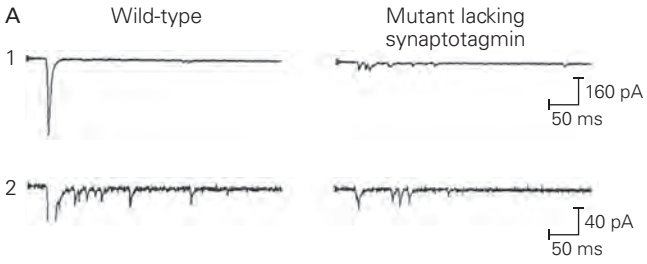
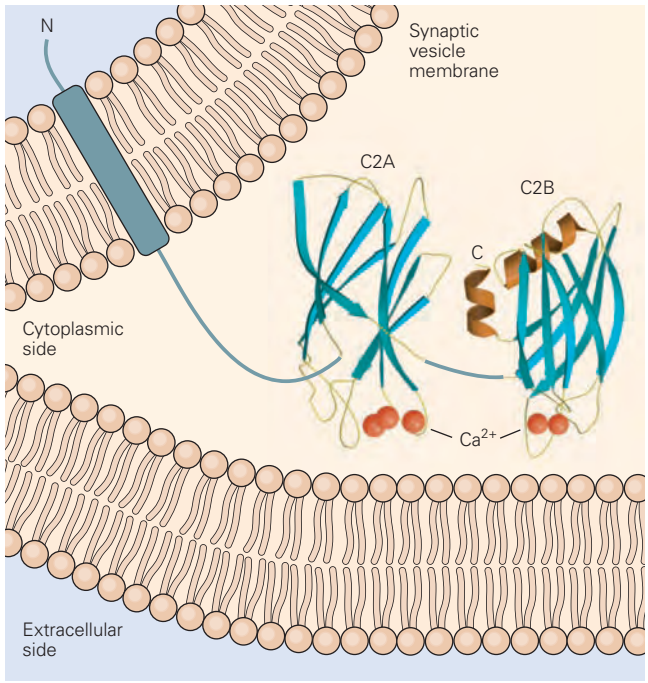
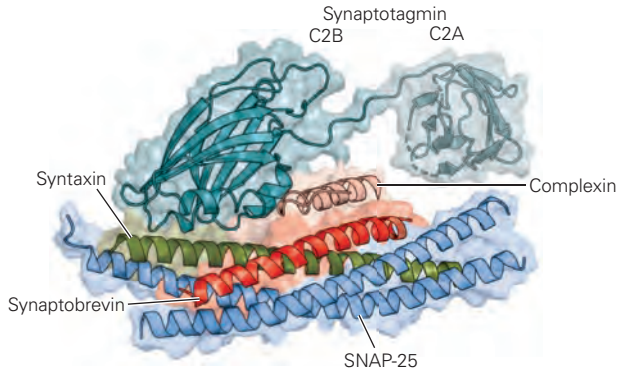
A



B1 Calcium-bound synaptotagmin

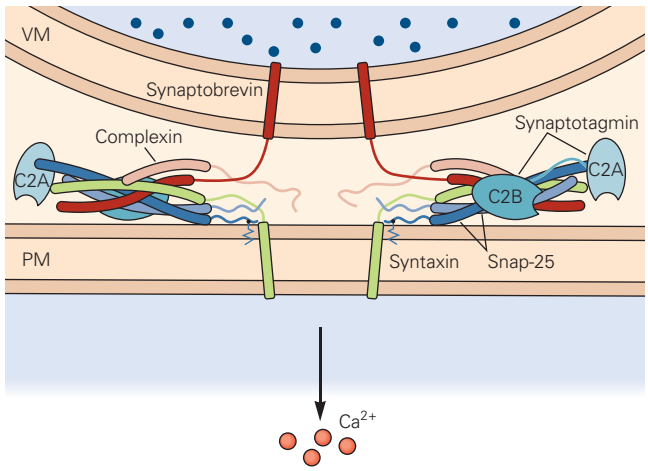


B2 Synaptotagmin/SNARE complex

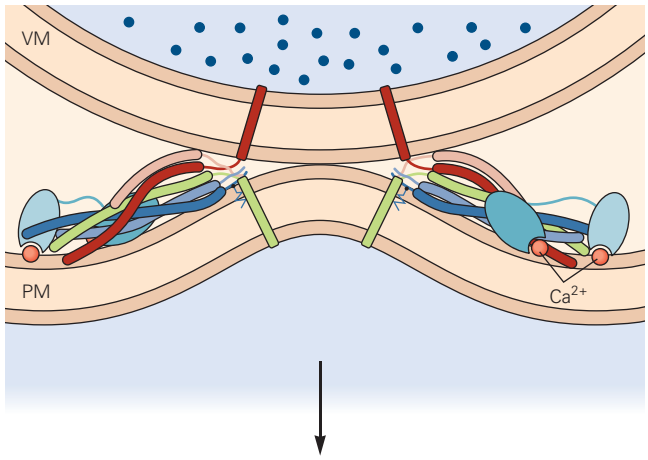


C

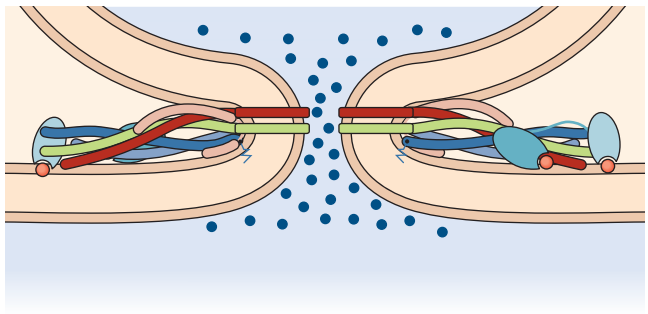
Locked



Unlocking and triggering



Fusion pore formation



exocytosis at the active zone. Other types of exocytosis, such as that which occurs in the adrenal medulla, do not require a specialized domain of the plasma membrane. The active zone is thought to coordinate and regulate the docking and priming of synaptic vesicles to enable the speed and tight regulation of release. This is accomplished through a conserved set of proteins that form one large macromolecular structure at active zones.

An exquisitely detailed view of the active zone at the frog neuromuscular junction was obtained by Jack MacMahan using a powerful ultrastructural technique called electron microscopic tomography. This technique has shown how synaptic vesicles are tethered to the membrane by a series of distinctive structural entities, termed *ribs* and *beams*, that attach to defined sites on the vesicles and to particles (*pegs*) in the presynaptic membrane that may correspond to voltage-gated Ca^{2+} channels (Figure 15–14).

A key goal in understanding how the various synaptic vesicle and active zone proteins are coordinated during exocytosis is to match up the various proteins that have been identified with elements of this electron microscopic structure. Several cytoplasmic proteins have been identified that are thought to be components of a structural matrix at the active zone. These include three large cytoplasmic multidomain proteins, *Munc13* (not related to the Munc18 protein discussed earlier), *RIM*, and *RIM-binding proteins* (RIM-BPs), which form a tight complex with each other and may comprise part of the ribs and beams. The binding of synaptic vesicles to RIM and Munc13 is essential for priming the vesicles for exocytosis. Phosphorylation of RIM by cAMP-dependent protein kinase is implicated in the enhancement of transmitter release associated with certain forms of long-term synaptic plasticity that may contribute to learning and memory. As we will see later, regulation of Munc13 by second messengers is involved in shorter-term forms of synaptic plasticity.

RIM binds the synaptic vesicle proteins *Rab3* and *Rab27*, members of the family of low-molecular-weight guanosine triphosphatases (GTPases). Rab3 and Rab27 proteins transiently associate with synaptic vesicles as a GTP-bound Rab3 complex (Figure 15–11B). The binding of RIM to Rab3 or Rab27 is thought to tether synaptic vesicles to the active zone during the vesicle cycle prior to SNARE-complex assembly. Moreover, RIM and RIM-BP together mediate the recruitment of Ca^{2+} channels to the active zone, allowing tight coupling of Ca^{2+} influx to vesicle release. This general machinery is conserved through evolution and is present in invertebrates, although with modifications.

At the *Drosophila* neuromuscular junction, Sigrist and colleagues identified another protein, Bruchpilot, as a major component of the electron-dense active zone "T-bar" structure; Bruchpilot is associated with the fly homolog of RIM-binding protein, which also serves to recruit Ca^{2+} channels to active zones in *Drosophila*. As coordinators of both presynaptic Ca^{2+} channels and synaptic vesicles, these proteins act as essential regulators of presynaptic release in the fly. In *Caenorhabditis elegans*, RIM plays a central role for the same processes.

Figure 15–13 (Opposite) Synaptotagmin mediates Ca^{2+}-dependent transmitter release by forming a protein complex that favors vesicle fusion.

A. Fast Ca^{2+}-triggered transmitter release is absent in mutant mice lacking synaptotagmin-1. Recordings show excitatory postsynaptic currents evoked in vitro by stimulation of cultured hippocampal neurons from wild-type mice and from mutant mice in which synaptotagmin-1 has been deleted by homologous recombination (**1**). Neurons from wild-type mice show large, fast excitatory postsynaptic currents evoked by presynaptic action potentials, reflecting the fact that synaptic transmission is dominated by the rapid synchronous release of transmitter from a large number of synaptic vesicles. In the **bottom trace** (**2**), where the synaptic current is shown at a highly expanded scale, one can see that a small, prolonged phase of asynchronous release of transmitter follows the fast phase of synchronous release. During this slow phase, there is a prolonged increase in frequency of individual quantal responses. In neurons from a mutant mouse, a presynaptic action potential triggers only the slow asynchronous phase of release; the rapid synchronous phase has been abolished. (Reproduced, with permission, from Geppert et al., 1994).

B. The X-ray crystal structure synaptotagmin. **B1.** A ribbon diagram shows that the C2A domain binds three Ca^{2+} ions and the C2B domain two Ca^{2+} ions. The **blue arrows** show β-strands. There are two short α-helixes (**orange**) at the C-terminus of the C2B domain. The structures of the other regions of synaptotagmin have not yet been determined and are drawn here for illustrative purposes. The membrane and structures are drawn to scale. (Adapted, with permission, from Fernandez et al., 2001). **B2.** The X-ray crystal structure of synaptotagmin (**light blue**) bound to the SNARE complex (synaptobrevin, syntaxin and SNAP-25) and complexin. The transmembrane domain of synaptotagmin is not shown. (Adapted, with permission, from Zhou et al, 2017.)

C. Zippering of the synaptotagmin-complexin-SNARE complex mediates vesicle fusion. **Top**, in the absence of Ca^{2+}, the α-helixes of the SNARE complex and complexin, with the bound synaptotagmin, are only partially zippered. **Middle**, binding of Ca^{2+} to the C2A and C2B domains of synaptotagmin allows them to interact with the plasma membrane, applying force to bring the vesicle and plasma membranes closer together. **Bottom**, synaptotagmin-mediated proximity and the final zippering of the complexin-SNARE-synaptotagmin complex triggers membrane fusion. (Adapted, with permission, from Zhou et al., 2017.)

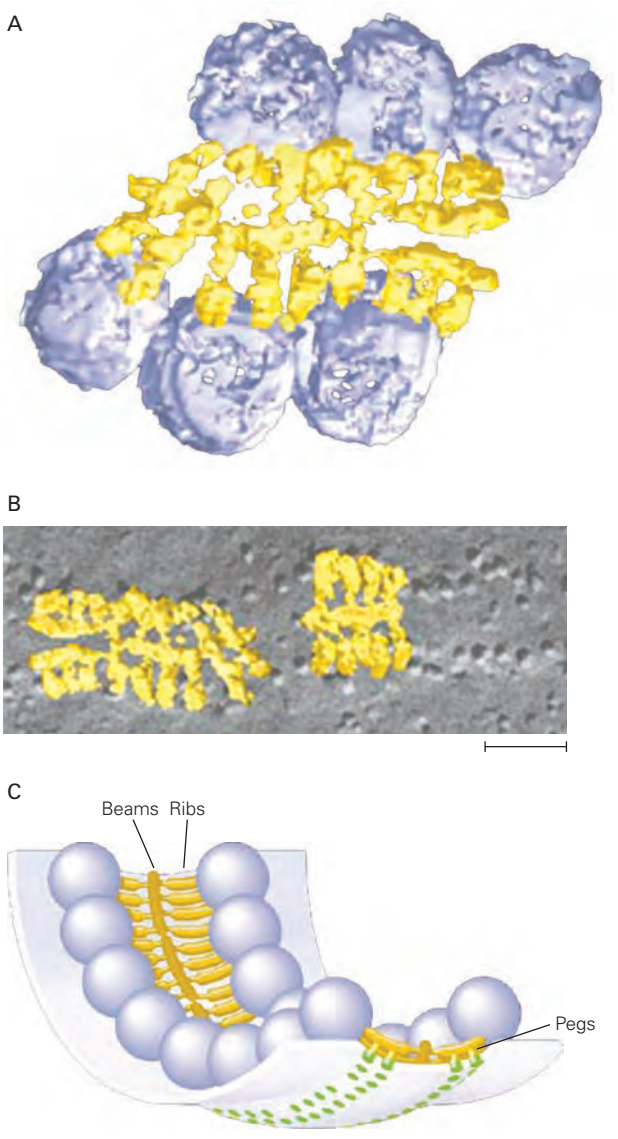


Figure 15–14 Synaptic vesicles at the active zone. The images are obtained from electron microscopic tomography. (Reproduced, with permission, from Harlow et al. 2001. Copyright © 2001 Springer Nature.)

A. Vesicles are tethered to filamentous proteins of the active zone. Three distinct filamentous structures are resolved: pegs, ribs, and beams. Ribs protruding from the vesicles are attached to long horizontal beams, which are anchored to the membrane by vertical pegs.

B. Ribs and beams superimposed on a freeze fracture view of intramembranous particles at the active zone show how the ribs are aligned with the particles, some of which are presumed to be voltage-gated Ca^{2+} channels. Scale bar = 100 nm.

C. A model for the structure of the active zone shows the relation between synaptic vesicles, pegs, ribs, and beams.

Modulation of Transmitter Release Underlies Synaptic Plasticity

The effectiveness of chemical synapses can be modulated dramatically and rapidly—by several-fold in a matter of seconds—and this change can be maintained for seconds, to hours, or even days or longer, a property called *synaptic plasticity.*

Synaptic strength can be modified presynaptically, by altering the release of neurotransmitter, postsynaptically, by modulating the response to transmitter (as discussed in Chapter 13), or both. Long-term changes in presynaptic and postsynaptic mechanisms are crucial for the refinement of synaptic connections during development (Chapter 49) and for storing information during learning and memory (Chapters 53 and 54). Here, we focus on how synaptic strength can be changed through modulation of the amount of transmitter released. In principle, changes in transmitter release can be mediated by two different mechanisms: changes in Ca^{2+} influx or changes in the amount of transmitter released in response to a given Ca^{2+} concentration. As we will see later, both types of mechanisms contribute to different forms of plasticity.

Synaptic strength is often altered by the pattern of activity of the presynaptic neuron. Trains of action potentials produce successively larger postsynaptic currents at some synapses and successively smaller currents in others (Figure 15–15A). A decrease in the size of the postsynaptic response to repeated stimulation is referred to as *synaptic depression* (Figure 15–15A, upper); the opposite, enhancement of transmission with repeated stimulation, is called *synaptic facilitation* or *potentiation* (Figure 15–15A, lower, 15–15E). Various synapses exhibit these disparate forms of *short-term synaptic plasticity*—sometimes overlapping and sometimes with one predominating—resulting in characteristic patterns of short-term dynamics in individual synapse types (Figure 15–15A).

Whether a synapse facilitates or depresses often is determined by the probability of release in response to the first action potential of a train. Synapses with an initial high probability of release normally undergo depression because the high rate of release transiently depletes docked vesicles at the active zone. Synapses with an initial low probability of release undergo synaptic facilitation, in part because the buildup in intracellular Ca^{2+} during the train increases the probability of release (see later). The importance of release probability in controlling the sign of plasticity can be seen by the effect of genetic mutations. Synapses formed by hippocampal neurons in cell culture have an initially high release probability and so normally depress in

response to 20-Hz stimulation. However, a mutation that reduces by approximately two-fold the Ca^{2+}-binding affinity of synaptotagmin-1, thus reducing the initial probability of release, converts the depressing synapse into a facilitating one (Figure 15–15B).

Mechanisms that affect the concentration of free Ca^{2+} in the presynaptic terminal also affect the amount of transmitter released. For example, the buildup of inactivation of certain voltage-gated K^+ channels during high-frequency firing leads to a gradual increase in the duration of the action potential. Prolongation of the action potential increases the time that voltage-gated Ca^{2+} channels stay open, which leads to enhanced entry of Ca^{2+} and a subsequent increase in transmitter release, resulting in a larger postsynaptic potential (Figure 15–15C).

Most studies of the functional implications of short-term synaptic dynamics have been performed in vitro or are based on computational results. However, recent in vivo experiments are beginning to shed light on the behavioral importance of short-term plasticity. For example, in vivo recordings in rodents from thalamocortical synapses have suggested that synaptic depression may contribute to sensory adaptation during repeated whisker stimulation. The time course of this sensory adaptation parallels the attenuation of cortical spiking to whisker stimulation and the synaptic depression of EPSPs at thalamocortical synapses (Figure 15–15D).

High-frequency stimulation of the presynaptic neuron, which in some cells can generate up to 500 to 1,000 action potentials per second, is called *tetanic stimulation.* Such intense stimulation can cause dramatic changes in synaptic strength. The increase in size of the EPSP during tetanic stimulation is called *potentiation*; the increase that persists after tetanic stimulation is called *posttetanic potentiation* (Figure 15–15E). In contrast to synaptic facilitation, which lasts milliseconds to seconds, posttetanic potentiation usually lasts several minutes, but it can persist for an hour or more at some synapses.

Synapses utilize a complex containing Munc13 and RIM, two of the active zone proteins discussed earlier, to counteract vesicle depletion during high-frequency stimulation. The rise in presynaptic Ca^{2+} during tetanic stimulation activates phospholipase C, which produces inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Diacylglycerol directly interacts with a protein domain on Munc13 called the C1 domain (homologous to the diacylglycerol-binding domain in protein kinase C but distinct from the C2 domain of synaptotagmin), thereby accelerating the rate of synaptic vesicle recycling. At the same time, IP_3 causes additional release of Ca^{2+} from intracellular stores, and the increase in Ca^{2+} further activates Munc13 by binding to its C2 domain, which resembles the C2 domain of synaptotagmin but acts as an agent of short-term synaptic plasticity.

Activity-Dependent Changes in Intracellular Free Calcium Can Produce Long-Lasting Changes in Release

Several Ca^{2+}-dependent mechanisms contribute to longer-lasting changes in transmitter release that persist after a high-frequency tetanus is terminated. Normally the rise in Ca^{2+} in the presynaptic terminal in response to an action potential is rapidly buffered by cytoplasmic Ca^{2+}-binding proteins and mitochondria. Calcium ions are also actively transported out of the neuron by pumps and transporters. However, during tetanic stimulation, so much Ca^{2+} flows into the axon terminals that the Ca^{2+} buffering and clearance systems can become saturated.

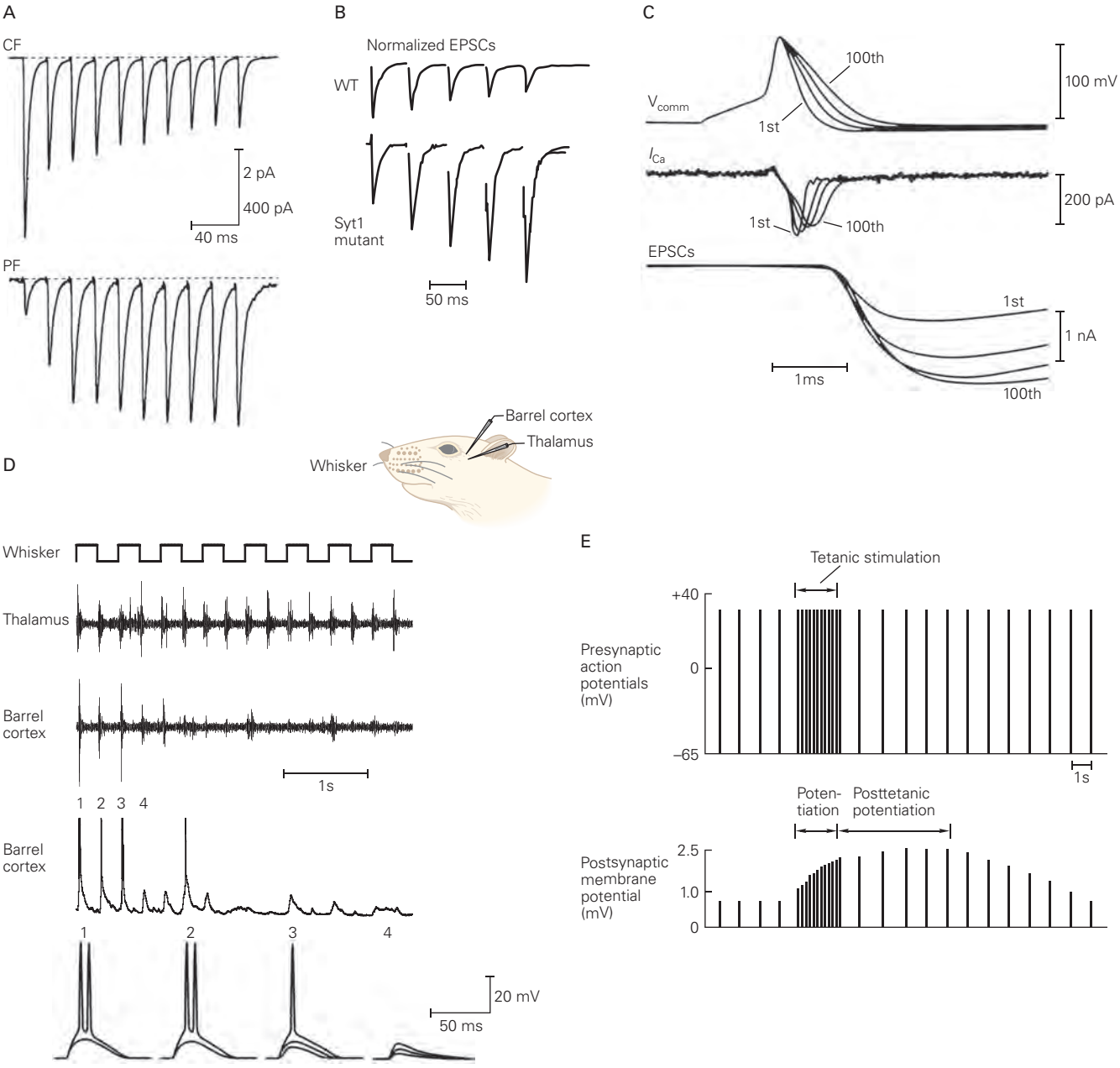
This leads to a temporary excess of Ca^{2+} called *residual* Ca^{2+}. The residual free Ca^{2+} enhances synaptic transmission for many minutes or longer by activating certain enzymes that are sensitive to enhanced levels of resting Ca^{2+}, including the Ca^{2+}/calmodulin-dependent protein kinases. Activation of such Ca^{2+}-dependent enzymatic pathways is thought to increase the priming of synaptic vesicles in the terminals. Here, then, is the simplest kind of cellular memory! The presynaptic cell can store information about the history of its activity in the form of residual free Ca^{2+} in its terminals (or residual Ca^{2+} bound to sensor proteins).

This Ca^{2+} acts by multiple pathways that have different half-times of decay. In Chapter 13, we saw how posttetanic potentiation at certain synapses is followed by an even longer-lasting process (also initiated by Ca^{2+} influx), called *long-term potentiation,* which can last for many hours or even days. The importance of long-term potentiation for learning and memory will be considered in Chapters 53 and 54.

Axo-axonic Synapses on Presynaptic Terminals Regulate Transmitter Release

Synapses are formed on axon terminals as well as the cell body and dendrites of neurons (see Chapter 13). Although axosomatic synaptic actions affect all branches of the postsynaptic neuron's axon (because they affect the probability that the neuron will fire an action potential), axo-axonic actions selectively control individual terminals of the axon. One important action of axo-axonic synapses is to increase or decrease Ca^{2+} influx into the presynaptic terminals of the postsynaptic cell, thereby enhancing or depressing transmitter release, respectively.

As we saw in Chapter 13, when one neuron releases transmitter that hyperpolarizes the cell body (or


A
CF
2 pA
400 pA
40 ms
PF
B
Normalized EPSCs
WT
Syt1
mutant
50 ms
C
100th
100 mV
V_{comm}
1st
I_{Ca}
200 pA
1st
100th
EPSCs
1st
1 nA
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100th
Barrel cortex
Thalamus
Whisker
D
Whisker
Thalamus
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1 2 3 4
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cortex
1
2
3
4
20 mV
50 ms
E
Tetanic stimulation
+40
0
–65
Presynaptic
action
potentials
(mV)
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tiation
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potentiation
2.5
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0
Postsynaptic
membrane
potential
(mV)

dendrites) of another, it decreases the likelihood that the postsynaptic cell will fire; this action is called *postsynaptic inhibition.* In contrast, when a neuron forms synapses on the axon terminal of another cell, it can reduce the amount of transmitter that will be released by the postsynaptic cell onto a third cell; this action is called *presynaptic inhibition* (Figure 15–16A). Other axo-axonic synaptic actions can increase the amount of transmitter released by the postsynaptic cell; this action is called *presynaptic facilitation* (Figure 15–16B). Both presynaptic inhibition and facilitation can occur in response to activation of ionotropic or metabotropic receptors in the membrane of the presynaptic terminals.

The best-analyzed mechanisms of presynaptic inhibition and facilitation are found in invertebrate neurons and vertebrate mechanoreceptor neurons (whose axons project to neurons in the spinal cord). Three mechanisms for presynaptic inhibition have been identified in these cells. One depends on the activation of inhibitory interneurons that form axo-axonic synapses on the sensory neuron presynaptic terminals, where they activate ionotropic $GABA_A$ receptor-channels. Because the Cl^- reversal potential in the presynaptic terminals is relatively positive, the increased Cl^- conductance resulting from activation of $GABA_A$ channels depolarizes the presynaptic terminal. This voltage change, termed the primary afferent depolarization, is thought to inactivate voltage-gated Na^+ channels, reducing the amplitude of the presynaptic action potential, which decreases the activation of voltage-gated Ca^{2+} channels and thereby decreases the amount of transmitter release.

The other two mechanisms for presynaptic inhibition both result from the activation of presynaptic G protein–coupled metabotropic receptors. One type of action results from the modulation of ion channels. As we saw in Chapter 14, the βγ-subunit complex of G proteins can simultaneously close voltage-gated Ca^{2+} channels and open K^+ channels. This decreases the influx of Ca^{2+} and enhances repolarization of the presynaptic terminal following an action potential, thus diminishing transmitter release. A second type of G protein–dependent action depends on a direct action by the βγ-subunit complex on the release machinery itself, independent of any changes in ion channel activity or Ca^{2+} influx. This second action is thought to involve a decrease in the Ca^{2+} sensitivity of the release machinery.

In contrast, presynaptic facilitation can be caused by enhanced influx of Ca^{2+}. In certain molluscan neurons, serotonin acts through cAMP-dependent protein phosphorylation to close K^+ channels in the presynaptic

Figure 15–15 (Opposite) Diversity of short-term plasticity in the central nervous system.

A. Excitatory postsynaptic currents (**EPSCs**) were recorded from a cerebellar Purkinje neuron under voltage clamp in response to repetitive stimulation of either the climbing fiber (**CF**) or parallel fiber (**PF**) inputs to the Purkinje cells. In both cases EPSCs were recorded while afferents were stimulated 10 times at 50 Hz. Note that the CF EPSC depresses whereas the PF EPSC facilitates during repetitive stimulation. (Reproduced, with permission, from Dittman et al. 2000. Copyright © 2000 Society for Neuroscience.)

B. EPSCs were recorded from hippocampal neurons in culture during stimulation at 20 Hz. EPSC size was normalized by dividing each response by peak amplitude of first EPSC in each individual train. The EPSC depresses in neurons cultured from wild-type mice (**WT**) whereas the EPSC facilitates in neurons from mice harboring a mutated form of synaptotagmin-1 that reduces its Ca^{2+} binding affinity (**Syt1 mutant**, R233Q). (Reproduced, with permission, from Fernandez-Chacon et al. 2001. Copyright © 2001 Springer Nature.)

C. The action potential recorded at the presynaptic terminals of dentate gyrus granule neurons broadens progressively during a 2-s long train of 50 Hz stimulation. This results in enhanced synaptic transmission from the granule neurons onto their CA3 postsynaptic target. The 1st, 25th, 50th, and 100th action potentials are shown. These action potential waveforms were then used as the command waveforms (**Vcomm, top**) to voltage clamp the presynaptic nerve terminal ("action potential clamp"), eliciting the voltage-gated Ca^{2+} current (I_{Ca}) recorded in the terminal (**middle**) and the EPSCs in a postsynaptic CA3 neuron (**bottom**). As the action potential, waveform increases in duration, the duration of I_{Ca} increases, increasing the amplitude of the EPSCs. (Adapted, with permission, from Geiger and Jonas, 2000. Copyright © 2000 Cell Press.)

D. Simultaneous extracellular multiunit recordings of action potentials from thalamus and barrel cortex (*second* and *third traces* from top) during a train of 4-Hz mechanical stimulation of the primary whisker (**top trace**). Cortical and thalamic responses both depress during stimulation, although cortical responses depress faster. Intracellular voltage responses of a cortical neuron in a whisker barrel to 4-Hz stimulation of the primary whisker (**bottom two traces**). The first of the two traces shows responses to successive whisker stimuli in one train. Time scale same as top traces. The bottom trace shows an expanded view of the first four responses to whisker stimulation in three separate trains. Note there is variability from trial to trial in the spiking responses to the second and third stimuli in the train, likely due to the probabilistic nature of transmitter release. (Reproduced from Chung et al. 2002. Copyright © 2002 Cell Press.)

E. A brief burst of high-frequency stimulation leads to sustained enhancement in transmitter release. The time scale of the experimental records here has been compressed (each presynaptic and postsynaptic potential appears as a single line indicating its amplitude). A stable excitatory postsynaptic potential (EPSP) of around 1 mV is produced when the presynaptic neuron is stimulated at a relatively low rate of one action potential per second. The presynaptic neuron is then stimulated for a few seconds at a higher rate of 50 action potentials per second. During this *tetanic stimulation*, the EPSP increases in size because of enhanced transmitter release, a phenomenon known as *potentiation.* After several seconds of stimulation, the presynaptic neuron is returned to the initial rate of stimulation (one per second). However, the EPSPs remain enhanced for minutes and, in some cells, for several hours. This persistent increase is called *posttetanic potentiation.*

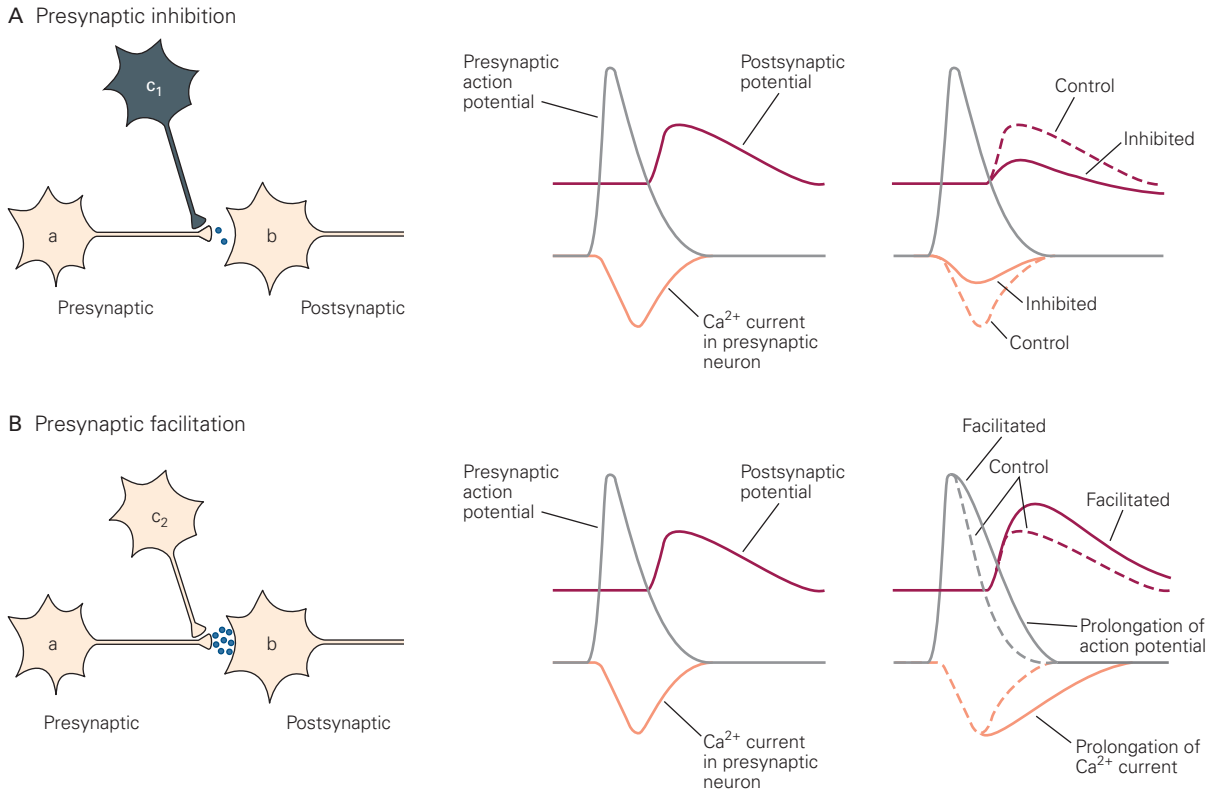

Figure 15–16 Axo-axonic synapses can inhibit or facilitate transmitter release by the presynaptic cell.

A. An inhibitory neuron (c_1) forms a synapse on an axon terminal of neuron **a**. Release of transmitter by cell c_1 activates metabotropic receptors on the terminal, thus inhibiting the Ca^{2+} current in the terminal and reducing the amount of transmitter released by cell a onto cell **b**. The reduction of transmitter release from cell a in turn reduces the amplitude of the excitatory postsynaptic potential in cell b, a process termed presynaptic inhibition.

B. A facilitating neuron (c_2) forms a synapse on an axon terminal of neuron **a**. Release of transmitter by cell c_2 activates metabotropic receptors on the terminal, thus decreasing a K^+ current in the terminals and thereby prolonging the action potential and increasing Ca^{2+} influx into cell a. This increases transmitter release from cell a onto cell **b**, thereby increasing the size of the EPSP in cell b, a process termed presynaptic facilitation.

terminal (including the *Aplysia* S-type K^+ channel discussed in Chapter 14). This action increases the duration of the presynaptic action potential, thereby increasing Ca^{2+} influx by enabling the voltage-dependent Ca^{2+} channels to remain open for a longer period. In other cells, activation of presynaptic ionotropic receptors increases transmitter release. Activation of presynaptic Ca^{2+}-permeable ionotropic receptor-channels, including presynaptic NMDA-type glutamate receptors, can increase release by directly enhancing Ca^{2+} influx. Activation of presynaptic ionotropic receptor-channels that are not permeable to Ca^{2+} can indirectly increase presynaptic Ca^{2+} levels by depolarizing the terminal and activating voltage-gated Ca^{2+} channels.

Thus, presynaptic terminals are endowed with a variety of mechanisms that allow for the fine-tuning of the strength of synaptic transmission. Although we know a fair amount about the mechanisms of short-term changes in synaptic strength—changes that last seconds, minutes, and hours—we are only beginning to learn about their functional roles. The mechanisms that support changes that persist for days, weeks, and longer also remain mysterious. These long-term changes often require alterations in gene expression and growth of presynaptic and postsynaptic structures in addition to alterations in Ca^{2+} influx and enhancement of transmitter release from existing terminals. We will discuss how such changes may contribute to different forms of long-term learning and memory in Chapters 53 and 54.

Highlights

1. Chemical neurotransmission is the primary mechanism by which neurons communicate, and process information; it occurs throughout the nervous system. Release of neurotransmitter is

stimulated by a series of electrical and biochemical processes in the presynaptic nerve terminal.

2. Neurotransmitter release is steeply dependent on depolarization of the presynaptic terminal. While the action potential is controlled by sodium and potassium conductances, it is the depolarization itself, rather than opening of either voltage-gated sodium or potassium channels, that triggers release.
3. Depolarization of the presynaptic terminal opens voltage-gated Ca^{2+} channels (VGCCs), resulting in Ca^{2+} influx. These channels are concentrated at presynaptic "active zones," very close to the sites at which release occurs. The relationship between Ca^{2+} influx and neurotransmitter release is tightly coupled and steeply nonlinear. The peak Ca^{2+} entry lags slightly behind the peak of the action potential and quickly produces a marked rise in the rate of transmitter release.
4. VGCCs are heterogeneous—five classes have been described with distinct biophysical, biochemical, and pharmacological properties. Multiple classes of VGCCs can contribute to neurotransmitter release at individual nerve terminals and are targets of disease-causing mutations. P/Q- and N-type Ca^{2+} channels are particularly prominent at active zones in the central nervous system.
5. Chemical transmission generally involves the release of quantal packets of neurotransmitter, with a quantum corresponding to the contents of a single synaptic vesicle. Under conditions that decrease transmitter release, such as lowered extracellular Ca^{2+}, a presynaptic action potential triggers the probabilistic release of a few quanta, which produce postsynaptic responses of variable amplitude that are integral multiples of the unitary response to a single quantum, interspersed by complete failures of transmission.
6. The unitary events are driven by the fusion of individual synaptic vesicles of relatively homogeneous size and transmitter content. Visualized as small, clear, spherical membrane organelles, single vesicles contain thousands of small-molecule neurotransmitters. Other neurotransmitters, including the biogenic amines and neuropeptides, are packaged into a distinct class of larger, dense-core vesicles that mediate slower forms of synaptic transmission. A typical presynaptic terminal in the mammalian central nervous system involved in fast synaptic transmission contains 100 to 200 vesicles. A small number of vesicles dock along the presynaptic membrane of the active zone and are the most ready to fuse.
7. At many synaptic connections, the amplitude of a postsynaptic potential can be described as a product of multiple factors: (1) the number of presynaptic sites occupied by a readily releasable vesicle (n), (2) the release probability of individual sites (p), and (3) the size of the postsynaptic response to the release of a single vesicle (a). On individual trials, the number of vesicles released can be described by a binomial distribution reflecting the likelihood of release of zero, one, two, or more vesicles, as if we were to count the number of heads when n coins were being flipped.
8. Exocytosis, the process by which vesicles fuse with the presynaptic membrane, and endocytosis, the process that retrieves vesicles, occur in rapid succession in nerve terminals and other secretory structures. These events are evident in morphological studies and are studied in real time by electrical measurements of membrane surface area.
9. Exocytosis is mediated by evolutionarily conserved SNARE proteins. Together, the presynaptic plasma membrane proteins syntaxin and SNAP-25 and the synaptic vesicle membrane protein synaptobrevin contribute to the SNARE complex, a set of four helical domains. Formation of this complex is critical for vesicle fusion as shown by the ability of various neurotoxins to block transmitter release through the cleavage of SNARE proteins. SNARE complex assembly is modulated by a family of SM proteins, exemplified by Munc18.
10. Synaptotagmins, such as synaptotagmin-1 (syt1), are abundant vesicular proteins that act as Ca^{2+} sensors for regulation of vesicle release. Syt1 binds multiple Ca^{2+} ions and thus forms a close association with the plasma membrane following Ca^{2+} influx. By binding to the SNARE complex even before the rise in presynaptic Ca^{2+}, it may enable that complex to cause fusion quickly.
11. Synaptic vesicle exocytosis is exquisitely precise and rapid because its molecular machinery is embedded in an active zone protein scaffold consisting of RIM, RIM-BP, and Munc-13. The complex: 1. Tethers vesicles to the plasma membrane through binding of RIM to Rab3 and Rab27 vesicle proteins; 2. Recruits calcium channels to the vicinity of tethered vesicles via binding to RIM and RIM-BP; and 3. Facilitates SNARE complex assembly via interaction with Munc13. The active zone complex also mediates many forms of short- and long-term synaptic plasticity.
12. Rapid endocytosis of vesicle membranes after release enables fast recycling of vesicles for a continuous supply during prolonged stimulation.
13. Synaptic terminals are diverse and vary in their release properties. Active zone scaffolding proteins differ across synapses and species, as does

expression of presynaptic Ca^{2+} channels and synaptotagmins. At some synapses, vesicles and Ca^{2+} channels appear aligned by an intricate structural network.

14. Transmitter release can be modulated intrinsically or extrinsically as an aspect of synaptic plasticity. Synaptic strength can be strongly influenced intrinsically by the pattern of firing in phenomena known as "depression" and "facilitation." In addition, extrinsic neuromodulators can alter the dynamics of release by regulation of Ca^{2+} channels or events downstream of Ca^{2+} entry.

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