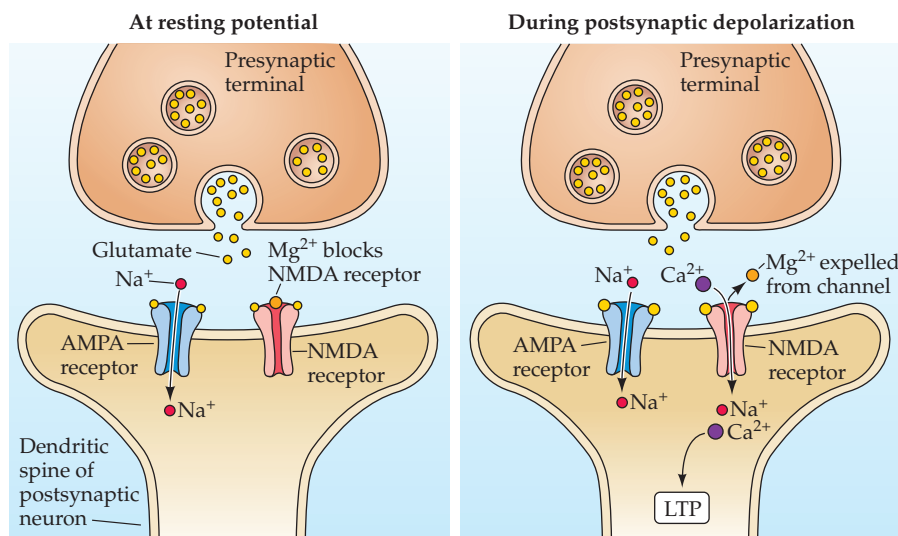


FIGURE 8.10 The NMDA receptor channel can open only during depolarization of the postsynaptic neuron from its normal resting potential. Depolarization expels Mg^{2+} from the NMDA channel, allowing current to flow into the postsynaptic cell. This leads to Ca^{2+} entry, which in turn triggers LTP. (After Nicoll et al., 1988.)

necessary to relieve the block. Thus, induction of LTP relies on activation of NMDA receptors.

Numerous observations have established that induction of LTP is due to accumulation of postsynaptic Ca^{2+} as a result of Ca^{2+} influx through NMDA receptors. Imaging studies have shown that activation of NMDA receptors increases postsynaptic Ca^{2+} levels and that these Ca^{2+} signals can be restricted to the dendritic spines of individual synapses. Furthermore, injection of Ca^{2+} chelators blocks LTP induction, whereas elevation of Ca^{2+} levels in postsynaptic neurons potentiates synaptic transmission. Thus, a rise in postsynaptic Ca^{2+} concentration serves as a second-messenger signal that induces LTP. The fact that these postsynaptic Ca^{2+} signals are highly localized (see Box 7B) can account for the input specificity of LTP (see Figure 8.9A).

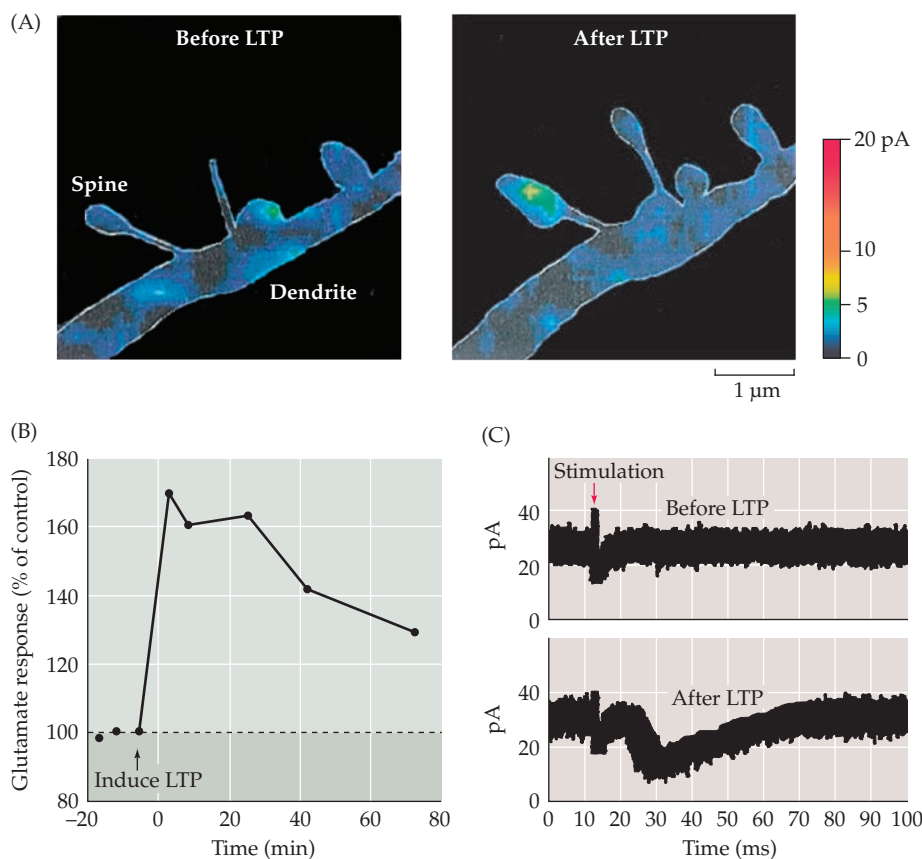
The subsequent *expression* of LTP relies on dynamic changes in AMPA receptors. Excitatory synapses can dynamically regulate their postsynaptic AMPA receptors via the same sorts of membrane trafficking processes that occur in presynaptic neurons during neurotransmitter release (see Chapter 5). LTP apparently is due to synaptotagmin-mediated insertion of AMPA receptors into the postsynaptic membrane. These AMPA receptors come from an intracellular organelle known as the recycling endosome. The resulting increase in the number of AMPA receptors

increases the response of the postsynaptic cell to released glutamate (Figure 8.11A), yielding a strengthening of synaptic transmission that can last for as long as LTP is maintained (Figure 8.11B). LTP does not affect the number of postsynaptic NMDA receptors; thus, while these receptors are crucial for induction of LTP, they do not play a major role in LTP expression. Stimulus-induced changes in AMPA receptor trafficking can even add new AMPA receptors to “silent” synapses that did not previously have postsynaptic AMPA receptors (Box 8B). At silent synapses, where synaptic activity generates no postsynaptic response at the normal resting potential, LTP adds AMPA receptors so that the synapse can produce postsynaptic responses (Figure 8.11C). Thus, the strengthening of synaptic transmission during LTP arises from an increase in the sensitivity of the postsynaptic cell to glutamate. Under some circumstances, LTP also can increase the ability of presynaptic terminals to release glutamate.

Ca^{2+} also activates complex postsynaptic signal transduction cascades that include at least two Ca^{2+} -activated protein kinases: Ca^{2+} /calmodulin-dependent protein kinase type II (CaMKII) and protein kinase C (PKC; see Chapter 7). CaMKII, which is the most abundant postsynaptic protein at Schaffer collateral synapses, seems to play an especially important role. This enzyme is activated by stimuli that induce

FIGURE 8.11 Addition of postsynaptic AMPA receptors during LTP. (A) Spatial maps of the glutamate sensitivity of a hippocampal neuron dendrite before and 120 minutes after induction of LTP.

The color scale indicates the amplitude of responses to highly localized glutamate application. LTP causes an increase in the glutamate response of a dendritic spine due to an increase in the number of AMPA receptors on the spine membrane. (B) Time course of changes in glutamate sensitivity of dendritic spines during LTP. Induction of LTP at time = 0 causes glutamate sensitivity to increase for more than 60 minutes. (C) LTP induces AMPA receptor responses at silent synapses in the hippocampus. Prior to inducing LTP, no excitatory postsynaptic currents (EPSCs) are elicited at -65 mV at this silent synapse (upper trace). After LTP induction, the same stimulus produces EPSCs that are mediated by AMPA receptors (lower trace). (A,B from Matsuzaki et al., 2004; C after Liao et al., 1995.)

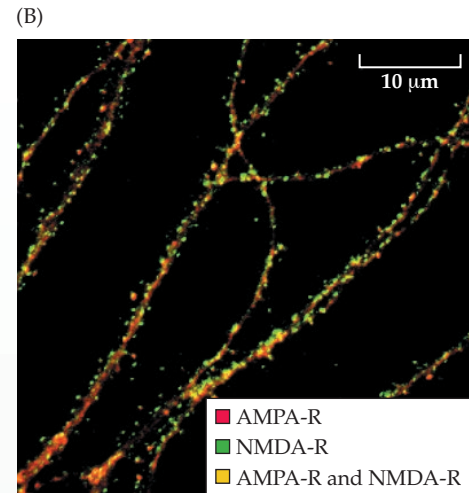
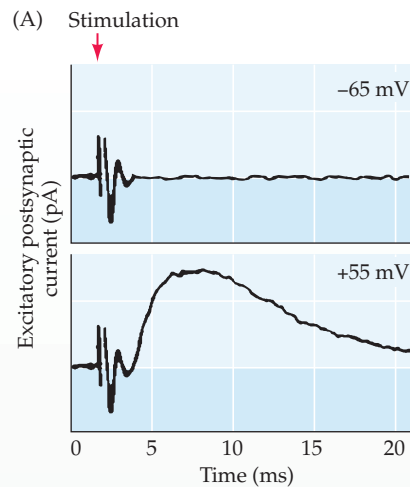


BOX 8B ■ Silent Synapses

Several observations indicate that postsynaptic glutamate receptors are dynamically regulated at excitatory synapses. Early insight into this process came from the finding that stimulation of some glutamatergic synapses generates no postsynaptic electrical signal when the postsynaptic cell is at its normal resting membrane potential (Figure A). However, once the postsynaptic cell is depolarized, these “silent synapses” can transmit robust postsynaptic electrical responses. The fact that transmission at such synapses can be turned on or off according to the postsynaptic membrane potential suggests an interesting and simple means of modifying neural circuitry.

Silent synapses are especially prevalent in development and have been found in many brain regions, including the hippocampus, cerebral cortex, and spinal cord. The silence of these synapses is evidently due to the voltage-dependent blockade of NMDA receptors by Mg^{2+} (see text and Chapter 6). At the normal resting membrane potential, presynaptic release of glutamate evokes no postsynaptic response at such synapses because their NMDA receptors are blocked by Mg^{2+} . However, depolarization of the postsynaptic neuron displaces the Mg^{2+} , allowing glutamate release to induce postsynaptic responses mediated by NMDA receptors.

Glutamate released at silent synapses evidently binds only to NMDA receptors. How, then, does glutamate release avoid activating AMPA receptors? The most likely explanation is that these synapses may have only NMDA receptors. Immunocytochemical experiments demonstrate that some excitatory synapses have only NMDA receptors (green spots in Figure B).



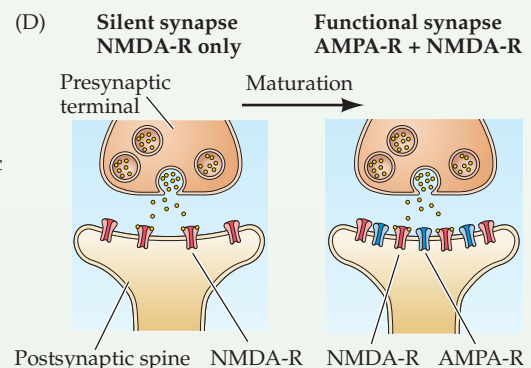
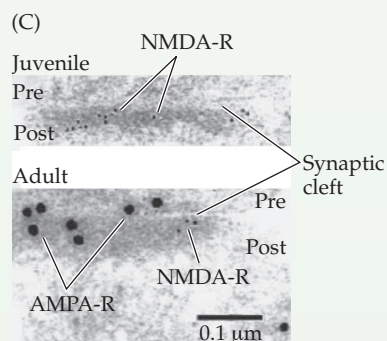
(A) Electrophysiological evidence for silent synapses. Stimulation of some axons fails to activate synapses when the postsynaptic cell is held at a negative potential (-65 mV, upper trace). However, when the postsynaptic cell is depolarized ($+55$ mV, lower trace), stimulation produces a robust response. (B) Immunofluorescent localization of NMDA receptors (green) and AMPA receptors (red) in a cultured hippocampal neuron. Many dendritic spines are positive for NMDA receptors but not AMPA receptors, indicating NMDA receptor-only synapses. (A after Liao et al., 1995; B courtesy of M. Ehlers.)

Such NMDA receptor-only synapses are particularly abundant early in postnatal development and decrease in adults (Figure C). Silencing of glutamatergic synapses has also been reported to result from the use of the recreational drug cocaine. Thus, at least some silent synapses are not a separate class of excitatory synapses that lack AMPA receptors, but rather an early stage in the ongoing maturation of the glutamatergic synapse (Figure D). Evidently, AMPA and NMDA receptors are not inextricably linked at excitatory synapses, but are targeted via

independent cellular mechanisms. Such synapse-specific glutamate receptor composition implies sophisticated mechanisms for regulating the localization of each type of receptor. Dynamic changes in the trafficking of AMPA and NMDA receptors can strengthen or weaken synaptic transmission and are important in LTP and LTD, as well as in the maturation of glutamatergic synapses.

In summary, although silent synapses have begun to whisper their secrets, much remains to be learned about their physiological importance.

(C) Electron microscopy of excitatory synapses in CA1 stratum radiatum of the hippocampus from 10-day-old (juvenile) or 5-week-old (adult) rats double-labeled for AMPA receptors and NMDA receptors. The presynaptic terminal (pre), synaptic cleft, and postsynaptic spine (post) are indicated. AMPA receptors are abundant at the adult synapse but are absent from the younger synapse. (D) Diagram of glutamatergic synapse maturation. Early in postnatal development, many excitatory synapses contain only NMDA receptors. As synapses mature, AMPA receptors are recruited. (C from Petralia et al., 1999)



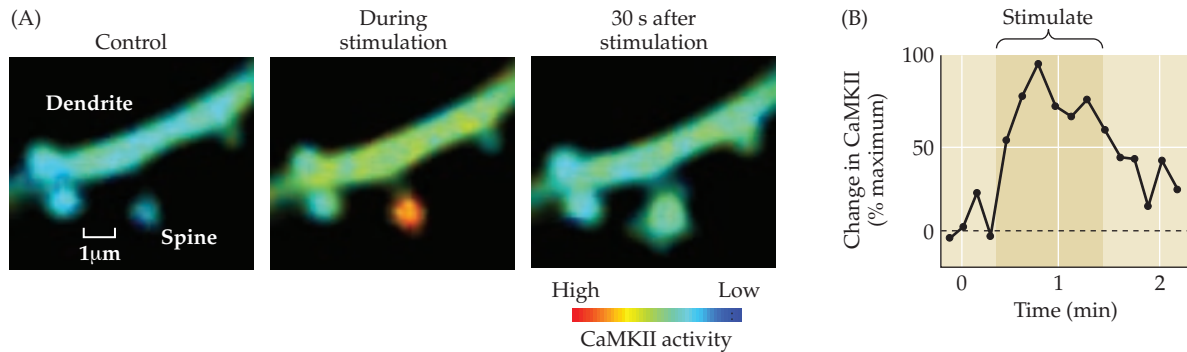


FIGURE 8.12 CaMKII activity in the dendrite of a CA1 pyramidal neuron during LTP. (A) The degree of CaMKII activation (indicated by the pseudocolor scale below) in a dendritic spine dramatically increases during stimulation that induces LTP. (B) Time course of transient changes in CaMKII activity associated with LTP. (From Lee et al., 2009.)

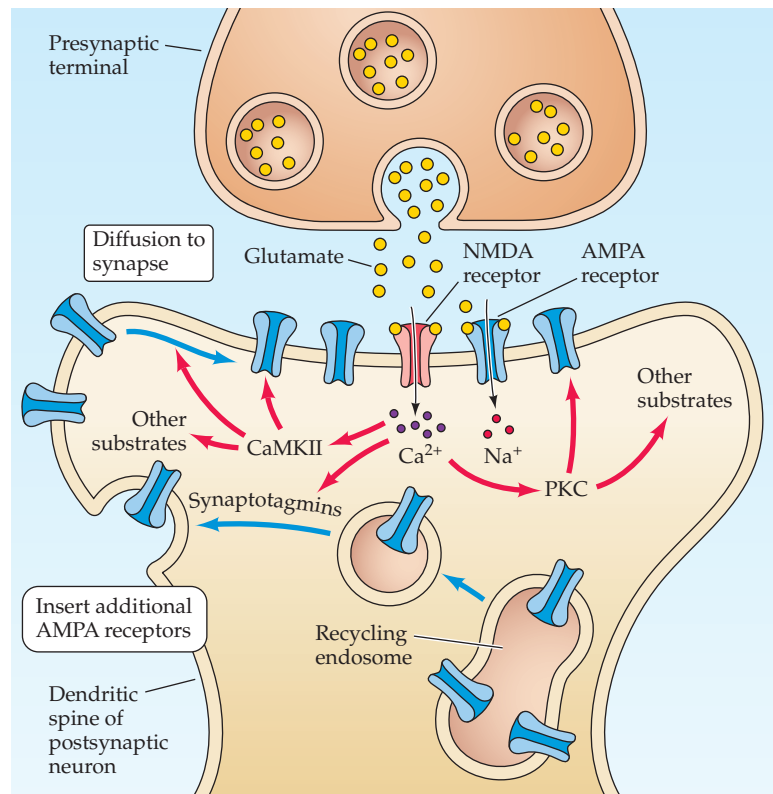
LTP (Figure 8.12), and pharmacological inhibition or genetic deletion of CaMKII prevents LTP. The autophosphorylation property of CaMKII (see Chapter 7) may also serve to prolong the duration of LTP. It is thought that CaMKII and PKC phosphorylate downstream targets, including both AMPA receptors and other targets, that collectively facilitate delivery of extrasynaptic AMPA receptors to the synapse.

In summary, the molecular signaling pathways involved in LTP at the Schaffer collateral–CA1 synapse are well understood. Ca^{2+} entering through postsynaptic NMDA receptors leads to activation of synaptotagmins and protein kinases that regulate trafficking of AMPA receptors, thereby enhancing the postsynaptic response to glutamate released from the presynaptic terminal (Figure 8.13). Still other forms of LTP are observed at other synapses and, in some cases, rely on signaling mechanisms different from those involved in LTP at the Schaffer collateral–CA1 synapse.

The scheme depicted in Figure 8.13 can account for the changes in synaptic transmission that occur over the first 1 to 2 hours after LTP is induced. However, there is also a later phase of LTP that depends on changes in gene expression and the synthesis of new proteins. The contributions of this late phase can

be observed by treating synapses with drugs that inhibit protein synthesis: Blocking protein synthesis prevents LTP measured several hours after a stimulus but does not affect LTP measured at earlier times (Figure 8.14). This late phase of LTP appears to be initiated by protein kinase A, which goes on to activate transcription factors such as CREB, which stimulate the expression of other proteins. Although most of these newly synthesized proteins have not yet been identified, they include other transcriptional regulators, protein kinases, and AMPA receptors (Figure 8.15A). How these proteins contribute to the late phase of LTP is not yet known. There is evidence that the number and size of synaptic contacts increase during LTP (Figure 8.15B,C). Thus, it is likely that some of the proteins newly synthesized during the late phase of LTP are involved in construction of new synaptic

FIGURE 8.13 Signaling mechanisms underlying LTP. During glutamate release, the NMDA receptor channel opens only if the postsynaptic cell is sufficiently depolarized. The Ca^{2+} ions that enter the cell through the channel activate postsynaptic protein kinases, such as CaMKII and PKC, that trigger a series of phosphorylation reactions. These reactions regulate trafficking of postsynaptic AMPA receptors through recycling endosomes, leading to insertion of new AMPA receptors into the postsynaptic spine. Subsequent diffusion of AMPA receptors to the subsynaptic region yields an increase in the spine's sensitivity to glutamate, which causes LTP.



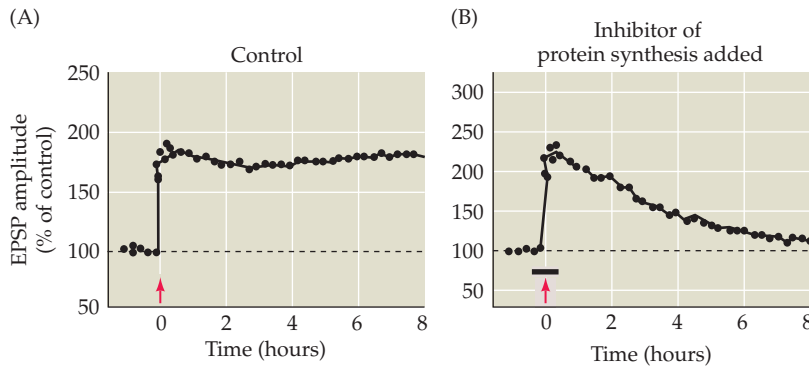


FIGURE 8.14 Role of protein synthesis in maintaining LTP. (A) Repetitive high-frequency stimulation (arrow) induces LTP that persists for many hours. (B) Treatment with anisomycin (at bar), an inhibitor of protein synthesis, causes LTP to decay within a few hours after the high-frequency stimulation (arrow). (After Frey and Morris, 1997.)

contacts that serve to make LTP essentially permanent (as in Figure 8.7D).

In conclusion, it appears that LTP in the mammalian hippocampus has many parallels to the long-term changes in synaptic transmission underlying behavioral sensitization in *Aplysia*. Both consist of an early, transient phase that relies on protein kinases to produce post-translational changes in membrane ion channels, and both have later, long-lasting phases that require changes in gene expression

mediated by CREB. Both forms of long-term synaptic plasticity are likely to be involved in long-term storage of information, although the role of LTP in memory storage in the hippocampus is not firmly established.

Mechanisms Underlying Long-Term Depression

If synapses simply continued to increase in strength as a result of long-term potentiation, eventually they would reach some level of maximum efficacy, making it difficult to encode new information. Thus, to make synaptic strengthening useful, other processes must selectively weaken specific sets of synapses, and LTD is such a process. In the late 1970s, LTD was found to occur at the synapses between the Schaffer collaterals and the CA1 pyramidal cells in the hippocampus. Whereas LTP at these synapses requires brief, high-frequency stimulation, LTD occurs when the Schaffer collaterals are stimulated at a low rate—about 1 Hz—for long periods (10–15 minutes). This pattern of activity depresses the EPSP for several hours and, like LTP, is specific to the activated synapses (Figure 8.16A,B). Moreover, LTD can erase the increase in EPSP size due to LTP, and conversely, LTP can erase the decrease in EPSP size due to LTD. This complementarity suggests that

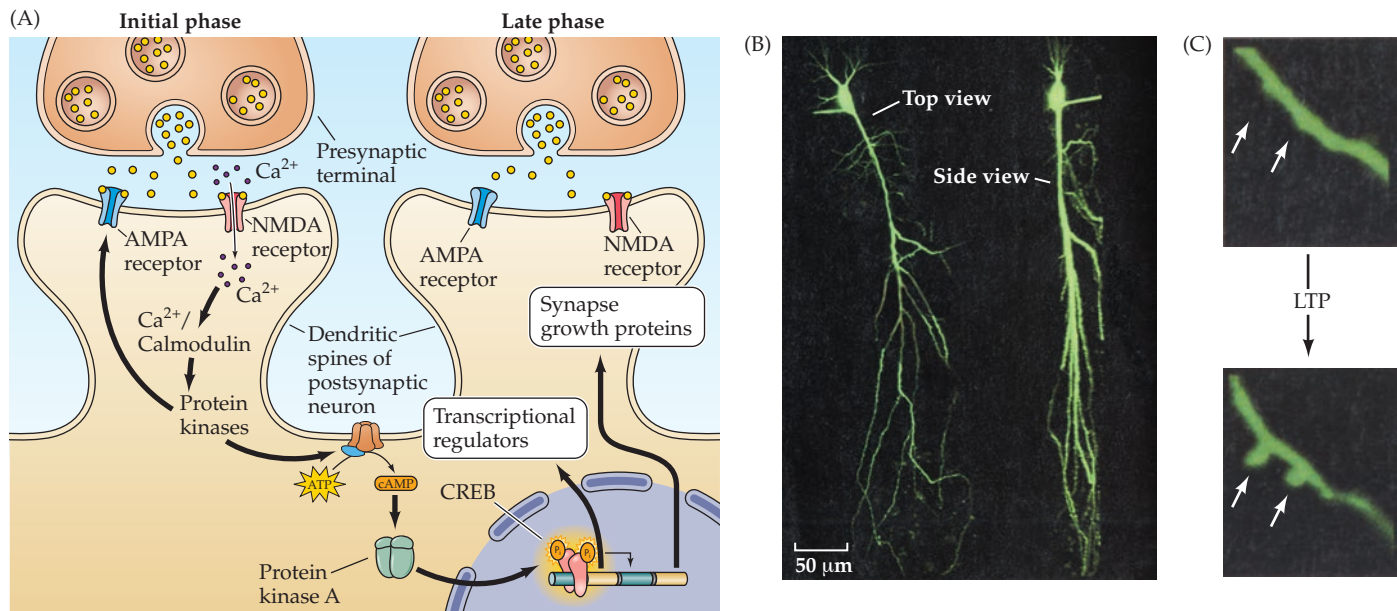


FIGURE 8.15 Mechanisms responsible for long-lasting changes in synaptic transmission during LTP. (A) The late component of LTP is the result of PKA activating the transcriptional regulator CREB, which turns on expression of several genes that produce long-lasting changes in PKA activity and synapse structure. (B,C) Structural changes associated with LTP in the hippocampus. (B) The dendrites of a CA1 pyramidal

neuron were visualized by filling the cell with a fluorescent dye. (C) New dendritic spines (white arrows) can be observed approximately 1 hour after a stimulus that induces LTP. The presence of novel spines raises the possibility that LTP may arise, in part, from formation of new synapses. (A after Squire and Kandel, 1999; B and C after Engert and Bonhoeffer, 1999.)

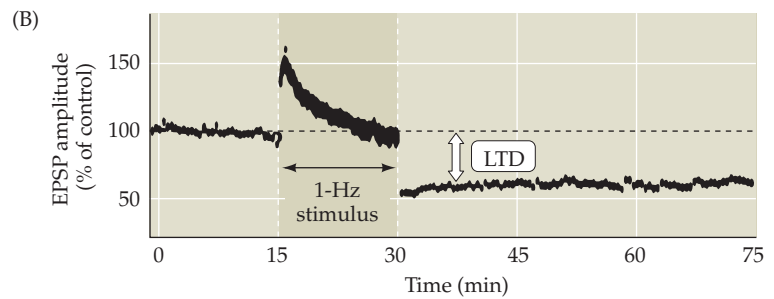
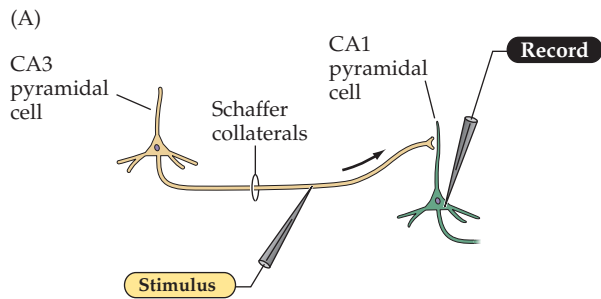
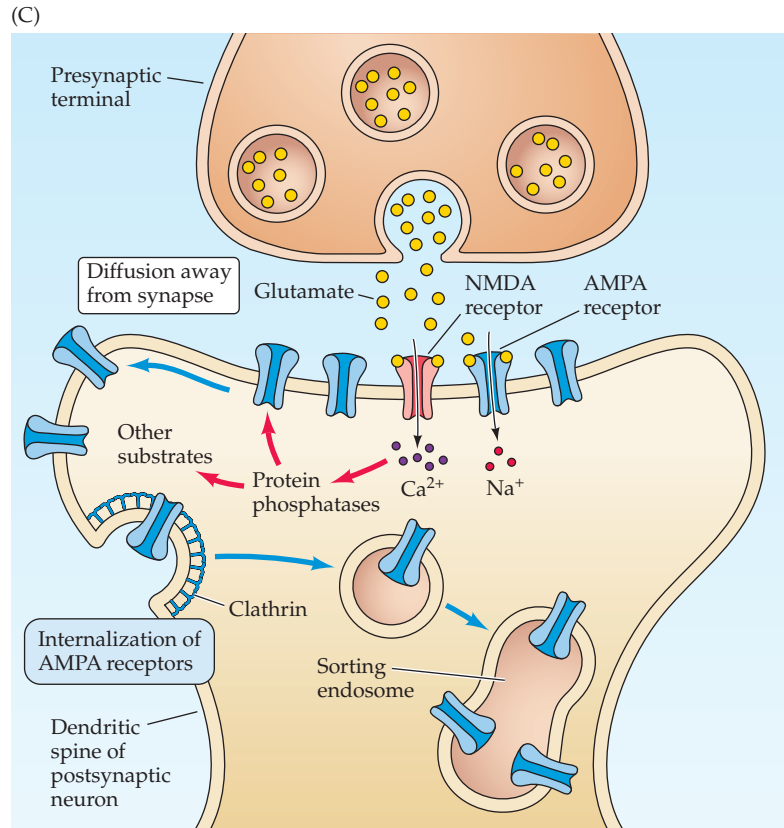


FIGURE 8.16 Long-term synaptic depression in the hippocampus. (A) Electrophysiological procedures used to monitor transmission at the Schaffer collateral synapses on CA1 pyramidal neurons. (B) Low-frequency stimulation (one per second) of the Schaffer collateral axons causes a long-lasting depression of synaptic transmission. (C) Mechanisms underlying LTD. A low-amplitude rise in Ca^{2+} concentration in the postsynaptic CA1 neuron activates postsynaptic protein phosphatases, which cause internalization of postsynaptic AMPA receptors, thereby decreasing the sensitivity to glutamate released from the Schaffer collateral terminals. (B after Mulkey et al., 1993.)

LTD and LTP reversibly affect synaptic efficiency by acting at a common site.

LTP and LTD at the Schaffer collateral–CA1 synapses actually share several key elements. Both require activation of NMDA-type glutamate receptors and the resulting entry of Ca^{2+} into the postsynaptic cell. The major determinant of whether LTP or LTD arises appears to be the nature of the Ca^{2+} signal in the postsynaptic cell: Small and slow rises in Ca^{2+} lead to depression, whereas large and fast increases in Ca^{2+} trigger potentiation. As noted above, LTP is at least partially due to activation of protein kinases, which phosphorylate their target proteins. LTD, in contrast, appears to result from activation of phosphatases, specifically PP1 and PP2B (calcineurin), a Ca^{2+} -dependent phosphatase (see Chapter 7). Evidence in support of this idea is that inhibitors of these phosphatases prevent LTD but do not block LTP. The different effects of Ca^{2+} during LTD and LTP may arise from the selective activation of protein phosphatases and kinases by the different types of postsynaptic Ca^{2+} signals occurring during these two forms of synaptic plasticity. Although the phosphatase substrates important for LTD have not yet been identified, it is possible that LTP and LTD phosphorylate and dephosphorylate the same set of regulatory proteins to control the efficacy of transmission at the Schaffer collateral–CA1 synapse. Just as LTP at this synapse is associated with insertion of AMPA receptors, LTD is often associated with a loss of synaptic AMPA receptors. This loss probably arises from internalization of AMPA receptors into sorting endosomes in the postsynaptic cell (Figure 8.16C), due to the same clathrin-dependent



endocytosis mechanisms important for synaptic vesicle recycling in the presynaptic terminal (see Chapter 5). As is also the case for LTP, there is a late phase of LTD that requires synthesis of new proteins.

A quite different form of LTD is observed in the cerebellum. LTD of synaptic inputs onto cerebellar Purkinje cells was first described by Masao Ito and Masanobu Kano in Japan in the early 1980s. Purkinje neurons in the cerebellum receive two distinct types of excitatory input: climbing fibers and parallel fibers (Figure 8.17A; see Chapter 19). LTD reduces the strength of transmission at the parallel fiber synapse (Figure 8.17B) and subsequently was found to depress transmission at the climbing fiber synapse as well. This form of LTD has been implicated in the motor learning that mediates the coordination, acquisition, and storage of complex movements in the cerebellum. Although the role of LTD in cerebellar motor learning remains controversial, it

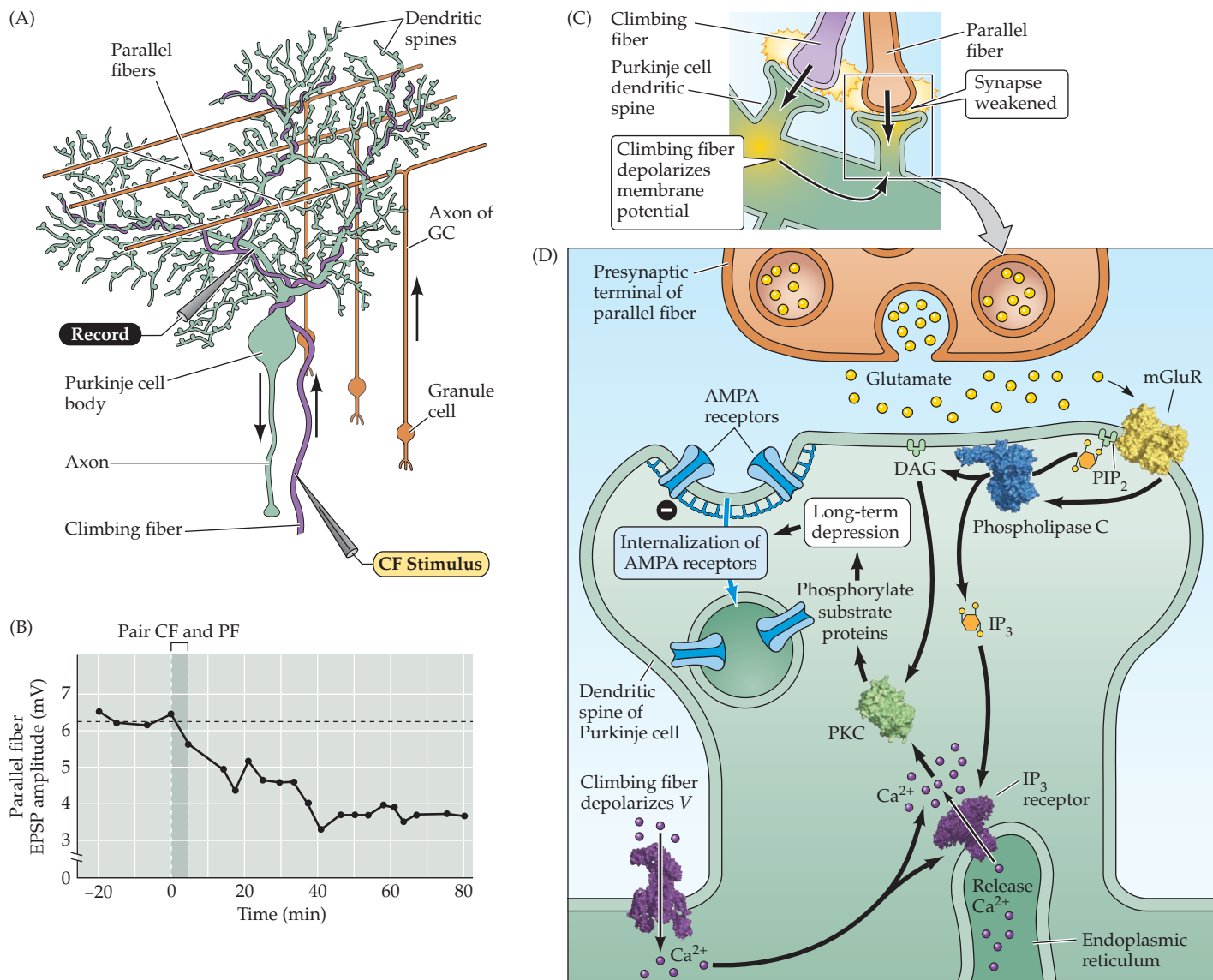


FIGURE 8.17 Long-term synaptic depression in the cerebellum. (A) Experimental arrangement. Synaptic responses were recorded from Purkinje cells following stimulation of parallel fibers and climbing fibers. (B) Pairing stimulation of climbing fibers (CF) and parallel fibers (PF) causes LTD that reduces the parallel fiber EPSP. (C) LTD requires depolarization of the Purkinje cell, produced by climbing fiber activation, as well as signals generated by active parallel fiber synapses. (D) Mechanism underlying cerebellar LTD. Glutamate released by parallel fibers

activates both AMPA receptors and metabotropic glutamate receptors (mGluRs). The activated mGluRs produce two second messengers, DAG and IP₃, which interact with Ca²⁺ that enters when climbing fiber activity opens voltage-gated Ca²⁺ channels. The resultant release of Ca²⁺ from the endoplasmic reticulum leads to a further rise in intracellular Ca²⁺ concentration and the activation of PKC, which triggers clathrin-dependent internalization of postsynaptic AMPA receptors, weakening the parallel fiber synapse. (B after Sakurai, 1987.)

has nonetheless been a useful model system for understanding the cellular mechanisms of long-term synaptic plasticity.

Like many forms of motor learning, cerebellar LTD is associative because it occurs only when climbing fibers and parallel fibers are activated at the same time (Figure 8.17C). In this case, associativity arises from the combined actions of two distinct intracellular signal transduction pathways that are activated in the postsynaptic Purkinje cell in response to activity of the climbing fiber and parallel fiber synapses. In the first pathway, glutamate released from the parallel fiber terminals activates two types of receptors, the AMPA-type and metabotropic glutamate receptors (see

Chapter 6). Glutamate binding to the AMPA receptor results in mild membrane depolarization, whereas binding to the metabotropic receptor produces the second messengers inositol triphosphate (IP₃) and diacylglycerol (DAG) (see Chapter 7). Glutamate released by climbing fibers also activates AMPA receptors, which strongly depolarizes the Purkinje cell membrane potential and initiates a second signal transduction pathway: an influx of Ca²⁺ through voltage-gated channels and a subsequent increase in intracellular Ca²⁺ concentration. These two second messengers—IP₃ and Ca²⁺—cause an amplified rise in intracellular Ca²⁺ concentration by acting together on IP₃ receptors. This

triggers release of Ca^{2+} from intracellular stores and leads to synergistic activation of PKC by Ca^{2+} and DAG (Figure 8.17D). Thus, the associative property of cerebellar LTD appears to arise from both IP_3 receptors and PKC serving as coincidence detectors. Other protein kinases also act to sustain the activation of PKC beyond the time that IP_3 and Ca^{2+} concentrations are elevated.

PKC phosphorylates several downstream substrate proteins, including AMPA receptors. The main consequence of PKC-dependent phosphorylation is to cause an internalization of AMPA receptors via clathrin-dependent endocytosis (see Figure 8.17D). This loss of AMPA receptors decreases the response of the postsynaptic Purkinje cell to glutamate released from the presynaptic terminals of the parallel fibers. Thus, in contrast to LTD in the hippocampus, cerebellar LTD requires the activity of protein kinases, rather than phosphatases, and does not involve Ca^{2+} entry through NMDA receptors. However, the net effect is the same in both cases: Internalization of postsynaptic AMPA receptors is a common mechanism for decreased efficacy of both hippocampal and cerebellar synapses during LTD. As is the case for LTP at the hippocampal Schaffer collateral synapse, as well as for long-term synaptic plasticity in *Aplysia*, CREB appears to be required for a late phase of cerebellar LTD. It is not yet known which proteins are synthesized as a consequence of CRE activation.

Spike Timing-Dependent Plasticity

The preceding sections show that LTP and LTD are preferentially initiated by different rates of repetitive synaptic activity, with LTP requiring high-frequency activity and LTD being induced by low-frequency activity. However, the precise temporal relationship between activity in the pre- and postsynaptic cells can also be an important determinant of the amount and direction of long-term synaptic plasticity. At a given (low) frequency of synaptic activity, LTD will occur if presynaptic activity is preceded by a postsynaptic action potential, while LTP will occur if the postsynaptic action potential follows presynaptic activity (Figure 8.18A,B). The relationship between the time interval and the magnitude of the synaptic change is a very sensitive function

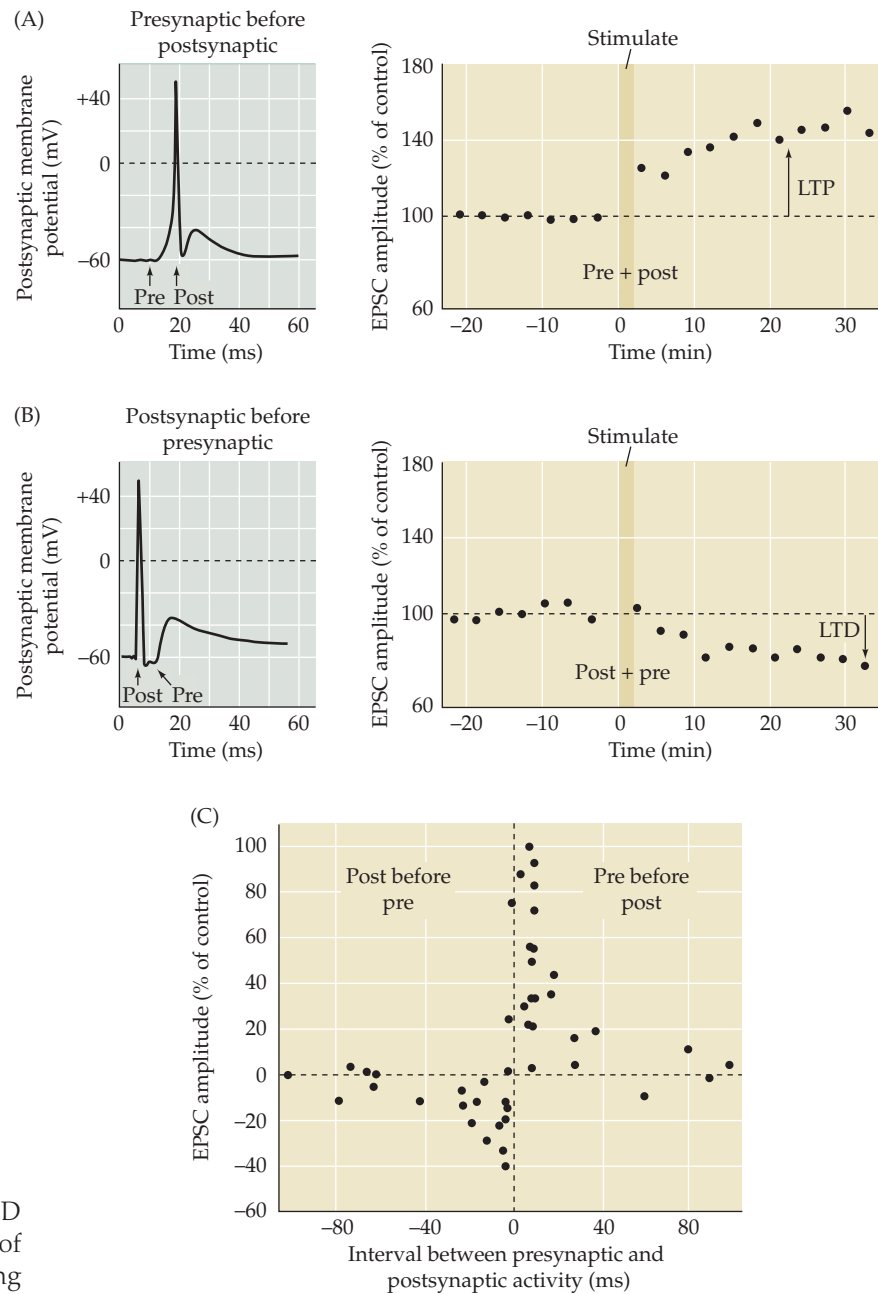


FIGURE 8.18 Spike timing-dependent synaptic plasticity in cultured hippocampal neurons. (A) Left: Stimulating a presynaptic neuron (Pre) causes an EPSP in the postsynaptic neuron; applying a subsequent stimulus to the postsynaptic neuron (Post) causes an action potential that is superimposed on the EPSP. Right: Repetitive application of this stimulus paradigm causes LTP of the EPSP. (B) Reversing the order of stimulation, so that the postsynaptic neuron is excited before the presynaptic neuron, causes LTD of the EPSP. (C) Complex dependence of STDP on the interval between presynaptic activity and postsynaptic activity. If the presynaptic neuron is activated 40 milliseconds or less before the postsynaptic neuron, then LTP occurs. Conversely, if the postsynaptic neuron is activated 40 milliseconds or less before the presynaptic neuron, LTD occurs. If the interval between the two events is longer than 40 milliseconds, no STDP is observed. (After Bi and Poo, 1998.)

of the time interval, with no changes observed if the presynaptic and postsynaptic activities are separated by 100 milliseconds or longer (Figure 8.18C).

Because precise timing of presynaptic and postsynaptic activity determines the polarity of these forms of long-lasting synaptic plasticity, they are called **spike timing-dependent plasticity (STDP)**. Although the mechanisms involved are not yet well understood, it appears that the properties of STDP arise from timing-dependent differences in postsynaptic Ca^{2+} signals. Specifically, if a postsynaptic action potential occurs *after* presynaptic activity, the resulting depolarization will relieve the block of NMDA receptors by Mg^{2+} and cause a relatively large amount of Ca^{2+} influx through postsynaptic NMDA receptors, yielding LTP. In contrast, if the postsynaptic action potential occurs *before* the presynaptic action potential, then the depolarization associated with the postsynaptic action potential will subside by the time an EPSP occurs. This sequence of events will reduce the amount of Ca^{2+} entry through the NMDA receptors, leading to LTD. It has been postulated that other signals, such as endocannabinoids (see Chapter 6), may also be required for LTD induction during STDP.

The requirement for a precise temporal relationship between presynaptic and postsynaptic activity means that STDP can perform several novel types of neuronal computation. STDP can provide a means of encoding information about causality. For example, if a synapse generates a suprathreshold EPSP, the resulting postsynaptic action potential

would rapidly follow presynaptic activity, and the resulting LTP would encode the fact that the postsynaptic action potential resulted from the activity of that synapse. STDP could also serve as a mechanism for competition between synaptic inputs: Stronger inputs would be more likely to produce suprathreshold EPSPs and be reinforced by the resulting LTP, whereas weaker inputs would not generate postsynaptic action potentials that were correlated with presynaptic activity. There is evidence that STDP is important for neural circuit function, such as determining orientation preference in the visual system (see Chapter 12).

In summary, activity-dependent forms of synaptic plasticity cause changes in synaptic transmission that modify the functional connections within and among neural circuits. These changes in the efficacy and local geometry of synaptic connectivity can provide a basis for learning, memory, and other forms of brain plasticity. Activity-dependent changes in synaptic transmission may also be involved in some pathologies. Abnormal patterns of neuronal activity, such as those that occur in epilepsy, can stimulate abnormal changes in synaptic connections that may further increase the frequency and severity of seizures (Clinical Applications). Despite the substantial advances in understanding the cellular and molecular bases of some forms of plasticity, the means by which selective changes of synaptic strength encode memories or other complex behavioral modifications in the mammalian brain are simply not known.

CLINICAL APPLICATIONS

Epilepsy: The Effect of Pathological Activity on Neural Circuitry

Epilepsy is a brain disorder characterized by periodic and unpredictable seizures. The behavioral manifestations of epileptic seizures in human patients range from mild twitching of an extremity to loss of consciousness and uncontrollable convulsions. Although many highly accomplished people have suffered from epilepsy (Alexander the Great, Julius Caesar, Napoleon, Dostoyevsky, and van Gogh, to name a few), seizures of sufficient intensity and frequency obviously can interfere with many aspects of daily life. Moreover, uncontrolled convulsions can lead to excitotoxicity. As much as 1% of the population is afflicted, making epilepsy one of the most common neurological problems.

Epilepsy is mediated by the rhythmic firing of large groups of neurons. It seems likely that this atypical neuronal

activity generates plastic changes in cortical circuitry that are critical to the pathogenesis of the disease. The importance of neuronal plasticity in epilepsy is indicated most clearly by an animal model of seizure production called *kindling*. To induce kindling, a stimulating electrode is implanted in the brain, often in the amygdala (a component of the limbic system that makes and receives connections with the cortex, thalamus, and other limbic structures, including the hippocampus; see Chapter 31). At the beginning of such an experiment, weak electrical stimulation, in the form of a low-amplitude train of electrical pulses, has no discernible effect on behavior or on the pattern of electrical activity in the brain. As this weak stimulation is repeated once a day for several weeks, it begins to produce behavioral and electrical indi-

cations of seizures. By the end of the experiment, the same weak stimulus that initially had no effect now causes full-blown seizures. This phenomenon is essentially permanent; even after an interval of a year, the same weak stimulus will again trigger a seizure. Thus, repetitive weak activation produces long-lasting changes in the excitability of the brain that time cannot reverse. The word *kindling* is therefore quite appropriate—a single match can start a devastating fire.

The changes in the electrical patterns of brain activity detected in kindled animals resemble those in human epilepsy. Modern thinking about the causes (and possible cures) of epilepsy has focused on where seizures originate and

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CLINICAL APPLICATIONS ■ (continued)

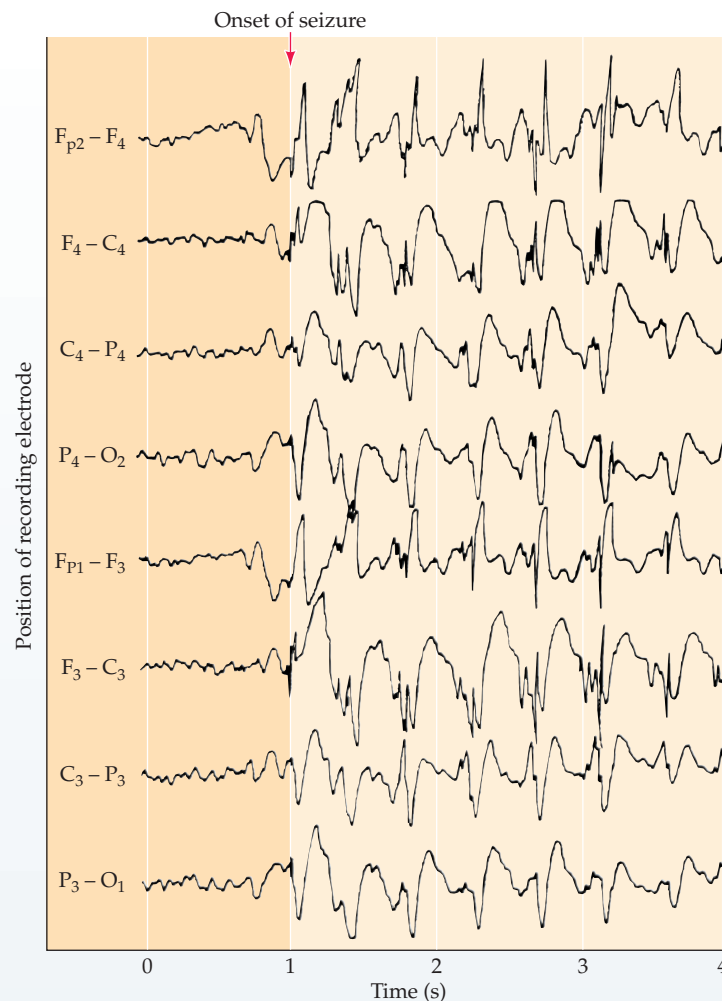
the mechanisms that make the affected region hyperexcitable. Most of the evidence suggests that atypical activity in small areas of the cerebral cortex (called foci) provide the triggers for a seizure that then spreads to other synaptically connected regions. For example, a seizure originating in the thumb area of the right motor cortex will first be evident as uncontrolled movement of the left thumb that subsequently extends to other more proximal limb muscles, whereas a seizure originating in the visual association cortex of the right hemisphere may be heralded by complex hallucinations in the left visual field. The behavioral manifestations of seizures therefore provide important clues for the neurologist seeking to pinpoint the atypical region of cerebral cortex.

Epileptic seizures can be caused by a variety of acquired or congenital factors, including cortical damage from trauma, stroke, tumors, congenital cortical dysgenesis (failure of the cortex to grow properly), and congenital vascular malformations. One rare form of epilepsy, Rasmussen's encephalitis, is an autoimmune disease that arises when the immune system attacks the brain, using both humoral agents (i.e., antibodies) and cellular agents (lymphocytes and macrophages) that can destroy neurons. Some forms of epilepsy are heritable, and more than a dozen distinct genes have been demonstrated to underlie unusual types of epilepsy (see Clinical Applications, Chapter 4). Most forms of familial epilepsy (such as juvenile myoclonic epilepsy and petit mal epilepsy) are caused by the simultaneous inheritance of more than one mutant gene.

No effective prevention or cure exists for epilepsy. Pharmacological therapies that successfully inhibit seizures are based on two general strategies. One approach is to enhance the function of inhibitory synapses that use the neurotransmitter GABA; the other is to limit action potential firing by acting on voltage-gated Na^+ channels. Commonly used antiseizure medications in-

clude carbamazepine, phenobarbital, phenytoin (Dilantin), and valproic acid. These agents, which must be taken daily, successfully inhibit seizures in 60% to 70% of patients. In a small fraction of patients, the epileptogenic region can be surgically excised. In extreme cases, physicians resort to cutting the corpus callosum to prevent the spread of sei-

zures (most of the "split-brain" patients described in Chapter 33 suffered from intractable epilepsy). One of the major reasons for controlling epileptic activity is to prevent the more permanent plastic changes that would ensue as a consequence of atypical and excessive neural activity.



Electroencephalogram (EEG) recorded during a seizure (at arrow). The traces show atypical rhythmic activity that reflects the synchronous firing of large numbers of cortical neurons. This pattern of activity persisted for much longer than the 3 seconds shown. Designations at left are various positions of electrodes on the head. (After Dyro, 1989.)