12
Transmitter Release

SOME OF THE BRAIN'S MOST remarkable abilities, such as learning and memory, are thought to emerge from the elementary properties of chemical synapses, where presynaptic terminals release chemical transmitters that activate receptors in the membrane of the postsynaptic cell. In the last three chapters we saw how postsynaptic receptors control ion channels that generate the postsynaptic potential. Here we consider how electrical and biochemical events in the presynaptic terminal lead to the secretion of neurotransmitters. In the next chapter we examine the chemistry of the neurotransmitters themselves.

Transmitter Release Is Regulated by Depolarization of the Presynaptic Terminal

What are the signals at the presynaptic terminal that lead to the release of transmitter? Bernard Katz and Ricardo Miledi first demonstrated the importance of depolarization of the presynaptic membrane through the firing of a presynaptic action potential. For this purpose they used the giant synapse of the squid, a synapse large enough to permit insertion of electrodes into both pre- and postsynaptic structures. Two electrodes are inserted into the presynaptic terminal—one for stimulating and one for recording—and one electrode is inserted into the postsynaptic cell for recording the excitatory postsynaptic potential (EPSP), which provides an index of transmitter release (Figure 12–1A).

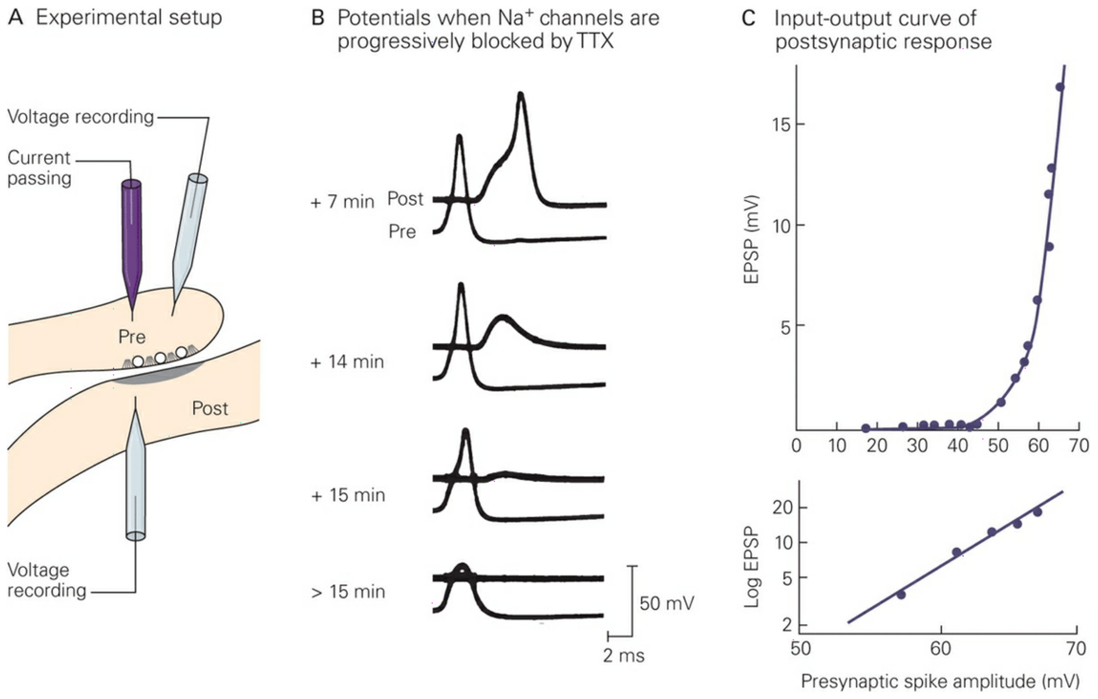


Figure 12-1 Transmitter release is triggered by changes in presynaptic membrane potential. (Adapted, with permission, from Katz and Miledi 1967a.)

A. Voltage recording electrodes are inserted in both the pre- and postsynaptic fibers of the giant synapse in the stellate ganglion of a squid. A current-passing electrode is also inserted presynaptically to elicit a presynaptic action potential.

B. Tetrodotoxin (TTX) is added to the solution bathing the cell to block the voltage-gated Na^+ channels that underlie the action potential. The amplitudes of both the presynaptic action potential and the excitatory postsynaptic potential (EPSP) gradually decrease as more and more Na^+ channels are blocked. After 7 min the presynaptic action potential can still produce a supra-threshold EPSP that triggers an action potential in the postsynaptic cell. After about 14 to 15 min the presynaptic spike gradually becomes smaller and produces smaller postsynaptic depolarizations. When the presynaptic spike is reduced to 40 mV or less, it fails to produce an EPSP. Thus the size of the presynaptic depolarization (here provided by the action potential) controls the magnitude of transmitter release.

C. An input–output curve for transmitter release is determined from the dependence of the amplitude of the EPSP on the amplitude of the presynaptic action potential. This relation is obtained by stimulating the presynaptic nerve during the onset of the blockade by TTX of the presynaptic Na^+ channels, when there is a progressive reduction in the amplitude of the presynaptic action potential and postsynaptic depolarization. The upper plot demonstrates that (1) a 40 mV presynaptic action potential is required to produce a postsynaptic potential. Beyond this threshold there is a steep increase in amplitude of the EPSP in response to small increases in the amplitude of the presynaptic potential and (2) the relationship between the presynaptic spike and the EPSP is logarithmic, as shown in the lower plot. A 10 mV increase in the presynaptic spike produces a 10-fold increase in the EPSP.

When the presynaptic neuron is stimulated it fires an action potential, and after a brief delay an EPSP large enough to trigger an action potential is recorded in the postsynaptic cell. Katz and Miledi then asked how the presynaptic action potential triggers transmitter release. They found that as voltage-gated Na^+ channels are blocked by application of tetrodotoxin, successive action potentials become progressively smaller. As the action potential is reduced in size, the EPSP decreases accordingly (Figure 12-1B). When the Na^+ channel blockade becomes so profound as to reduce the amplitude of the presynaptic spike below 40 mV (positive to the resting potential), the EPSP disappears altogether. Thus the amount of transmitter release (as measured by the size of the postsynaptic depolarization) is a steep function of the amount of presynaptic depolarization (Figure 12-1C).

Katz and Miledi next investigated how presynaptic depolarization triggers transmitter release. The action potential is produced by an influx of Na^+, and an efflux of K^+ through voltage-gated channels. To determine whether Na^+ influx or K^+ efflux is required to trigger transmitter release, Katz and Miledi first blocked the Na^+ channels with tetrodotoxin. They then asked whether direct depolarization of the presynaptic membrane, by current injection, would still trigger transmitter release. Indeed, depolarization of the presynaptic membrane beyond a threshold of about 40 mV positive to the resting potential elicits an EPSP in the postsynaptic cell. Beyond that threshold, progressively greater depolarization leads to progressively greater amounts of transmitter release. This result shows

that during a normal action potential presynaptic Na^+ influx is not necessary for release. Rather Na^+ influx is important only insofar as it depolarizes the membrane enough for transmitter release to occur (Figure 12–2B).

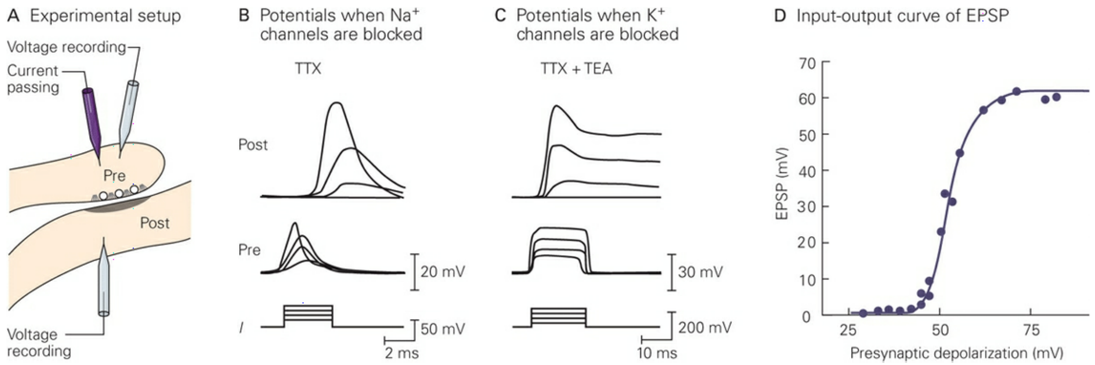


Figure 12-2 Transmitter release is not directly triggered by the opening of presynaptic voltage-gated Na^+ or K^+ channels. (Adapted, with permission, from Katz and Miledi 1967a.)

A. Voltage recording electrodes are inserted in both the pre- and postsynaptic fibers of the giant synapse in the stellate ganglion of a squid. A current-passing electrode has also been inserted into the presynaptic cell.

B. Depolarizing the presynaptic terminal with direct current injection through a microelectrode can trigger transmitter release even after the voltage-gated Na^+ channels are completely blocked by adding tetrodotoxin (TTX) to the cell-bathing solution. Three sets of traces represent (from bottom to top) the depolarizing current pulse injected into the presynaptic terminal (*I*), the resulting potential in the presynaptic terminal (**Pre**), and the EPSP generated by the release of transmitter onto the postsynaptic cell (**Post**). Progressively stronger current pulses in the presynaptic cell produce correspondingly greater depolarizations of the presynaptic terminal. The greater the presynaptic depolarization, the larger the EPSP. The presynaptic depolarizations are not maintained throughout the duration of the depolarizing current pulse because delayed activation of the voltage-gated K^+ channels causes repolarization.

C. Transmitter release occurs even after the voltage-gated Na^+ channels have been blocked with TTX *and* the voltage-gated K^+ channels have been blocked with tetraethylammonium (TEA). In this experiment TEA was injected into the presynaptic terminal. The three sets of traces represent the same measurements as in part B. Because the presynaptic K^+ channels are blocked, the presynaptic depolarization is maintained throughout the current pulse. The large sustained presynaptic depolarization produces large sustained EPSPs.

D. Blocking both the Na^+, and K^+ channels permits accurate control of presynaptic voltage and the determination of a complete input–output curve. Beyond a certain threshold (40 mV positive to the resting potential) there is a steep relationship between presynaptic depolarization and transmitter release, as measured from the size of the EPSP. Depolarizations greater than a certain level do not cause any additional release of transmitter. The initial presynaptic resting membrane potential was approximately -70 mV.

To examine the contribution of K^+ efflux to transmitter release, Katz and Miledi blocked the voltage-gated K^+ channels with tetraethylammonium at the same time they blocked the voltage-sensitive Na^+ channels with tetrodotoxin. They then injected a depolarizing current into the presynaptic terminals and found that the EPSPs were of normal size, indicating that normal transmitter release occurred (Figure 12-2C). Thus neither Na^+ nor K^+ flux is required for transmitter release.

In the presence of tetraethylammonium the current pulse elicits a maintained presynaptic depolarization because the K^+ current that normally repolarizes the presynaptic membrane is blocked. As a result, transmitter release is sustained throughout the current pulse as reflected in the prolonged depolarization of the postsynaptic cell. The sustained depolarization increased the accuracy of the measurements and permitted Katz and Miledi to determine a complete input–output curve relating presynaptic depolarization to transmitter release (Figure 12-2D). They confirmed the steep dependence of transmitter release on presynaptic depolarization. In the range of depolarization over which transmitter release increases (40–70 mV positive to the resting level) a 10 mV increase in depolarization produces a 10-fold increase in transmitter release. Depolarization of the presynaptic membrane above an upper limit produces no further increase in the postsynaptic potential.

Release Is Triggered by Calcium Influx

Katz and Miledi next turned their attention to Ca^{2+} ions. Earlier, Katz and José del Castillo had found that increasing the extracellular Ca^{2+} concentration enhanced transmitter release, whereas lowering the concentration reduced and ultimately blocked synaptic transmission. Because transmitter release is an intracellular process, these findings implied that Ca^{2+} must enter the cell to influence transmitter release.

Previous work on the squid giant axon membrane had identified a class of voltage-gated Ca^{2+} channels, the opening of which results in a large Ca^{2+} influx because of the large inward electrochemical driving force on Ca^{2+}. The extracellular Ca^{2+} concentration, approximately 2 mM in vertebrates, is normally four orders of magnitude greater than the intracellular concentration, approximately 10^{-7} M at rest. However, because these Ca^{2+} channels are sparsely distributed along the axon they cannot, by themselves, provide enough current to produce a regenerative action potential.

Katz and Miledi found that the Ca^{2+} channels were much more abundant at the presynaptic terminal. There, in the presence of tetraethylammonium and tetrodotoxin, a depolarizing current pulse was sometimes able to trigger a regenerative depolarization that required extracellular calcium, a *calcium spike*. Katz and Miledi therefore proposed that Ca^{2+} serves dual functions. It is a carrier of depolarizing charge during the action potential (like Na^+), and it is a special chemical signal—a second messenger—conveying information about changes in membrane potential to the intracellular machinery responsible for transmitter release. Calcium ions are able to serve as an efficient chemical signal because of their low resting concentration, approximately 10^5 fold lower than the resting concentration of Na^+. As a result, the small amounts of ions that enter or leave a cell during an action potential can lead to large percentage changes in intracellular Ca^{2+} that can trigger various biochemical reactions. Proof of the importance of Ca^{2+} channels in release has come from more recent experiments showing that specific toxins that block Ca^{2+} channels also block release.

The properties of the voltage-gated Ca^{2+} channels at the squid presynaptic terminal were measured by Rodolfo Llinás and his colleagues. Using a voltage clamp, Llinás depolarized the terminal while blocking the voltage-gated Na^+ channels with tetrodotoxin and the K^+ channels with tetraethylammonium. He found that graded depolarizations activated a graded inward Ca^{2+} current, which in turn resulted in graded release of transmitter (Figure 12–3). The Ca^{2+} current is graded because the Ca^{2+} channels are voltage-dependent like the voltage-gated Na^+, and K^+ channels. Calcium ion channels in squid terminals differ from Na^+ channels, however, in that they do not inactivate quickly but stay open as long as the presynaptic depolarization lasts.

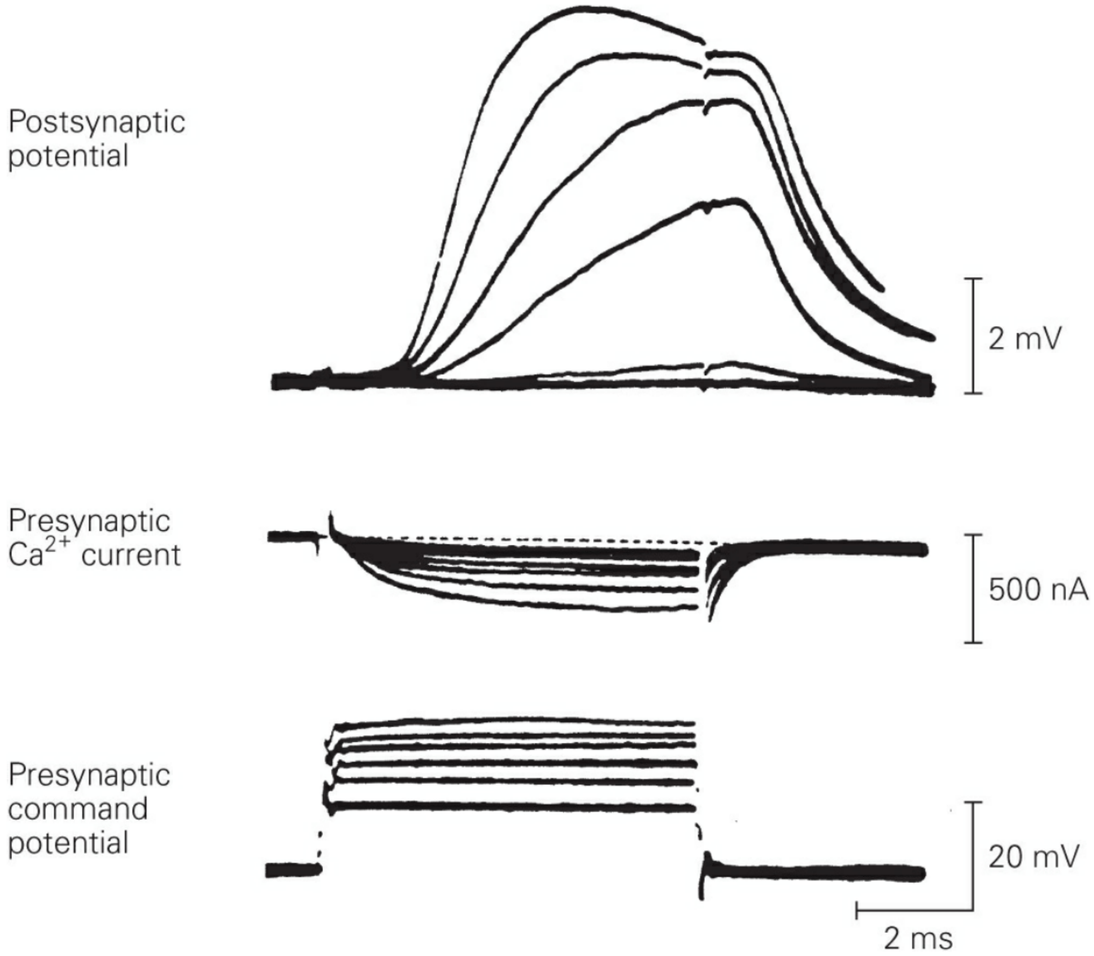


Figure 12-3 Transmitter release is regulated by Ca^{2+} influx into the presynaptic terminals through voltage-gated Ca^{2+} channels. The voltage-sensitive Na^+, and K^+ channels in a squid giant synapse were blocked by tetrodotoxin and tetraethyl-ammonium. The membrane of the presynap-

tic terminal was voltage-clamped and membrane potential stepped to six different command levels of depolarization (**bottom**). The amplitude of the postsynaptic depolarization (**top**) varies with the size of the presynaptic inward Ca^{2+} current (**middle**) because the amount of transmitter release is a function of the concentration of Ca^{2+} in the presynaptic terminal. The notch in the postsynaptic potential trace is an artifact that results from turning off the presynaptic command potential. (Adapted, with permission, from Llinás and Heuser 1977.)

Calcium ion channels are concentrated in pre-synaptic terminals at *active zones*, the sites where neurotransmitter is released, exactly opposite the postsynaptic receptors (Figure 12–4). Calcium ions do not diffuse long distances from their site of entry because free Ca^{2+} ions are rapidly buffered by calcium-binding proteins. As a result, Ca^{2+} influx creates a sharp local rise in Ca^{2+} concentration at the active zones. This rise in Ca^{2+} in the presynaptic terminals can be visualized using Ca^{2+}-sensitive fluorescent dyes (Figure 12-4B). One striking feature of transmitter release at all synapses is its steep and nonlinear dependence on Ca^{2+} influx; a twofold increase in Ca^{2+} influx can increase the amount of transmitter released by 16-fold. This relationship indicates that at some site, the *calcium sensor*, the cooperative binding of several Ca^{2+} ions is required to trigger release.

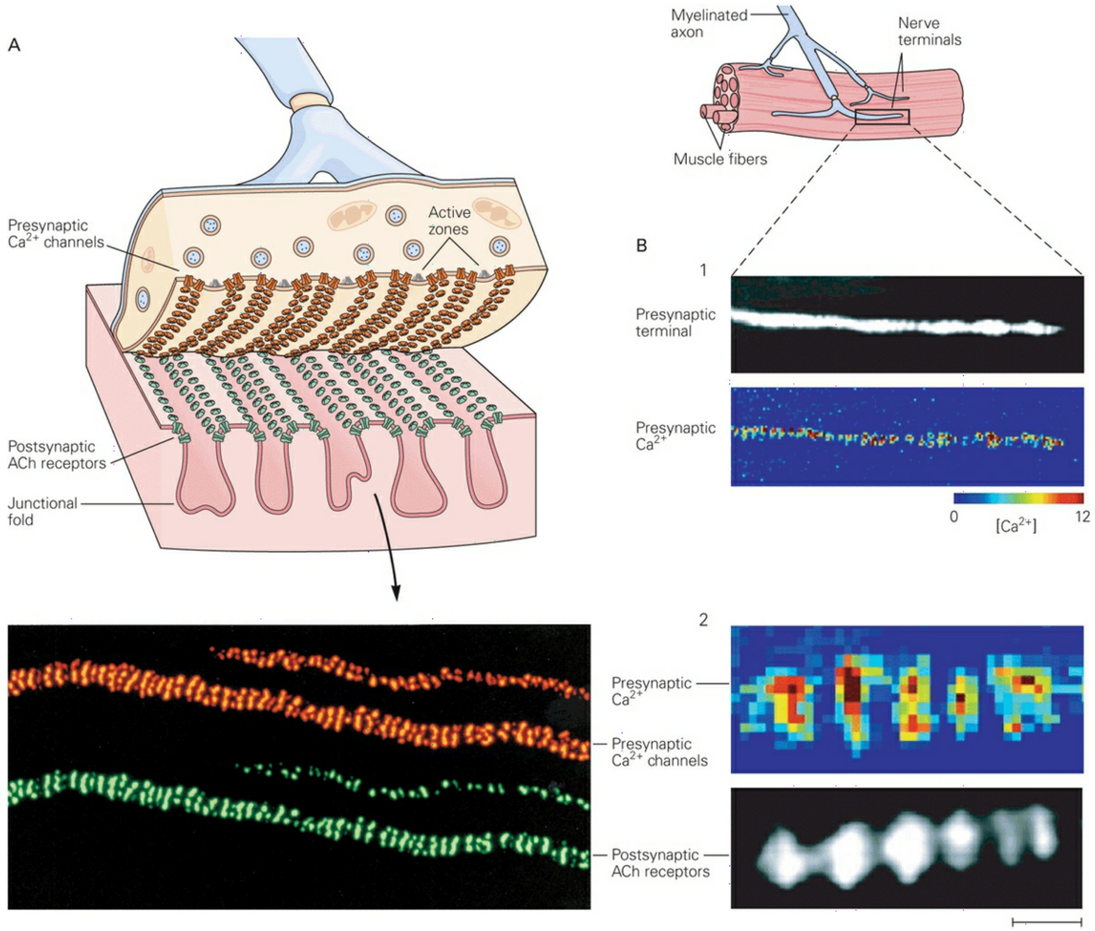


Figure 12-4 Calcium flowing into the presynaptic nerve terminal during synaptic transmission at the neuromuscular junction is concentrated at the active zone. Calcium channels in presynaptic terminals at the endplate are concentrated opposite clusters of nicotinic acetylcholine (ACh) receptors on the postsynaptic muscle membrane. Two drawings show the frog neuromuscular junction.

A. The enlarged view shows the microanatomy of the neuromuscular junction with the presynaptic terminal peeled back. A fluorescent image shows the presynaptic Ca^{2+} channels (labeled with a Texas red-coupled marine snail toxin that binds to Ca^{2+} channels), and postsynaptic ACh receptors (labeled with fluorescently tagged α-bungarotoxin, which binds selectively to ACh receptors). The two images are normally superimposed but have been separated for clarity. The patterns of labeling with both probes are in almost precise register, indicating that

the active zone of the presynaptic neuron is in almost perfect alignment with the postsynaptic membrane containing the high concentration of ACh receptors. (Reproduced, with permission, from Robitaille, Adler, and Charlton 1990.)

B. Calcium influx in presynaptic terminals is localized at active zones. Calcium can be visualized using calcium-sensitive fluorescent dyes. 1. A presynaptic terminal at a neuromuscular junction filled with the dye fura-2 under resting conditions is shown in the black and white image. The fluorescence intensity of the dye changes as it binds Ca^{2+}. In the color image, color-coded fluorescence intensity changes show local hot-spots of intracellular Ca^{2+} in response to a single presynaptic action potential. **Red** indicates regions with a large increase in Ca^{2+} **blue** indicates regions with little increase in Ca^{2+}. Regular peaks of Ca^{2+} are seen along the terminal, corresponding to the localization of Ca^{2+} channels at the active zones. 2. The color image shows a high-magnification view of the peak increase in terminal Ca^{2+} levels. The corresponding black-and-white image shows fluorescence labeling of nicotinic ACh receptors in the postsynaptic membrane, illustrating the close spatial correspondence between areas of presynaptic Ca^{2+} influx and areas of postsynaptic receptors. The scale bar represents 2 μm. (Reproduced, with permission, from Wachman et al. 2004.)

The Relation Between Presynaptic Calcium Concentration and Release

How much Ca^{2+} is necessary to induce release of neurotransmitters? To address this question Bert Sakmann and Erwin Neher and their colleagues measured synaptic transmission in the calyx of Held, a large synapse in the mammalian brain stem that is part of the auditory pathway. This synapse is specialized for very rapid and reliable transmission to allow for precise localization of sound in the environment.

The calyx forms a cup-like presynaptic terminal that engulfs a postsynaptic cell body (Figure 12–5A). To ensure that every action potential results in reliable synaptic transmission, the calyx synapse includes almost a thousand active zones that function as independent synapses. In contrast, synaptic terminals of a typical neuron in the brain contain only a single active zone. Because the calyx terminal is large, it is possible to insert electrodes into both the pre- and postsynaptic structures, much as with the squid giant synapse, and directly measure the synaptic coupling between the two compartments. This paired recording allows a precise determination of the time course of activity in the presynaptic and postsynaptic cells (Figure 12-5B).

These recordings revealed a brief lag of 1 to 2 ms between the onset of the presynaptic action potential and the postsynaptic excitatory synaptic potential, which accounts for what Sherrington termed the *synaptic delay*. Because Ca^{2+} channels open more slowly than Na^{+} channels, Ca^{2+} does not begin to enter the presynaptic terminal until the membrane has begun to repolarize. Surprisingly, once Ca^{2+} enters the terminal, transmitter is rapidly released with a delay of only a few hundred microseconds. Thus the synaptic delay is largely attributable to the time required to open Ca^{2+} channels. The astonishing speed of Ca^{2+} action indicates that, prior to Ca^{2+} influx, the biochemical machinery underlying the release process must already exist in a primed and ready state.

A presynaptic action potential normally produces only a brief rise in presynaptic Ca^{2+} concentration because the Ca^{2+} channels open only for a short time. In addition, Ca^{2+} influx is localized at the active zone. These two properties contribute to a concentrated local pulse of Ca^{2+} that induces a burst of transmitter release (Figure 12-5B). As we shall see later in this chapter, the duration of the action potential regulates the amount of Ca^{2+} that flows into the terminal and thus the amount of transmitter release.

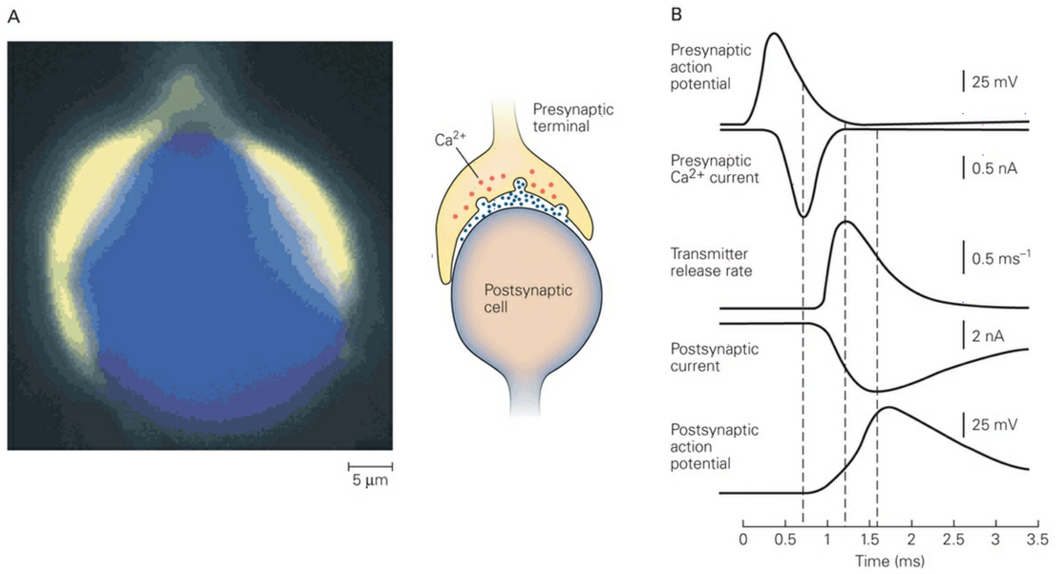


Figure 12-5 The precise relation between presynaptic Ca^{2+}, and transmitter release at a central synapse has been measured. (Reproduced, with permission, from Meinrenken, Borst, and Sakmann 2003, and Sun et al. 2007.)

A. The large presynaptic terminal of the calyx of Held in the mammalian brain stem (**yellow**) engulfs a postsynaptic cell body (**blue**). Fluorescence image on left shows a calyx filled with a calcium-sensitive dye.

B. Time courses of the presynaptic action potential, presynaptic Ca^{2+} current, transmitter release rate, postsynaptic current through glutamate receptors, and the postsynaptic action potential. The **dashed lines** indicate the timing of the peak responses for the Ca^{2+} current, transmitter release, and postsynaptic action potential.

C. Transmitter release is steeply dependent on the Ca^{2+} concentration in the presynaptic terminal. The calyx was loaded with a caged Ca^{2+} compound that releases its bound Ca^{2+} in response to a flash of ultraviolet light, and with a Ca^{2+} sensitive dye that allows measuring how much Ca^{2+} is released. By controlling the intensity of light one can regulate the increase in Ca^{2+} in the presynaptic terminal. The plot, on a logarithmic scale, shows the relation between the rate of vesicle release and intracellular Ca^{2+} concentration. The circles depict measurements from individual experiments, and the blue line depicts a fit of the data by a model that assumes that release is triggered by a major Ca^{2+} sensor that binds five Ca^{2+} ions, resulting in a Ca^{2+} cooperativity of five. Due to the non-linear relationship between Ca^{2+}, and release small increments in Ca^{2+} at concentrations of more than one μm cause massive increases in release.

D. The release of transmitter from a vesicle requires the binding of five Ca^{2+} ions to a calcium-sensing synaptic vesicle protein. In the figure Ca^{2+} binding is shown to trigger exocytosis by binding to five sensors present on a single vesicle. In reality, a single sensor binds several Ca^{2+} ions to trigger release.

To determine how much Ca^{2+} is needed to trigger release, the Neher and Sakmann groups introduced into the presynaptic terminal an inactive form of Ca^{2+} that was complexed within a light-sensitive *chemical cage*. They also loaded the terminals with a fluorescent dye that alters its fluorescence upon binding Ca^{2+}, and so can be used to assay the intracellular Ca^{2+} concentration. By uncaging the Ca^{2+} ions with a flash of light they could trigger transmitter release through a uniform, known increase in Ca^{2+} concentration. These experiments revealed that a rise in Ca^{2+} concentration of less than 1 μM is sufficient to induce release of some transmitter, but approximately 10 to 30 μM Ca^{2+} is required to release the amount normally observed during an action potential. Here again the relationship between Ca^{2+} concentration and transmitter release is highly nonlinear, consistent with a model in which four or five Ca^{2+} ions must bind to the Ca^{2+} sensor to trigger release (Figure 12–5C,D).

Several Classes of Calcium Channels Mediate Transmitter

Release

Calcium channels are found in all nerve cells and in many non-neuronal cells. In skeletal and cardiac muscle cells Ca^{2+} channels are important for excitation-contraction coupling; in endocrine cells they mediate release of hormones. Neurons contain five classes of voltage-gated Ca^{2+} channels: the L-type, P/Q-type, N-type, R-type, and T-type, each encoded by distinct genes or gene families. Each type has specific biophysical and pharmacological properties and physiological functions (Table 12–1).

Calcium channels are multimeric proteins whose distinct properties are determined by their pore-forming subunit, the α_1-subunit. The α_1-subunit is homologous to the α-subunit of the voltage-gated Na^+ channel, comprised of four repeats of a domain with six membrane-spanning segments that includes the S4 voltage-sensor and pore-lining P region (see Figure 7-14). Calcium channels also have auxiliary subunits (termed α_2, β, γ, and Δ) that modify the properties of the channel formed by the α_1-subunit.

Four of the voltage-gated Ca^{2+} channels—the L-type, P/Q-type, N-type, and R-type—require fairly strong depolarization to be activated (voltages positive to -40 to -20 mV are required) and thus are often referred to as *high-voltage-activated* Ca^{2+} channels. In contrast, T-type channels open in response to small depolarizations around the threshold for generating an action potential (-60 to -40 mV) and are therefore called *low-voltage-activated* Ca^{2+} channels. Because they are activated by small changes in membrane potential, the T-type channels help control excitability at the resting potential and are an important source of the excitatory current that drives the rhythmic pacemaker activity of certain cells in both brain and heart.

In neurons the rapid release of conventional transmitters associated with fast synaptic transmission is mediated mainly by the P/Q-type and N-type Ca^{2+} channels because these are the channel types concentrated at the active zone. The localization of N-type Ca^{2+} channels at the frog neuromuscular junction has been visualized using a fluorescence-labeled snail toxin that binds selectively to these channels (see Figure 12-4A). The L-type channels are not found in the active zone and thus do not normally contribute to the fast release of conventional transmitters such as ACh and glutamate. However, Ca^{2+} influx through L-type channels is important for slower forms of release that do not occur at specialized active zones, such as the release of neuropeptides from neurons and of hormones from endocrine cells. As we shall see below, regulation of Ca^{2+} influx into presynaptic terminals controls the amount of transmitter release and hence the strength of synaptic transmission.

Voltage-gated Ca^{2+} channels are responsible for certain acquired and genetic diseases. A point mutation in the α_1-subunit of P/Q-type channels underlies an inherited form of migraine. A mutation in the α_1-subunit of L-type channels inhibits the voltage-dependent inactivation of these channels, and underlies Timothy syndrome, a pervasive developmental disorder involving both impaired cognitive function and a severe form of autism. Patients with Lambert-Eaton syndrome, an autoimmune disease associated with muscle weakness, make antibodies to the L-type channel α_1-subunit that decrease total Ca^{2+} current.

Transmitter Is Released in Quantal Units

How does the influx of Ca^{2+} trigger release? Katz and his colleagues provided the key insight into this question by showing that transmitter is released in discrete amounts they called *quanta*. Each quantum of transmitter produces a postsynaptic potential of fixed size, called the *quantal synaptic potential*. The total postsynaptic potential is made up of a large number of quantal potentials. EPSPs seem smoothly graded in amplitude only because each quantal (or unit) potential is small relative to the total potential.

Katz and Fatt obtained the first clue as to the quantal nature of synaptic transmission in 1951 when they observed spontaneous postsynaptic potentials of approximately 0.5 mV in the nerve-muscle synapse of the frog. Like end-plate potentials evoked by nerve stimulation, these small depolarizing responses were largest at the site of nerve-muscle contact and decayed electrotonically with distance (see Figure 9-5). Small spontaneous potentials have since been observed in mammalian muscle and in central neurons. Because the postsynaptic potentials at vertebrate nerve-muscle synapses are called *end-plate potentials*, Fatt and Katz called these spontaneous potentials *miniature end-plate potentials*.

Table 12–1 Voltage-Gated Ca^{2+} Channels of Neurons

Gene	Former name	Ca^{2+} channel type	Tissue	Blocker	Voltage-dependence[1]	Function
$Ca_V1.1$ –1.4	$\alpha_{1C,D,F,S}$	L	Muscle, neurons	Dihydropyridines	HVA	Contraction, slow release
$Ca_V2.1$	α_{1A}	P/Q	Neurons	ω-Agatoxin (spider venom)	HVA	Fast release +++
$Ca_V2.2$	α_{1B}	N	Neurons	ω-Conotoxin (cone snail venom)	HVA	Fast release ++
$Ca_V2.3$	α_{1E}	R	Neurons	SNX-482 (tarantula venom)	HVA	Fast release +
$Ca_V3.1$ – 3.3	$\alpha_{1G,H,I}$	T	Muscle, neurons	Mibefradil (limited selectivity)	LVA	Pacemaker firing

[1]**HVA**, high voltage activated; **LVA**, low voltage activated.

Several results convinced Fatt and Katz that the miniature end-plate potentials represented responses to the release of small amounts of ACh, the neurotransmitter used at the nerve-muscle synapse. The time course of the miniature end-plate potentials and the effects of various drugs on them are indistinguishable from the properties of the end-plate potential. Like the end-plate potentials, the miniature end-plate potentials are enhanced and prolonged by prostigmine, a drug that blocks hydrolysis of ACh by acetylcholinesterase. Conversely, they are reduced and finally abolished by agents that block the ACh receptor. The miniature end-plate potentials represent responses to small packets of transmitter that are spontaneously released from the presynaptic nerve terminal in the absence of an action potential. Their frequency can be increased by a small depolarization of the presynaptic terminal. They disappear if the presynaptic motor nerve degenerates and reappear when a new motor synapse is formed.

What could account for the small, fixed size of the miniature end-plate potential (around 0.5–1 mV)? Del Castillo and Katz first tested the possibility that each event represents a response to the opening of a *single* ACh receptor-channel. Small amounts of ACh applied to the frog muscle end-plate elicited depolarizing postsynaptic responses that were much smaller than the 0.5 mV response of a miniature end-plate potential. This finding made it clear that the miniature end-plate potential represents the opening of more than one ACh receptor-channel. In fact, Katz and Miledi were later able to estimate the voltage response to the elementary current through a single ACh receptor-channel as being only approximately 0.3 μV (see Chapter 9). Based on this estimate a miniature end-plate potential of 0.5 mV would represent the summation of the elementary currents of approximately 2,000 channels. Later work showed that a miniature end-plate potential is the response to the synchronous release of approximately 5,000 molecules of ACh.

What is the relationship of the large end-plate potential evoked by nerve stimulation and the small, spontaneous miniature end-plate responses? This question was addressed by del Castillo and Katz in a study of synaptic signaling at the nerve-muscle synapse bathed in a solution low in Ca^{2+}. Under this condition the end-plate potential is reduced markedly, from the normal 70 mV to about 0.5 to 2.5 mV. Moreover, the amplitude of each successive end-plate potential now varies randomly from one stimulus to the next; often no response can be detected at all (termed *failures*). However, the minimum response above zero—the unit EPSP in response to a presynaptic action potential—is identical in amplitude (approximately 0.5 mV) and shape to the spontaneous miniature end-plate potentials. Importantly, the amplitude of each end-plate potential is an integral multiple of the unit potential (Figure 12–6).

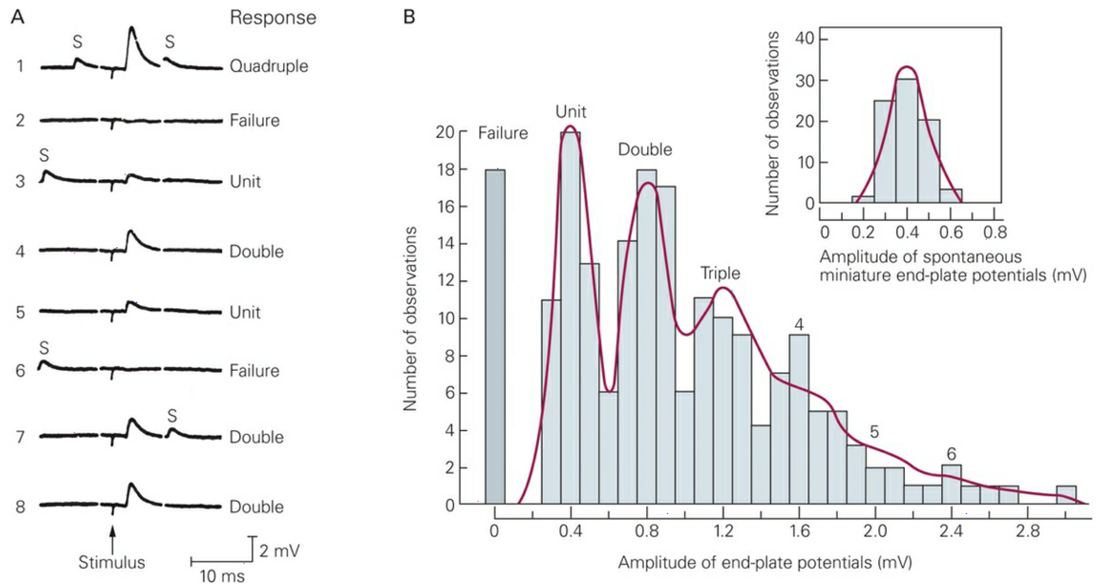


Figure 12-6 Neurotransmitter is released in fixed increments. Each increment or quantum of transmitter produces a unit excitatory postsynaptic potential (EPSP) of fixed amplitude. The amplitude of the EPSP evoked by nerve stimulation is thus equal to the amplitude of the unit EPSP multi-

plied by the number of quanta of transmitter released.

A. Intracellular recordings from a muscle fiber at the end-plate show the change in postsynaptic potential when eight consecutive stimuli of the same size are applied to the motor nerve. To reduce transmitter release and to keep the end-plate potentials small, the tissue is bathed in a calcium-deficient (and magnesium-rich) solution. The postsynaptic responses to the nerve stimulus vary. Two presynaptic impulses elicit no EPSP (failures), two produce unit potentials, and the others produce EPSPs that are approximately two to four times the amplitude of the unit potential. Note that the spontaneous miniature end-plate potentials (**S**), which occur at random intervals in the traces, are the same size as the unit potential. (Adapted, with permission, from Liley 1956.)

B. After many end-plate potentials are recorded, the number of EPSPs corresponding to a given amplitude is plotted as a function of this amplitude in the histogram shown here. The distribution of responses falls into a number of peaks. The first peak, at 0 mV, represents failures. The first peak of responses, at 0.4 mV, represents the unit potential, the smallest elicited response. The unit response has the same amplitude as the spontaneous miniature end-plate potentials (inset), indicating that the unit response is caused by the release of a single quantum of transmitter. The other peaks in the histogram are integral multiples of the amplitude of the unit potential; that is, responses are composed of two, three, four, or more quantal events.

The number of responses under each peak divided by the total number of events in the entire histogram is the probability that a single presynaptic action potential triggers the release of the number of quanta that corresponds to the peak. For example, if there are 30 events in the peak corresponding to the release of two quanta out of a total of 100 events recorded, the probability that a presynaptic action potential releases exactly two quanta is 30/100 or 0.3. This probability follows a Poisson distribution (**red curve**). This theoretical distribution is composed of the sum of several Gaussian functions. The spread of the unit peak (standard deviation of the Gaussian function) reflects the fact that the amount of transmitter in a quantum, and hence the amplitude of the quantal postsynaptic response, varies randomly about a mean value. The successive Gaussian peaks widen progressively because the variability associated with each quantal event sums linearly with the number of quanta. The distribution of amplitudes of the spontaneous miniature potentials (inset) is fit by a Gaussian curve whose width is identical to that of the Gaussian curve for the unit synaptic responses. (Adapted, with permission, from Boyd and Martin 1956.)

Now del Castillo and Katz could ask: How does the rise of intracellular Ca^{2+} that accompanies each action potential affect the release of transmitter? They found that increasing the external Ca^{2+} concentration does not change the amplitude of the unit synaptic potential. However, the proportion of failures decreases and the incidence of higher-amplitude responses (composed of multiple quantal units) increases. These observations show that an increase in external Ca^{2+} concentration does not enhance the *size* of a quantum of transmitter (that is, the number of ACh molecules in each quantum) but rather acts to increase the average number of quanta that are released in response to a presynaptic action potential (Box 12–1). The greater the Ca^{2+} influx into the terminal, the larger the number of transmitter quanta released.

Thus three findings led del Castillo and Katz to conclude that transmitter is released in packets with a fixed amount of transmitter, a quantum: The amplitude of the end-plate potential varies in a stepwise manner at low levels of ACh release, the amplitude of each step increase is an integral multiple of the unit potential, and the unit potential has the same mean amplitude as that of the spontaneous miniature end-plate potentials.

In the absence of an action potential the rate of quantal release is low —only one quantum per second is released spontaneously at the end-plate. In the presence of a normal concentration of extracellular Ca^{2+} the firing of an action potential in the presynaptic terminal at the vertebrate nerve-muscle synapse releases approximately 150 quanta, each approximately 0.5 mV in amplitude, resulting in a large end-plate potential. Thus when Ca^{2+} enters the presynaptic terminal during an action potential, it dramatically increases the rate of quantal release by a factor of 150,000, triggering the synchronous release of about 150 quanta in about one millisecond.

Transmitter Is Stored and Released by Synaptic Vesicles

lease p also varies, ranging from as high as 0.7 at the neuromuscular junction in the frog and 0.9 in the crab down to around 0.1 at some central synapses. Estimates for n range from 1,000 (at the vertebrate nerve-muscle synapse) to 1 (at single terminals of central neurons).

The parameters n and p are statistical terms; the physical processes represented by them are not yet completely understood. As discussed in this chapter, transmitter is packaged in synaptic vesicles and a quantum of transmitter corresponds to the all-or-none release of the contents of a vesicle. Although the parameter n was initially assumed to represent the number of readily releasable (or available) quanta of transmitter, it is now thought to reflect the number of release sites or active zones in the presynaptic terminals that are loaded with vesicles containing neurotransmitter.

Although an active zone has a number of docked vesicles, there is evidence that a presynaptic action potential can trigger the exocytosis of at most one vesicle per active zone. Although the number of release sites is thought to be fixed, the fraction that is loaded with vesicles is thought to be variable.

The parameter p probably represents a compound probability depending on at least two processes: the number of vesicles that have been loaded or docked onto a release site (a process referred to as vesicle mobilization) and the probability that an action potential will discharge a quantum of transmitter from a given vesicle docked at the active zone. This probability is thought to depend on Ca^{2+} influx during an action potential.

The quantal size a is the response of the postsynaptic membrane to the release of a single quantum of transmitter. Quantal size depends largely on the properties of the postsynaptic cell, such as the input resistance and capacitance (which can be independently estimated) and the responsiveness of the postsynaptic membrane to the transmitter substance. This can also be measured by the postsynaptic membrane's response to the application of a constant amount of transmitter.

The mean size of a synaptic response E evoked by an action potential thus depends on the product of the total number of quanta present, the probability that an individual quantum is released, and the size of the response to a quantum: $E = n \cdot p \cdot a$

Quantal transmission has been demonstrated at all chemical synapses so far examined with only one exception: at the synapse between photoreceptors and bipolar neurons in the retina (Chapter 26). Nevertheless, the efficacy of transmitter release from a single presynaptic cell onto a single postsynaptic cell varies widely in the nervous system and depends on several factors: (1) the number of synapses between a pair of presynaptic and postsynaptic cells (ie, the number of presynaptic boutons that contact the postsynaptic cell); (2) the number of active zones in an individual synaptic terminal; and (3) the probability that a presynaptic action potential will trigger release of one or more quanta of transmitter at an active zone (Box 12–1).

In the central nervous system most presynaptic terminals have only a single active zone where an action potential usually releases at most a single quantum of transmitter in an all-or-none manner. However at some central synapses, such as the calyx of Held, the presynaptic terminal may contain many active zones and thus can release a large number of quanta in response to a single presynaptic action potential. Central neurons also differ in the number of synapses that a typical presynaptic cell makes with a typical postsynaptic cell. Whereas most central neurons form only a few synapses with any one postsynaptic cell, a single climbing fiber forms up to 10,000 terminals on a single Purkinje neuron in the cerebellum! Finally, the mean probability of transmitter release from a single active zone also varies widely among different presynaptic terminals, from less than 0.1 (that is, a 10% chance that a presynaptic action potential will trigger release of a vesicle) to greater than 0.9. This wide range of probabilities can even be seen among the individual boutons at different synapses between one specific type of presynaptic cell and one specific type of postsynaptic cell.

Thus central neurons vary widely in the efficacy and reliability of synaptic transmission. Synaptic reliability is defined as the probability that an action potential in a presynaptic cell leads to some measurable response in the postsynaptic cell—that is, the probability that a pre-synaptic action potential releases one or more quanta of transmitter. Efficacy refers to the mean amplitude of the synaptic response, which depends on both the reliability of synaptic transmission and on the mean size of the

response when synaptic transmission does occur.

Most central neurons communicate at synapses that have a low probability of transmitter release. The high failure rate of release at most central synapses (ie, their low release probability) is not a *design defect* but serves a purpose. As we discuss below, this feature allows transmitter release to be regulated over a wide dynamic range, which is important for learning and memory. In synaptic connections where a low probability of release is deleterious for function, this limitation is overcome by simply having many active zones in one synapse, as is the case at the calyx of Held and the nerve-muscle synapse. Both contain hundreds of independent active zones, so an action potential reliably releases 150 to 250 quanta, ensuring that a presynaptic signal is always followed by a postsynaptic action potential. Reliable transmission at the neuromuscular junction is essential for survival. An animal would not survive if its ability to move away from a predator was hampered by a low-probability response.

Not all chemical signaling between neurons depends on synapses. Some substances, such as certain lipid metabolites and the gas nitric oxide (see Chapter 11), can diffuse across the lipid bilayer of the membrane. Others can be moved out of nerve endings by carrier proteins if their intracellular concentration is sufficiently high. Plasma membrane transporters for glutamate or γ-aminobutyric acid (GABA) normally take up transmitter into a cell from the synaptic cleft following a presynaptic action potential (see Chapter 13). However, in certain glial cells of the retina, the direction of glutamate transport can be reversed under certain conditions, causing glutamate to leave the cell through the transporter into the synaptic cleft. Still other substances simply leak out of nerve terminals at a low rate. Surprisingly, approximately 90% of the ACh that leaves the pre-synaptic terminals at the neuromuscular junction does so through continuous leakage. This leakage is ineffective, because it is diffuse and not targeted to receptors at the end-plate region, and because it is continuous and low level rather than synchronous and concentrated.

Synaptic Vesicles Discharge Transmitter by Exocytosis and Are Recycled by Endocytosis

The quantal hypothesis of del Castillo and Katz has been amply confirmed by direct experimental evidence that synaptic vesicles do indeed package neurotransmitter and that they release their contents by directly fusing with the presynaptic membrane, a process termed *exocytosis*.

Forty years ago Victor Whittaker discovered that the synaptic vesicles in the motor nerve terminals of the electric organ of the fish *Torpedo* contain a high concentration of ACh. Later, Thomas Reese and John Heuser and their colleagues obtained electron micrographs that caught vesicles in the act of exocytosis. To observe the brief exocytotic event, they rapidly froze the nerve-muscle synapse by immersing it in liquid helium at precisely defined intervals after the presynaptic nerve was stimulated. In addition, they increased the number of quanta of transmitter discharged with each nerve impulse by applying the drug 4-aminopyridine, a compound that blocks certain voltage-gated K^+ channels, which increases the duration of the action potential, thereby enhancing Ca^{2+} influx.

These techniques provided clear images of synaptic vesicles at the active zone during exocytosis. Using a technique called *freeze-fracture electron microscopy*, Reese and Heuser noted deformations of the presynaptic membrane along the active zone immediately after synaptic activity, which they interpreted as invaginations of the cell membrane caused by fusion of synaptic vesicles. These deformations lay along one or two rows of unusually large intramembranous particles, visible along both margins of the presynaptic density and now thought to be the voltage-gated Ca^{2+} channels (Figure 12–7). The particle density (approximately 1,500 per μm^2) is approximately that of the Ca^{2+} channels essential for transmitter release. Moreover, the proximity of the particles to the release site is consistent with the short time interval between the onset of the Ca^{2+} current and the release of transmitter.

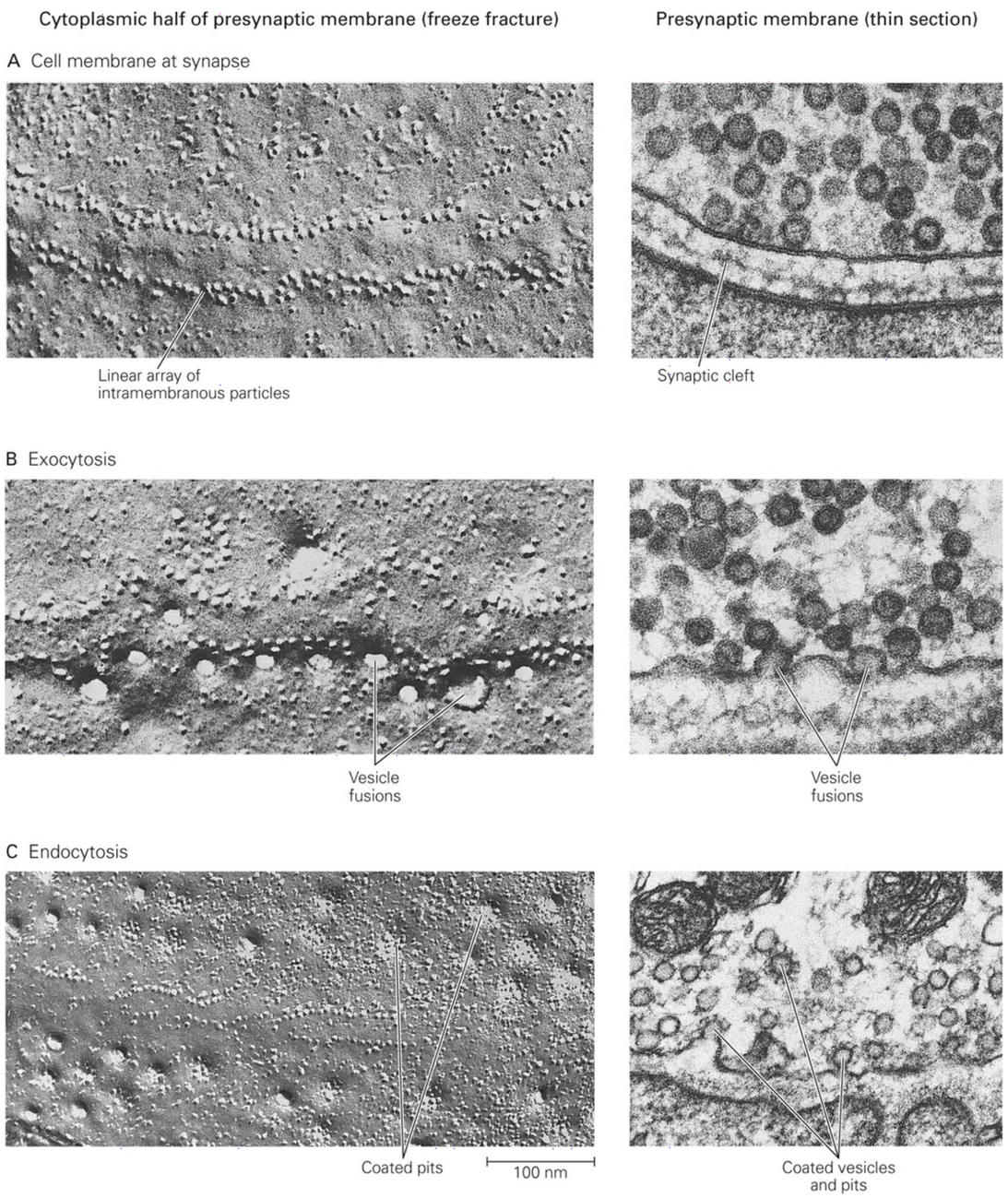


Figure 12-7 Synaptic vesicles release transmitter by exocytosis and are retrieved by endocytosis. The images on the left are freeze-fracture electron micrographs at a neuromuscular junction. The views shown are of the cytoplasmic leaflet of the presynaptic membrane looking up from the synaptic cleft. The images on the right are thin-section electron micrographs showing cross-section views of the presynaptic terminal, synaptic cleft, and postsynaptic muscle membrane. (Reproduced, with permission, from Heuser and Reese 1981.)

A. Parallel rows of intramembranous particles arrayed on either side of an active zone are thought to be the voltage-gated Ca^{2+} channels essential for transmitter release. The thin-section image at right shows the synaptic vesicles adjacent to the active zone.

B. Synaptic vesicles release transmitter by fusing with the plasma membrane (exocytosis). Here synaptic vesicles are caught in the act of fusing with the plasma membrane by rapid freezing of the tissue within 5 ms after a depolarizing stimulus. Each depression in the plasma membrane represents the fusion of one synaptic vesicle. In the micrograph at right vesicle fusion is observed as Ω-shaped structures.

C. After exocytosis, synaptic vesicle membrane is retrieved by endocytosis. Within approximately 10 seconds after fusion of the vesicles with the presynaptic membrane, coated pits form. After another 10 seconds the coated pits begin to pinch off by endocytosis to form coated vesicles. These vesicles include the membrane proteins of the original synaptic vesicle and also molecules captured from the external medium. The vesicles are recycled at the terminals or are transported to the cell body, where the membrane constituents are degraded or recycled (see Chapter 4).

Finally, Heuser and Reese found that these deformations are transient; they occur only when vesicles are discharged and do not persist after transmitter has been released. Thin-section electron micrographs revealed a number of omega-shaped (Ω) structures that have the appearance of synaptic vesicles that have just fused with the membrane, prior to the complete collapse of the vesicle membrane into the plasma membrane (Figure 12-7B). Heuser and Reese confirmed this idea by showing that the number of Ω-shaped structures is directly correlated with the size of the EPSP when they varied the concentration of 4-aminopyridine to alter the amount of transmitter release. These morphological studies provide striking evidence that transmitter is released from synaptic vesicles by means of exocytosis.

Following exocytosis, the excess membrane added to the presynaptic terminal is retrieved. When Heuser and Reese obtained images of presynaptic terminals 10 to 20 seconds after stimulation, they observed new

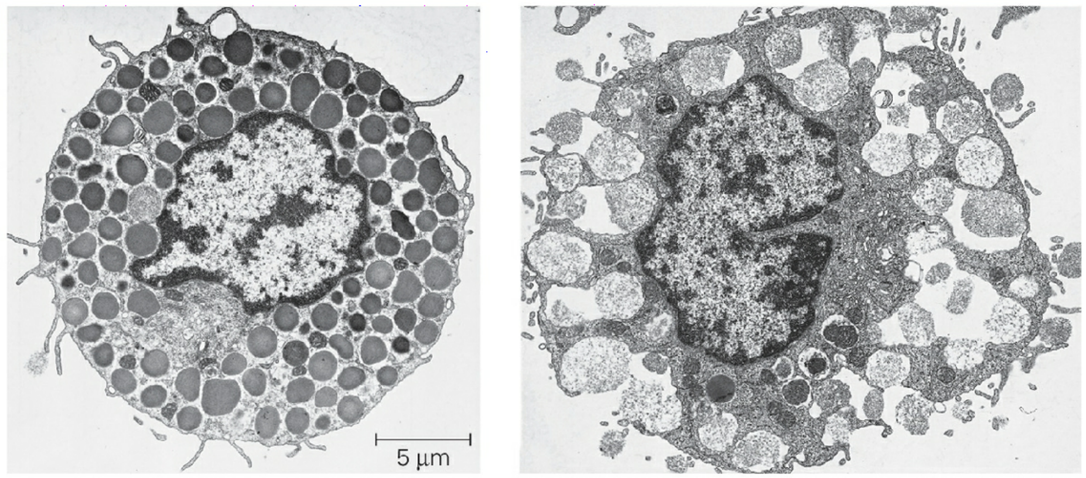
structures at the plasma membrane, the coated pits, which represent membrane retrieval through the process of endocytosis (Figure 12-7C). Several seconds later the coated pits are seen to pinch off from the membrane and appear as coated vesicles in the cytoplasm. As we will see below, endocytosis through coated pit formation represents one of several means of vesicle membrane retrieval.

Capacitance Measurements Provide Insight into the Kinetics of Exocytosis and Endocytosis

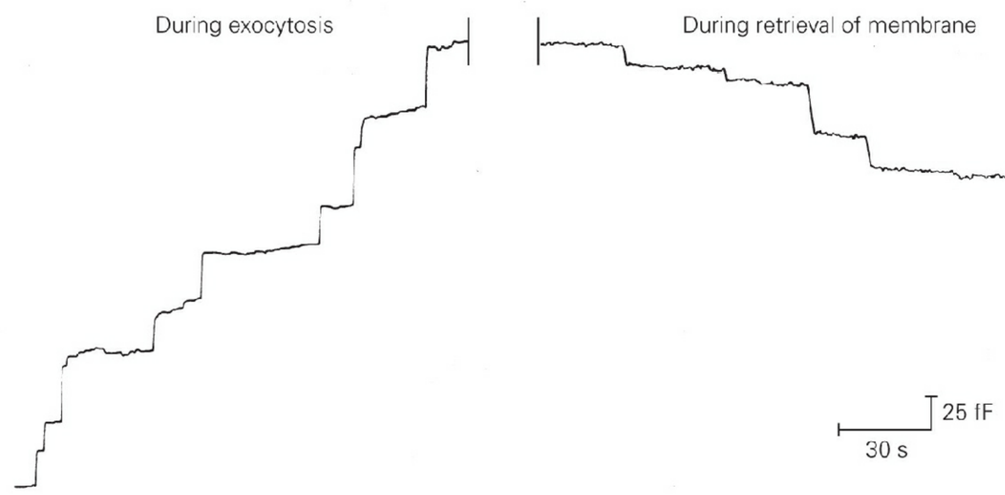
In certain neurons with large presynaptic terminals the increase in surface area of the plasma membrane during exocytosis can be detected in electrical measurements as increases in membrane capacitance. As we saw in Chapter 6, the capacitance of the membrane is proportional to its surface area. Neher discovered that one could use measurements of capacitance to monitor exocytosis in secretory cells.

In adrenal chromaffin cells (which release epinephrine and norepinephrine) and in mast cells of the rat peritoneum (which release histamine and serotonin) individual dense-core vesicles are large enough to permit measurement of the increase in capacitance associated with fusion of a single vesicle. Release of transmitter in these cells is accompanied by stepwise increases in capacitance, followed somewhat later by stepwise decreases, which reflect the retrieval and recycling of the excess membrane (Figure 12–8).

A Mast cell before and after exocytosis of secretory vesicles



B Membrane capacitance during and after exocytosis of mast cell vesicles



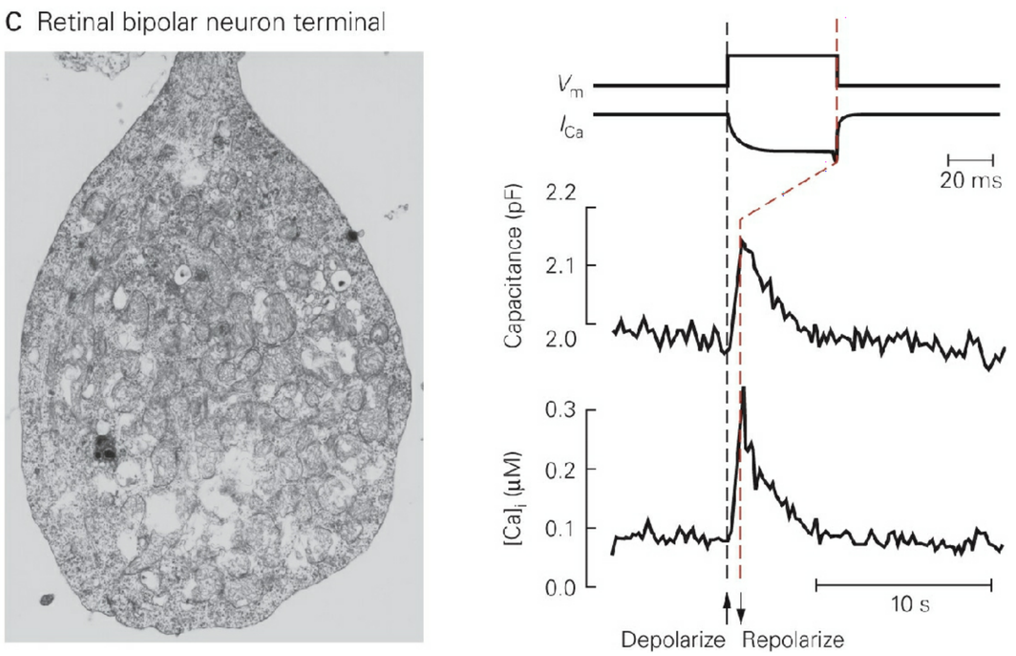


Figure 12-8 (Opposite) Changes in capacitance reveal the time course of exocytosis and endocytosis.

A. Electron micrographs show a mast cell before (**left**) and after (right) inducing exocytosis. Mast cells are secretory cells of the immune system that contain large dense-core vesicles filled with the transmitter histamine. Exocytosis of the secretory vesicles is normally triggered by the binding of antigen complexed to an immunoglobulin (IgE). Under experimental conditions massive exocytosis can be triggered by the inclusion of a nonhydrolyzable analog of guanosine triphosphate (GTP) in an intracellular recording electrode. (Reproduced, with permission, from Lawson et al. 1977.)

B. Stepwise increases in capacitance reflect the successive fusion of individual secretory vesicles with the mast cell membrane. The step increases are unequal because of variability in the membrane area of the vesicles. After exocytosis the membrane added through fusion is retrieved through endocytosis. Endocytosis of individual vesicles gives rise to the stepwise decreases in membrane capacitance. In this way the cell maintains a constant size. (The units are in femtofarads, fF, where 1 fF = 0.1 μm^2 of membrane area.) (Adapted, with permission, from Fernandez, Neher, and Gomperts 1984.)

C. The giant presynaptic terminals of bipolar neurons in the retina are more than 5 μm in diameter, permitting direct patch-clamp recordings of membrane capacitance and Ca^{2+} current. A brief depolarizing voltage-clamp step in membrane potential (V_m) elicits a large sustained Ca^{2+} current (I_{Ca}) and a rise in the cytoplasmic Ca^{2+} concentration, $[Ca]_i$. This results in the fusion of several thousand small synaptic vesicles with the cell membrane, leading to an increase in total membrane capacitance. The increments in capacitance caused by fusion of individual vesicles are too small to resolve. As the internal Ca^{2+} concentration falls back to its resting level upon repolarization, the extra membrane area is retrieved and capacitance returns to its baseline value. The increases in capacitance and Ca^{2+} concentration outlast the brief depolarization and Ca^{2+} current (note different time scales) because of the relative slowness of endocytosis and Ca^{2+} metabolism. (Micrograph reproduced, with permission, from Zenisek et al. 2004; traces adapted, with permission, from von Gersdorff and Matthews 1994.)

In neurons the changes in capacitance caused by fusion of single, small synaptic vesicles are usually too low to resolve. In certain favorable synaptic preparations that release large numbers of vesicles (such as the giant presynaptic terminals of bipolar neurons in the retina), membrane depolarization triggers a transient smooth rise in the total capacitance of the terminal as a result of the exocytosis and retrieval of the membrane from hundreds of individual synaptic vesicles (Figure 12-8C). These results provide direct measurements of the rates of membrane fusion and retrieval.

Exocytosis Involves the Formation of a Temporary Fusion Pore

Morphological studies of mast cells using rapid freezing suggest that exocytosis depends on the formation of a temporary fusion pore that spans the membranes of the vesicle and plasma membranes. In electrophysiological studies of capacitance increases in mast cells, a channel-like fusion pore was detected in the electrophysiological recordings prior to

complete fusion of vesicles and cell membranes. This fusion pore starts out with a single-channel conductance of approximately 200 pS, similar to that of gap-junction channels, which also bridge two membranes. During exocytosis the pore rapidly dilates, probably from around 5 to 50 nm in diameter, and the conductance increases dramatically (Figure 12–9A).

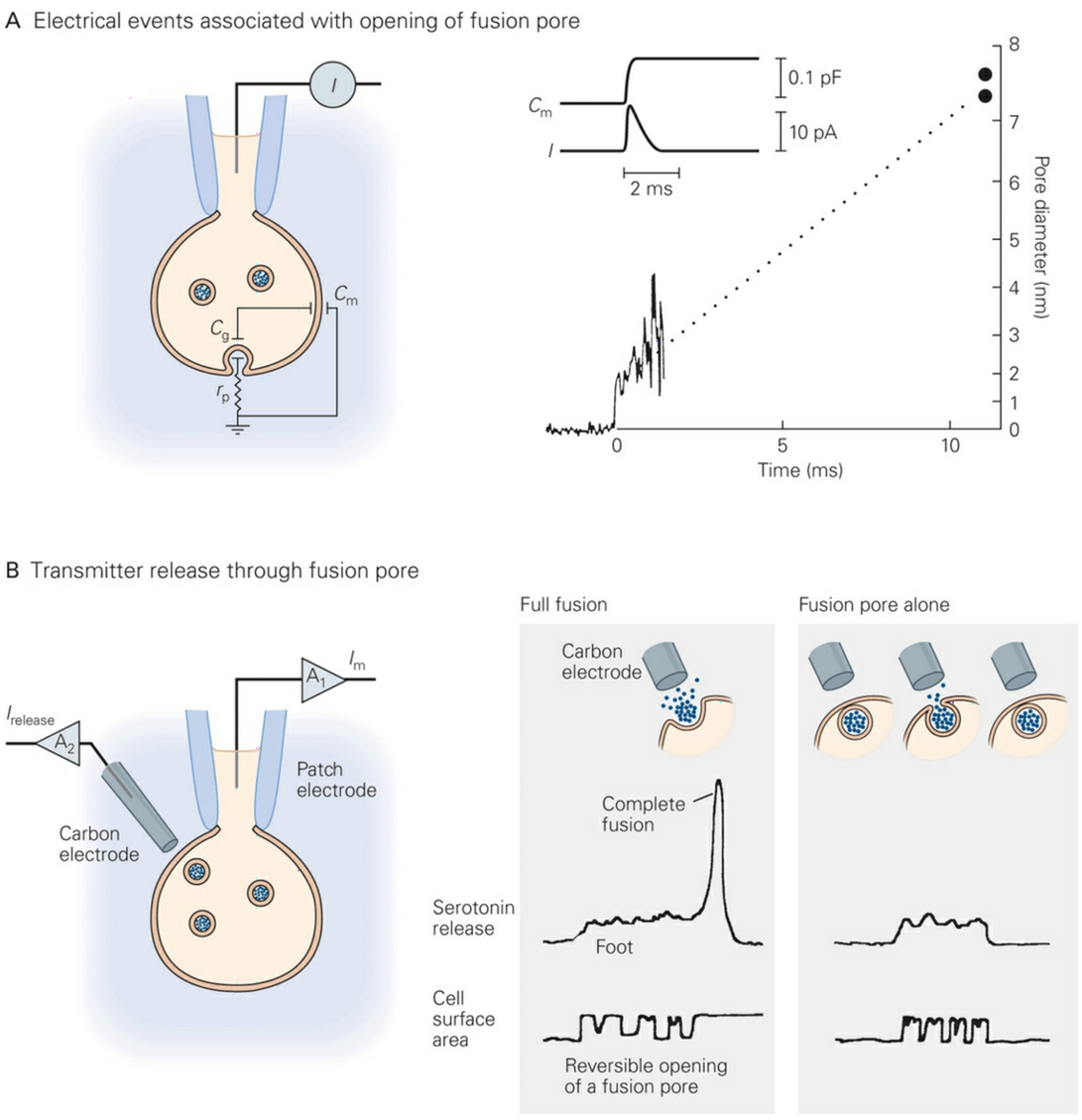


Figure 12-9 Some transmitter is released through temporary fusion pores.

A. A patch clamp is used to record membrane current associated with the opening of a fusion pore. As a vesicle fuses with the plasma membrane, the capacitance of the vesicle (C_g) is initially connected to the capacitance of the rest of the cell membrane (C_m) through the high resistance of the fusion pore (r_p). Because the membrane potential of the vesicle (lumenal side negative) is normally much more negative than the membrane potential of the cell, charge flows from the vesicle to the cell membrane during fusion. This transient current (I) is associated with the increase in membrane capacitance (C_m). The magnitude of the conductance of the fusion pore (g_p) can be calculated from the time constant of the transient current according to $\tau = C_g r_p = C_g / g_p$. The pore diameter can be calculated from the pore conductance, assuming that the pore spans two lipid bilayers and is filled with a solution whose resistivity is equal to that of the cytoplasm. In the plot on the right the pore shows an initial conductance of approximately 200 pS, similar to the conductance of a gap-junction channel, corresponding to a pore diameter of approximately 2 nm. The poor diameter and conductance rapidly increase as the pore dilates to approximately 7 to 8 nm in 10 ms (**filled circles**). (Adapted, with permission, from Monck and Fernandez 1992; Spruce et al. 1990.)

B. Transmitter release is measured by amperometry. A cell is voltage-clamped with an intracellular patch electrode while an extracellular carbon fiber is pressed against the cell surface. A large voltage applied to the tip of the carbon electrode oxidizes certain amine transmitters (such as serotonin or norepinephrine). This oxidation of one molecule generates one or more free electrons, which results in an electrical current that is proportional to the amount of transmitter release. The current can be recorded through an amplifier (A_2) connected to the carbon electrode. Membrane current and capacitance are recorded through the patch electrode amplifier (A_1). Recordings of transmitter release and capacitance measurements from mast cell secretory vesicles are shown at the right. These records indicate that serotonin may be released through the reversible opening and closing of the fusion pore prior to full fusion or by reversible fusion pore opening and closing through the fusion pore alone, that is without full fusion. During the brief fusion pore openings small amounts of transmitter escape through the pore, resulting in a low-level signal (a *foot*) that precedes a large spike of transmitter release upon full fusion (see inset for illustration). During the foot the cell membrane capacitance (proportional to cell surface

area) undergoes reversible step-like changes as the fusion pore opens and closes. Sometimes the reversible opening and closing is not followed by full fusion, such that transmitter is released through the fusion pore alone. (Adapted, with permission, from Neher 1993.)

The fusion pore is not just an intermediate structure leading to exocytosis of transmitter, as transmitter can be released through the pore prior to full fusion. This was first shown by amperometry, a method that uses an extracellular carbon-fiber electrode to detect certain amine neurotransmitters, such as serotonin, based on an electrochemical reaction between the transmitter and the electrode that generates an electrical current proportional to the local transmitter concentration. Firing of an action potential in serotonergic cells leads to a large transient increase in electrode current, corresponding to the exocytosis of the contents of a single dense-core vesicle. In some instances these large transient increases are preceded by smaller, longer-lasting current signals that reflect leakage of transmitter through a fusion pore that flickers open and closed several times prior to complete fusion (Figure 12-9B).

Transmitter can also be released solely through transient fusion pores, that is, without full collapse of the vesicle membrane into the plasma membrane. Capacitance measurements for exocytosis of both large dense-core vesicles and small clear vesicles in neuroendocrine cells show that the fusion pore can open and close rapidly and reversibly. The reversible opening and closing of a fusion pore represents a very rapid method of membrane retrieval. The circumstances under which the small clear vesicles at fast synapses discharge transmitter through a fusion pore, as opposed to full membrane collapse, are uncertain.

The Synaptic Vesicle Cycle Involves Several Steps

When firing at high frequency, a typical neuron is able to maintain a high rate of transmitter release. This can result in the exocytosis of a large number of vesicles, more than the number originally present within the presynaptic terminal. To prevent the supply of vesicles from being rapidly depleted, used vesicles are rapidly retrieved and recycled. Because nerve terminals are usually some distance from the cell body, replenishing vesicles by synthesis in the cell body and transport to the terminals would be too slow to be practical.

Synaptic vesicles are released and reused in a simple cycle (Figure 12–10A). Vesicles fill with neurotransmitter and cluster in the nerve terminal. They then dock at the active zone where they undergo a complex *priming* process that makes vesicles competent to respond to the Ca^{2+} signal that triggers the fusion process.

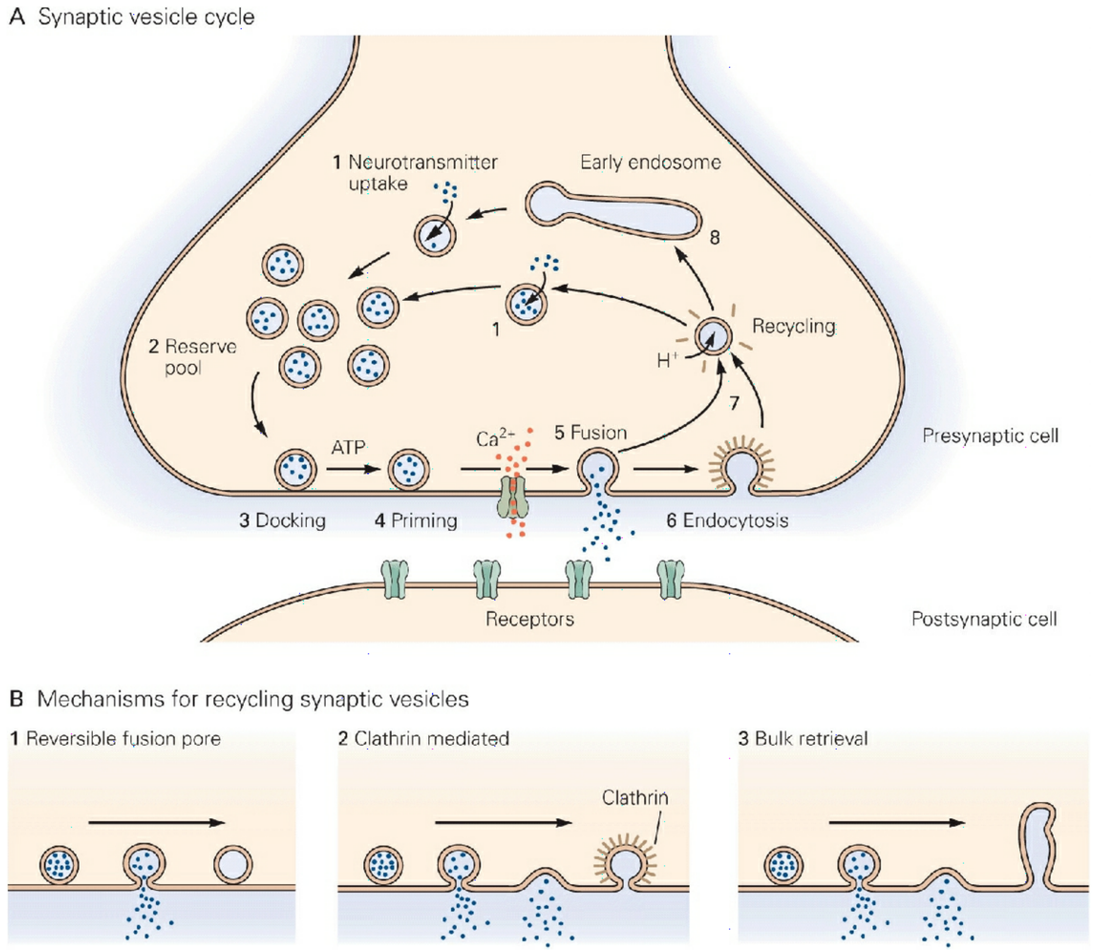


Figure 12-10 The synaptic vesicle cycle.

A. Synaptic vesicles are filled with neurotransmitters by active transport (**step 1**) and join the vesicle cluster that may represent a reserve pool (**step 2**). Filled vesicles dock at the active zone (**step 3**) where they undergo an ATP-dependent priming reaction (**step 4**) that makes them competent for calcium-triggered fusion (**step 5**). After discharging their contents, synaptic vesicles are recycled through one of several

routes (see part B). In one common route, vesicle membrane is retrieved via clathrin-mediated endocytosis (**step 6**) and recycled directly (**step 7**) or by endosomes (**step 8**).

B. Retrieval of vesicles after transmitter discharge is thought to occur via three mechanisms, each with distinct kinetics. **1**. A reversible fusion pore is the most rapid mechanism for reusing vesicles. The vesicle membrane does not completely fuse with the plasma membrane and transmitter is released through the fusion pore. Vesicle retrieval requires only the closure of the fusion pore and thus can occur rapidly, in tens to hundreds of milliseconds. This pathway may predominate at lower to normal release rates. The spent vesicle may either remain at the membrane (kiss-and-stay) or relocate from the membrane to the reserve pool of vesicles (kiss-and-run). **2**. In the classical pathway excess membrane is retrieved through endocytosis by means of clathrin-coated pits. These pits are found throughout the axon terminal except at the active zones. This pathway may be important at normal to high rates of release. **3**. In the bulk retrieval pathway, excess membrane reenters the terminal by budding from uncoated pits. These uncoated cisternae are formed primarily at the active zones. This pathway may be used only after high rates of release and not during the usual functioning of the synapse. (Adapted, with permission, from Schweizer, Betz, and Augustine 1995; Südhof 2004.)

Three mechanisms exist for retrieving the synaptic vesicle membrane following exocytosis and each has a distinct time course. The first, most rapid mechanism involves the reversible opening and closing of the fusion pore, without the full collapse of the vesicle membrane into the plasma membrane (Figure 12–10B1). In the *kiss-and-stay* pathway the vesicle remains at the active zone after the fusion pore closes, ready for a second release event. In the *kiss-and-run* pathway the vesicle leaves the active zone after the fusion pore closes, but is competent for rapid re-release. Vesicles are thought to be preferentially recycled through these pathways during stimulation at low frequencies.

Stimulation at higher frequencies recruits a second, slower recycling pathway that uses clathrin to retrieve the vesicle membrane after fusion with the plasma membrane (see Figure 12-10B2). (The clathrin-coated vesicle membranes are the coated pits observed by Heuser and Reese.) In this pathway the retrieved vesicular membrane must be recycled through an endosomal compartment before the vesicles can be reused. Clathrin-mediated recycling requires up to a minute for completion, and appears to shift from the active zone to the membrane surrounding the active zone (see Figure 12-7).

A third mechanism operates after prolonged high-frequency stimulation. Under these conditions large membranous invaginations into the presynaptic terminal are visible, which are thought to reflect membrane recycling through a process called *bulk retrieval* (Figure 12–10B3).

Exocytosis of Synaptic Vesicles Relies on a Highly Conserved Protein Machinery

Biochemists have isolated and purified many key proteins of synaptic vesicles, as well as their interacting partners in the plasma membrane (Figure 12–11). One key class of vesicle proteins are the neurotransmitter transporters (Chapter 13). These transmembrane proteins use energy stored in a proton gradient across the vesicle membrane to pump transmitter molecules into the vesicle from the cytoplasm, against their concentration gradient.

Other synaptic vesicle proteins target vesicles to their release sites, participate in the discharge of transmitter by exocytosis, and mediate recycling of the vesicle membrane. The protein machinery involved in these three steps is conserved from worms to humans, and forms the basis for the regulated release of neurotransmitter. We consider each of these steps in turn.

The Synapsins Are Important for Vesicle Restraint and Mobilization

The vesicles outside the active zone represent a reserve pool of transmitter. Paul Greengard discovered a family of proteins, *synapsins*, that are important regulators of the reserve pool of vesicles (Figure 12–11). Synapsins are peripheral membrane proteins that are bound to the cytoplasmic surface of synaptic vesicles. They also bind adenosine triphosphate (ATP)

and actin.

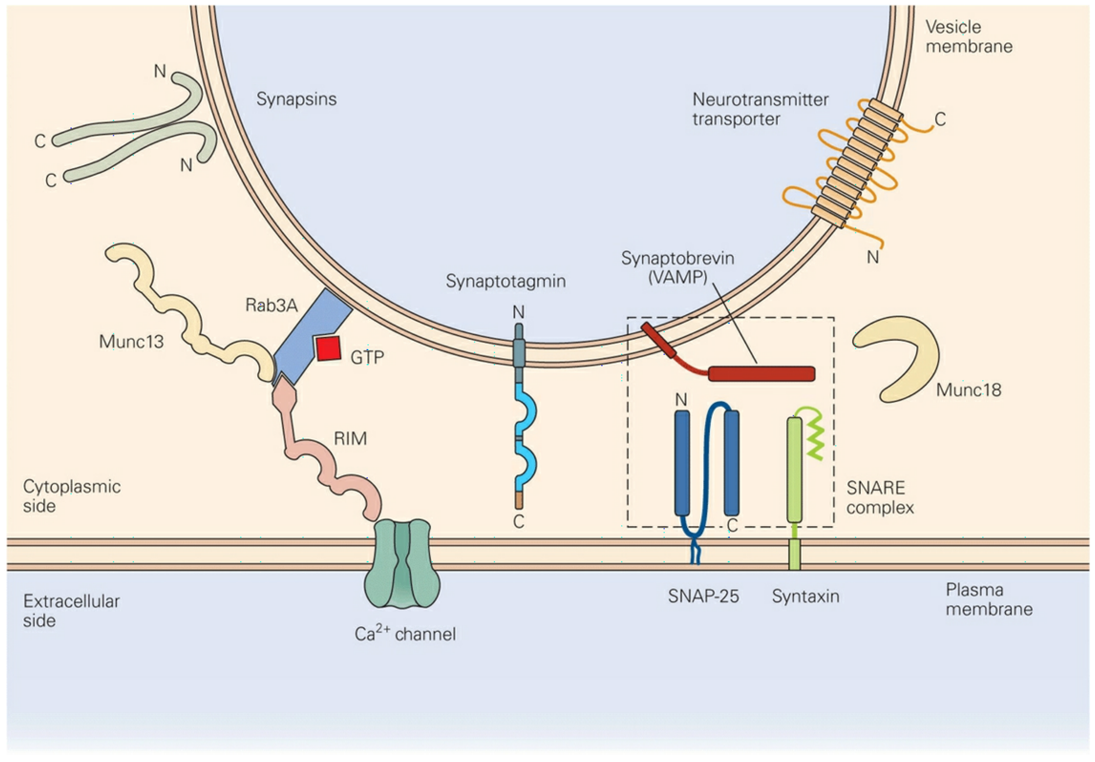


Figure 12-11 A protein network regulates synaptic vesicle exocytosis and membrane cycling. The drawing shows some of the key synaptic vesicle and plasma membrane proteins at the active zone and their interactions. Exocytosis is mediated by the formation of the SNARE complex (**dotted lines**), which results from the tight interaction between the protein synaptobrevin (or VAMP) in the vesicle membrane and the proteins syntaxin and SNAP-25 in the plasma membrane. Munc18 interacts with the SNARE complex and is essential for vesicle fusion. A second protein complex occurs when the vesicle protein Rab3 binds to the cytoplasmic proteins Munc13 and RIM. The SNARE and Rab3 complexes are functionally linked because of an interaction between syntaxin and Munc13. The syntaxin-Munc13 complex likely inhibits the formation of the SNARE complex. The vesicle protein synaptotagmin serves as the Ca^{2+} sensor for exocytosis and may also interact with RIM. Synaptic vesicles also contain membrane transporters necessary for neurotransmitter uptake. A peripheral vesicle protein, synapsin, is important in regulating the availability of vesicles from the reserve pool.

The synapsins are substrates for both protein kinase A and Ca^{2+}/calmodulin-dependent protein kinase I. When the nerve terminal is depolarized and Ca^{2+} enters, the synapsins become phosphorylated by the kinase and are thus released from the vesicles, a step that is thought to mobilize the reserve pool of vesicles for transmitter release. Indeed, genetic deletion of synapsins or application of a synapsin antibody leads to a decrease in the number of synaptic vesicles in the nerve terminal and a decrease in the ability of a terminal to maintain a high rate of transmitter release during repetitive stimulation.

SNARE Proteins Catalyze Fusion of Vesicles with the Plasma Membrane

Because a membrane bilayer is a stable structure, fusion of the synaptic vesicle and plasma membrane must overcome a large unfavorable activation energy. This is accomplished by a family of fusion proteins now referred to as SNAREs (soluble *N*-ethylmaleimide–sensitive factor attachment receptors) (Figure 12–12).

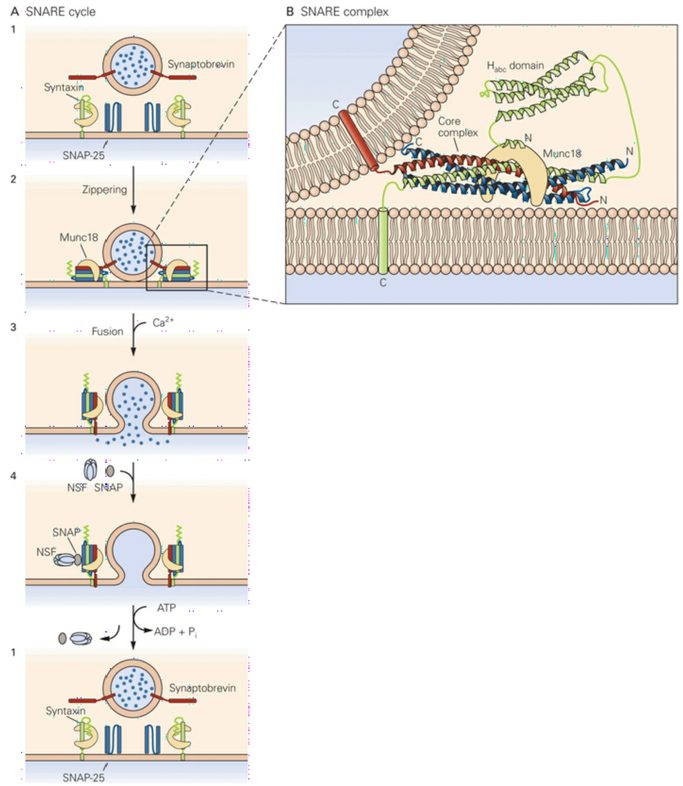


Figure 12-12 Formation and dissociation of the SNARE complex drives fusion of the synaptic vesicle and plasma membranes. (Adapted, with permission, from Rizo and Südhof 2002.)

A. The SNARE cycle. **1**. Synaptobrevin interacts with two plasma membrane target proteins, the transmembrane protein syntaxin and the peripheral membrane protein SNAP-25. **2**. The three proteins form a tight complex bringing the vesicle and presynaptic membranes in close apposition (see part B). Munc18 binds to the SNARE complex. **3**. Calcium influx triggers rapid fusion of the vesicle and plasma membranes; the SNARE complex now resides in the plasma membrane. **4**. Two proteins, NSF and SNAP (unrelated to SNAP-25), bind to the SNARE complex and cause it to dissociate in an ATP-dependent reaction.

B. The SNARE complex consists of a bundle of four α-helixes, one each from synaptobrevin and syntaxin and two from SNAP-25. The structure shown here is for the docked vesicle prior to fusion. The actual structure of the transmembrane domains has not been determined and is drawn here along with the vesicle and plasma membranes for illustrative purposes.

SNAREs are universally involved in membrane fusion, from yeast to humans. They mediate both constitutive membrane trafficking during the movement of proteins from the endoplasmic reticulum to the Golgi apparatus to the plasma membrane, as well as synaptic vesicle trafficking important for regulated exocytosis. SNAREs have a conserved protein sequence, the SNARE motif, that is 60 residues long. They come in two forms. Vesicle SNAREs or v-SNAREs (also referred to as R-SNAREs because they contain an important central arginine residue) reside in the vesicle membranes. Target-membrane SNAREs or t-SNAREs (also referred to as Q-SNAREs because they contain an important glutamine residue) are present in target membranes, such as the plasma membrane. In biochemical experiments using purified v-SNAREs and t-SNAREs in solution, four SNARE motifs bind tightly to each other to form an α-helical coiled-coil complex (Figure 12-12B).

Each synaptic vesicle contains a single type of v-SNARE called synaptobrevin (also called vesicle-associated membrane protein or VAMP). By contrast, the presynaptic active zone contains two types of t-SNARE proteins, syntaxin and SNAP-25. (Synaptobrevin and syntaxin have one SNARE motif; SNAP-25 has two.) The first clue that synaptobrevin, syntaxin, and SNAP-25 are all involved in fusion of the synaptic vesicle with the plasma membrane came from the finding that all three proteins are substrates for botulinum and tetanus toxins, bacterial proteases that are potent inhibitors of transmitter release. James Rothman then provided the crucial insight that these three proteins interact in a tight biochemical complex.

How does formation of the SNARE complex drive synaptic vesicle fusion? During exocytosis the SNARE motif of synaptobrevin, on the synaptic vesicle, forms a tight complex with the SNARE motifs of SNAP-25 and syntaxin, on the plasma membrane (Figure 12–12). The crystal structure of the SNARE complex suggests that this complex draws the membranes together. The ternary complex of synaptobrevin, syntaxin, and SNAP-25 is extraordinarily stable. The energy released in its assembly is thought to draw the negatively charged phospholipids of the vesicle and plasma membranes in close apposition, forcing them into a prefusion intermediate state (Figure 12–12). Such an unstable state may start the formation of the fusion pore and account for the rapid opening and closing (flickering) of the fusion pore observed in electrophysiological measurements. The SNARE proteins may form part of the fusion pore based upon studies that have shown that mutations in the transmembrane region of syntaxin alter the single-channel conductance of the fusion pore.

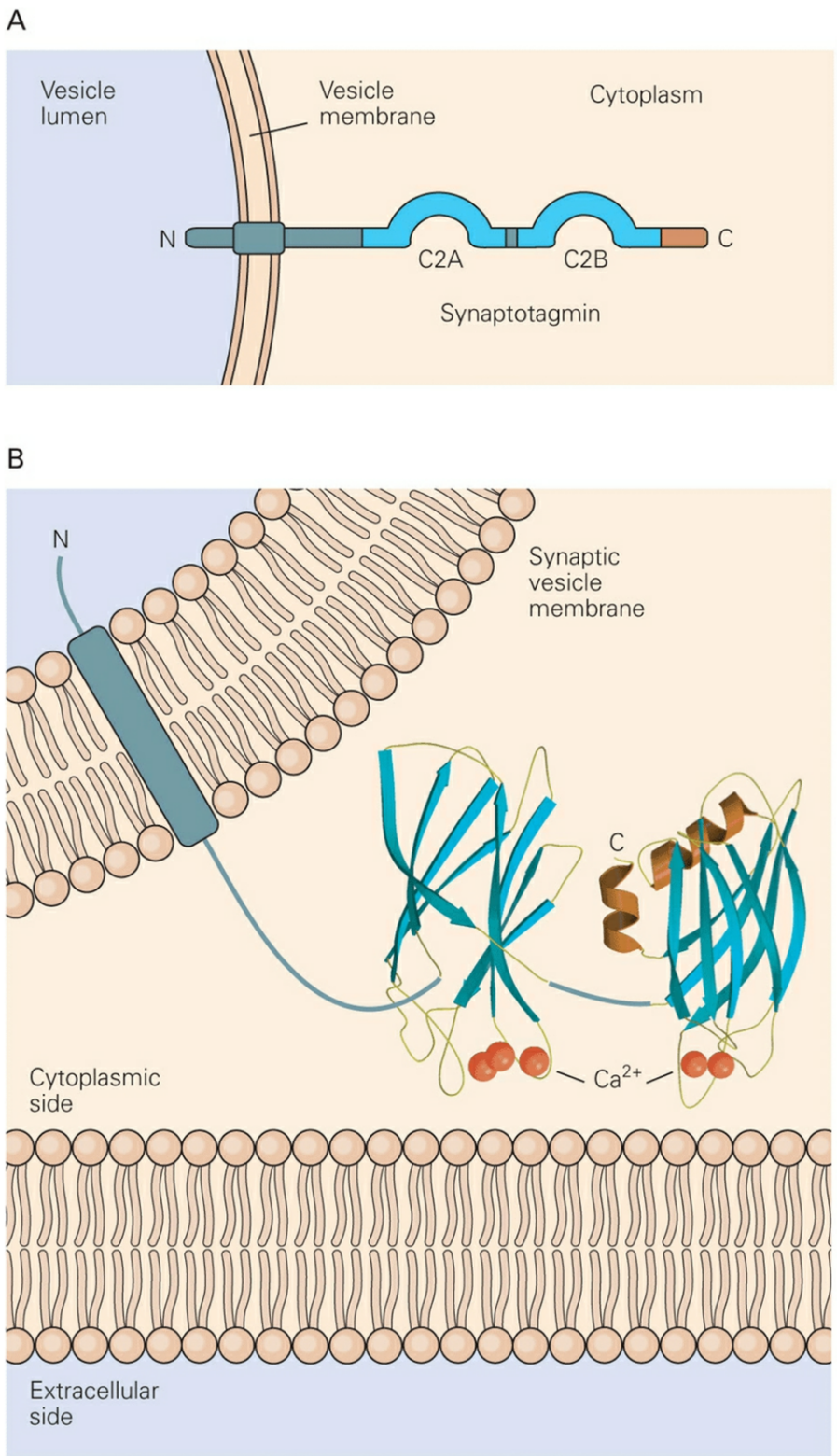
However, the SNAREs do not fully account for fusion of the synaptic vesicle and plasma membranes. Reconstitution experiments with purified proteins in lipid vesicles indicate that synaptobrevin, syntaxin, and SNAP-25 can catalyze fusion; but the in vitro reaction shows little regulation by Ca^{2+}, and the reaction is much slower and less efficient than vesicle fusion in a real synapse. One important additional protein required for exocytosis of synaptic vesicles is Munc18 (mammalian unc18 homolog). Homologs of Munc18, referred to as SM proteins (sec1/Munc18-like proteins), are essential for intracellular fusion reactions in general. Munc18 binds to syntaxin before the SNARE complex assembles by an unknown mechanism. Deletion of Munc18 prevents all synaptic fusion in neurons. The core fusion machinery is thus composed of SNARE and SM proteins that are modulated by various accessory factors specific for particular fusion reactions.

After fusion, the SNARE complex must be disassembled for efficient vesicle recycling to occur. Rothman discovered that a cytoplasmic ATPase called NSF (*N*-ethylmaleimide-sensitive fusion protein) binds to SNARE complexes via an adaptor protein called SNAP (soluble NSF-attachment protein, not related to the SNARE protein SNAP-25). NSF and SNAP use the energy of ATP hydrolysis to dissociate SNARE complexes, thereby regenerating free SNARE (Figure 12-12A). NSF also participates in the cycling of AMPA-type glutamate receptors in dendritic spines.

Calcium Binding to Synaptotagmin Triggers Transmitter Release

Because fusion of synaptic vesicles with the plasma membrane must occur within a fraction of a millisecond, it is thought that most proteins responsible for fusion are assembled prior to Ca^{2+} influx. According to this view, once Ca^{2+} enters the presynaptic terminal it binds a Ca^{2+} sensor on the vesicle, triggering immediate fusion of the membranes.

A family of closely related synaptic vesicle proteins, the synaptotagmins, has been identified as the major Ca^{2+} sensors that trigger fusion. The synaptotagmins are membrane proteins with a single N-terminal transmembrane region that anchors them to the synaptic vesicle (Figure 12–13A). The cytoplasmic region of each synaptotagmin protein is largely composed of two domains, the C2 domains, which are a common protein motif homologous to the Ca^{2+}, and phospholipid-binding C2 domain of protein kinase C. That the C2 domains bind not only Ca^{2+} but also phospholipids is consistent with their importance in calcium-dependent exocytosis. Moreover, the synaptotagmins bind Ca^{2+} over a concentration range similar to the Ca^{2+} concentration required to trigger transmitter release.



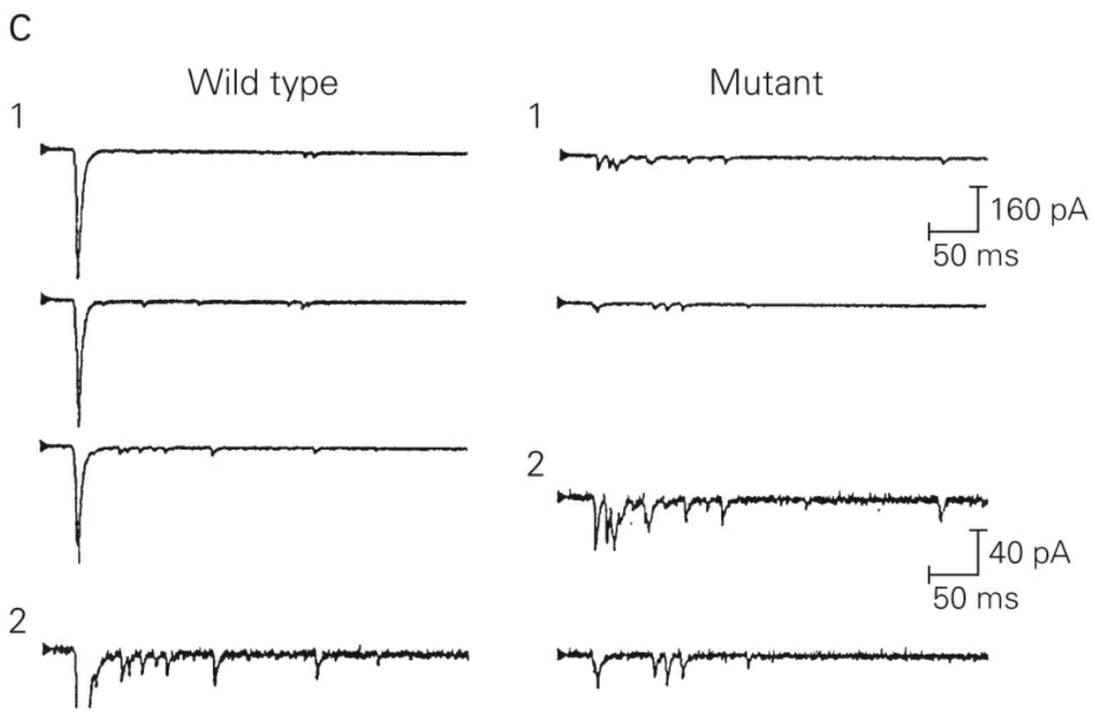


Figure 12-13 The structure of synaptotagmin and genetic evidence for its role in transmitter release.

A. Synaptotagmin is an integral membrane protein of synaptic vesicles. The short N-terminal tail, which resides in the vesicle lumen, is followed by a single hydrophobic domain that spans the vesicle membrane and a long cytoplasmic tail that contains two C2 domains (C2A and C2B) near the C terminus. The C2 domains are calcium- and phospholipid-binding motifs found in many other proteins, including PKC.

B. The X-ray crystal structure of the two C2 domains is shown here. The C2A domain binds three Ca^{2+} ions and the C2B domain two Ca^{2+} ions. 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.

C. Fast calcium-triggered transmitter release is absent in mutant mice lacking synaptotagmin. Recordings show excitatory postsynaptic currents evoked in vitro by stimulation of cultured hippocampal neurons from wildtype mice or mutant mice in which synaptotagmin has been deleted by homologous recombination (1). Neurons from wildtype mice show large, fast excitatory postsynaptic currents evoked by three successive 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, where the synaptic current is shown at a highly expanded current scale (2), 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.

The two C2 domains bind a total of five Ca^{2+} ions, the same number of Ca^{2+} ions that electrophysiological experiments reveal are required to trigger release of a quantum of transmitter (Figure 12-13B). The binding of the Ca^{2+} ions to synaptotagmin is thought to act as a switch, promoting the interaction of the C2 domains with phospholipids. The C2 domains of synaptotagmin also interact with SNARE proteins, through both calcium-independent and calcium-dependent reactions.

Studies with mutant mice in which synaptotagmin 1 is deleted or its Ca^{2+} affinity is altered provide further evidence that synaptotagmin is the physiological Ca^{2+} sensor. When the affinity of synaptotagmin for Ca^{2+} is decreased twofold, the Ca^{2+} required for transmitter release is changed by the same amount. When synaptotagmin 1 is deleted in mice, flies, or worms, an action potential is no longer able to trigger fast synchronous release. However, Ca^{2+} is still capable of stimulating a slow form of release referred to as asynchronous release (Figure 12-13C). Thus, although synaptotagmin 1 is not required for all forms of calcium-mediated transmitter release, it is essential for fast synaptic transmission.

The Fusion Machinery Is Embedded in a Conserved Protein Scaffold at the Active Zone

A defining feature of fast synaptic transmission is that neurotransmitter is released by 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 ensure the speed and tight regulation of release. This is accomplished through a conserved set of proteins that form one large macromolecular structure.

An exceedingly detailed view of the structure of the active zone at the frog neuromuscular junction has been obtained by Jack MacMahan using a powerful ultrastructural technique called scanning electron microscopic tomography. This technique has shown how synaptic vesicles are tethered to the membrane by a series of electron-dense projections, 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 12–14).

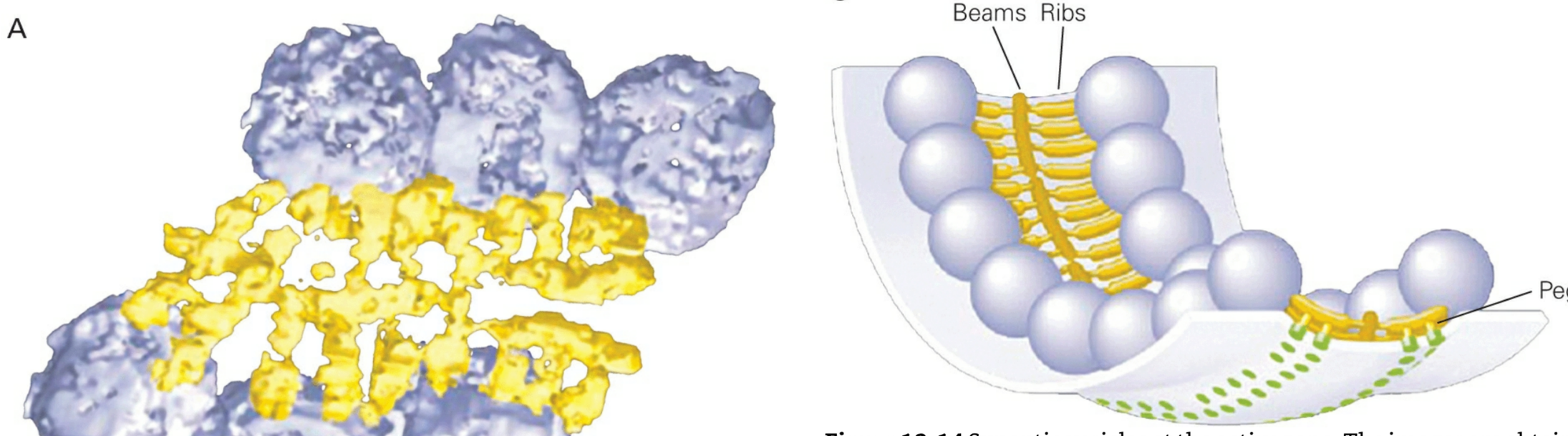


Figure 12-14 Synaptic vesicles at the active zone. The images are obtained from electron microscopic tomography. (Reproduced, with permission, from Harlow et al. 2001.)

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.

A key goal in understanding how the various synaptic vesicle and active zone proteins are coordinated during exocytosis is to fit the various proteins that have been identified into this high-resolution electron micrograph. Several cytoplasmic proteins have been identified that are thought to be components of a cytoskeletal matrix at the active zone. These include two large cytoplasmic multidomain proteins, Munc13 (not related to the Munc18 protein discussed above) and RIM, which form a tight complex with each other and may well comprise part of the ribs and beams. The binding of RIM and Munc13 is an important component of the priming of synaptic vesicles for exocytosis. Phosphorylation of RIM by cAMP-dependent protein kinase is likely to be a key regulatory mechanism in the long-term enhancement of transmitter release implicated in certain forms of learning and memory. As we will see below, regulation of Munc13 by second messengers is involved in short-term forms of synaptic plasticity. RIM binds several other synaptic vesicle proteins, including the Rab3 family of low molecular weight guanosine triphosphatases (GTPases). There are four Rab3 isoforms (Rab3A, B, C, and D) that transiently associate with synaptic vesicles as the GTP–Rab3 complex (Figure 12–11). The binding of RIM and Rab3 is thought to regulate the interaction of synaptic vesicles with the active zone during the vesicle cycle.

Modulation of Transmitter Release Underlies Synaptic Plasticity

The effectiveness of chemical synapses can be modified for short and long periods, a property called *synaptic plasticity*. Such functional modification can be effected by intrinsic or extrinsic signals. An example of an intrinsic signal is rapid firing; extrinsic signals include direct synaptic input from other neurons and more diffuse actions of neuromodulators.

Synaptic strength can be modified presynaptically, by altering the release of neurotransmitter, or postsynaptically, by modulating the response to transmitter, or both. Long-term changes in presynaptic and postsynaptic mechanisms are crucial to development and learning (Chapters 66 and 67). Here we focus on how synaptic strength changes through modulation of the amount of transmitter released.

Transmitter release 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. In principle, such changes 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.

Synaptic strength commonly is enhanced by intense activity. In many neurons a high-frequency train of action potentials is followed by a period during which a single action potential produces successively larger postsynaptic potentials (Figure 12–15A). High-frequency stimulation of the presynaptic neuron, which in some cells can generate 500 to 1,000 action potentials per second, is called *tetanic stimulation*. The increase in size of the EPSP during tetanic stimulation is called *potentiation*; the increase that persists after tetanic stimulation is called *post-tetanic potentiation* (Figure 12-15A). This enhancement usually lasts several minutes, but it can persist for an hour or more at some synapses. The opposite effect, a decrease in the size of postsynaptic potentials, occurs in response to more prolonged periods of high-frequency stimulation. This effect is referred to as synaptic *depression* (Figure 12-15B).

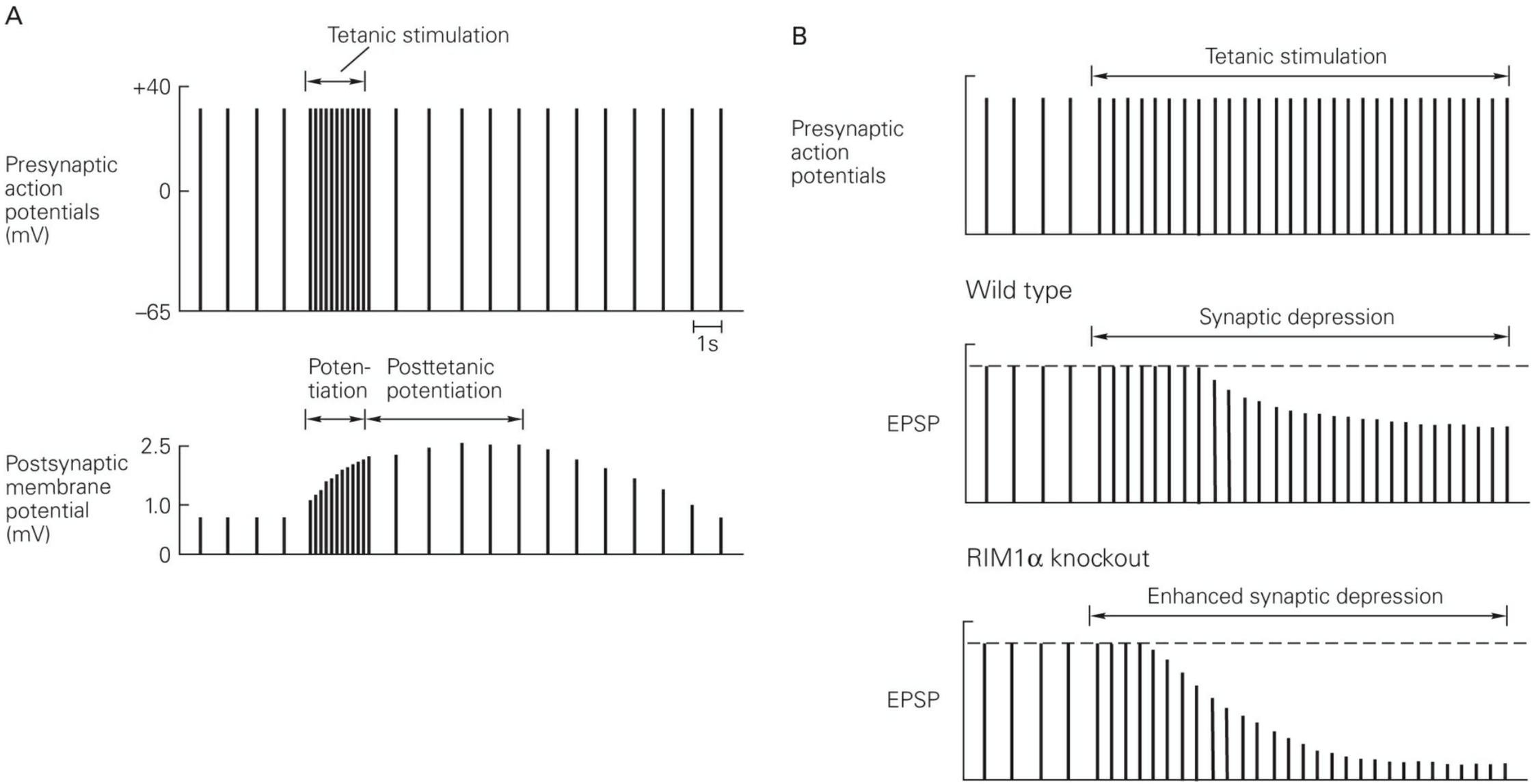


Figure 12-15 Repetitive presynaptic firing produces persistent changes in transmitter release.

A. A brief burst of high-frequency stimulation leads to a sustained enhancement in transmitter release. The time scale of the experimental records here has been compressed (each presynaptic and postsynaptic potential appears as a simple line indicating its amplitude). A stable excitatory postsynaptic potential (EPSP) of around one 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 release, a phenomenon known as *potentiation*. After several seconds of stimulation, the presynaptic neuron is returned to the initial rate of stimulation (1 per second). However, the EPSPs remain enhanced for minutes, and in some cells for several hours. This persistent increase is called *posttetanic potentiation*.

B. Prolonged tetanic stimulation can decrease the amplitude of the EPSP, a phenomenon called *synaptic depression*. In this example the presynaptic neuron produces a stable postsynaptic response of 1 mV when stimulated at a rate of 1 action potential per second. When the rate of stimulation is increased to 15 action potentials per second, the EPSP eventually declines in amplitude. This synaptic depression is thought to result from the temporary depletion of the store of releasable synaptic vesicles. Deletion of the active zone protein RIM1α enhances synaptic depression. (Adapted, with permission, from Schoch et al. 2002.)

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

Because transmitter release depends strongly on the intracellular Ca^{2+} concentration, mechanisms that affect the concentration of free Ca^{2+} in the presynaptic terminal also affect the amount of transmitter released. Normally the rise in Ca^{2+} in the presynaptic terminal in response to an action potential is rapidly buffered by cytoplasmic calcium-binding proteins and mitochondria; Ca^{2+} is 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 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+}, such as, the Ca^{2+}/calmodulin-dependent protein kinase. Activation of such calcium-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 stores information about the history of its activity in the form of residual Ca^{2+} in its terminals. This Ca^{2+} acts by multiple pathways that have different halftimes of decay. In Chapters 66 and 67 we shall see 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.

During prolonged tetanic stimulation synaptic vesicles become depleted at the active zone, resulting in synaptic depression. To counteract this depression, the synapse utilizes multiple mechanisms that often involve a complex of two of the active zone proteins discussed above, Munc13 and RIM. For example, prolonged 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 (no relation to the C2 domain) homologous to the diacylglycerol-binding domain in protein kinase C. Binding of diacylglycerol to Munc13 during prolonged repetitive stimulation helps maintain a high rate of transmitter release even in the face of depression by accelerating the rate of synaptic vesicle recycling. As a result, deletion of Munc13 or RIM results, among other actions, in an enhancement in depression (Figure 12-15B).

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 10). 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 10, when one neuron releases transmitter that hyperpolarizes the cell body (or dendrites) of another, it decreases the likelihood that the postsynaptic cell will fire; this action is called *postsyn-*

aptic inhibition. In contrast, when a neuron makes synapses onto 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 12–16A). Other axo-axonic synaptic actions can increase the amount of transmitter released by the postsynaptic cell; this action is called *presynaptic facilitation* (Figure 12-16B). Both presynaptic inhibition and facilitation can occur in response to activation of ionotropic or metabotropic receptors in the membrane of the presynaptic terminals. For reasons that are not well understood, presynaptic modulation usually occurs early in sensory pathways.

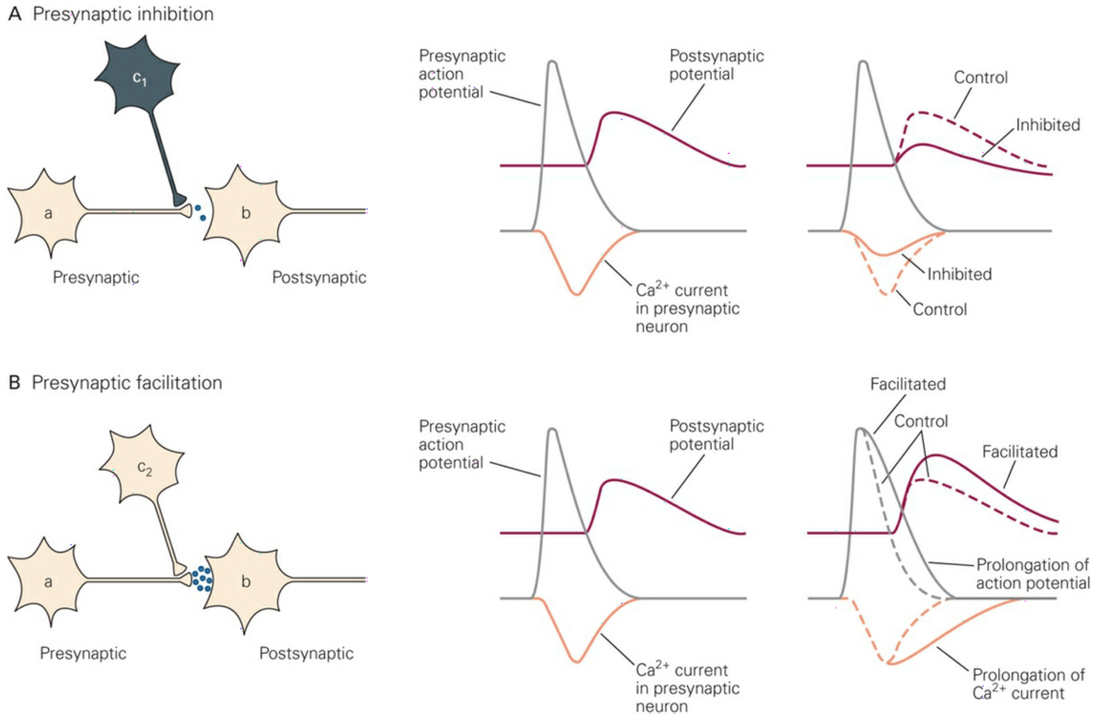


Figure 12-16 Axoaxonic synapses can inhibit or facilitate transmitter release by the presynaptic cell.

A. An inhibitory neuron (c_1) forms a synapse on the axon terminal of neuron a. Release of transmitter by cell c_1 activates a metabotropic receptor on the terminals of cell a, which inhibits the Ca^{2+} current in these terminals, thereby 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 the axon terminal of neuron a. Release of transmitter by cell c_2 activates a metabotropic receptor on the terminals of cell a, which decreases a K^+ current in the terminals, 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.

The best-analyzed mechanisms of presynaptic inhibition and facilitation are in invertebrate neurons and vertebrate mechanoreceptor neurons (whose cell bodies lie in dorsal root ganglia). Three mechanisms for presynaptic inhibition have been identified in these cells. One mechanism depends on the activation of ionotropic GABA receptor-channels in the presynaptic terminal. The opening of these channels leads to an increased conductance to Cl^-, which decreases (or short-circuits) the amplitude of the action potential in the presynaptic terminal. The smaller depolarization activates fewer Ca^{2+} channels, thereby decreasing transmitter release.

The other two mechanisms both result from the activation of presynaptic G protein-coupled metabo-tropic receptors. One type of action results from the modulation of ion channels. As we saw in Chapter 11, 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, thus diminishing transmitter release. The 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 terminal (including the *Aplysia* S-type K^+ channel discussed in Chapter 11). 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. This facilitation can be caused directly in the case of Ca^{2+}-permeable receptor-

channels by directly enhancing Ca^{2+} influx, or indirectly in the case of voltage-gated Ca^{2+} channels through depolarization of the presynaptic terminal, which leads to increased Ca^{2+} influx.

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 short-term changes in synaptic strength—changes that last minutes and hours—we are only beginning to learn about mechanisms that support changes that persist for days, weeks, and longer. 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.

An Overall View

In his book *Ionic Channels of Excitable Membranes*, Bertil Hille summarizes the importance of calcium in neuronal function as follows:

> Electricity is used to gate channels and channels are used to make electricity. However, the nervous system is not primarily an electrical device. Most excitable cells ultimately translate their electrical excitation into another form of activity. As a broad generalization, excitable cells translate their electricity into action by Ca^{2+} fluxes modulated by voltage-sensitive Ca^{2+} channels. Calcium ions are intracellular messengers capable of activating many cell functions. Calcium channels… serve as the only link to transduce depolarization into all the non-electrical activities controlled by excitation. Without Ca^{2+} channels our nervous system would have no outputs.

Neither Na^+ influx nor K^+ efflux is required to release neurotransmitter at a synapse. Only Ca^{2+}, which enters the cell through voltage-gated channels in the presynaptic terminal, is essential. Synaptic delay—the time between the onset of the action potential and the release of transmitter—largely reflects the time it takes for voltage-gated Ca^{2+} channels to open and for Ca^{2+} to trigger the discharge of transmitter from synaptic vesicles.

Transmitter is packaged in vesicles that each contain approximately 5,000 transmitter molecules. The all-or-none release of transmitter from a single vesicle results in a quantal synaptic potential. Synaptic potentials evoked by nerve stimulation are comprised of integral multiples of the quantal potential caused by the release of transmitter from multiple synaptic vesicles. Increasing the extracellular Ca^{2+} does not change the size of the quantal synaptic potential. Rather, it increases the probability that a vesicle will discharge its transmitter. As a result, the number of vesicles that release transmitter is increased, leading to a larger postsynaptic potential. In addition, transmitter is spontaneously released at low rates from synaptic vesicles, producing what are called *spontaneous miniature synaptic potentials*.

Electron microscopic images of presynaptic terminals that have been rapidly frozen following electrical stimulation reveal that synaptic vesicles release transmitter by exocytosis. The vesicle membrane fuses with the plasma membrane in the vicinity of the active zone, allowing the transmitter to flow out of the cell toward receptors on a postsynaptic cell. A conserved protein machinery mediates the exocytosis and rapid retrieval of synaptic vesicles that allows nerve terminals to maintain a high rate of transmitter release, even during prolonged trains of action potentials.

Vesicle fusion is thought to be catalyzed by the assembly of a synaptic vesicle SNARE protein (v-SNARE) with a pair of SNARE proteins in the presynaptic target membrane (t-SNARES) into a tight complex that forces the synaptic vesicle and plasma membrane into close proximity. Two other proteins, Munc13 and Munc18, are essential for synaptic vesicle fusion, probably because they enable SNARE complex assembly. Calcium triggers the opening of the vesicles during a late step in the synaptic vesicle cycle by binding to synaptotagmin, a synaptic vesicle protein that serves as the major Ca^{2+} sensor in exocytosis. The machinery that executes fusion and Ca^{2+} triggering is embedded in the active zone by a conserved protein scaffold that also regulates transmitter release. A critical component of this scaffold is a large multidomain protein called RIM that binds to Rab3, a GTP-binding protein on synaptic vesicles, and also interacts with Munc13.

The amount of transmitter released from a neuron is not fixed but can be modified by both intrinsic and extrinsic modulatory processes. High-frequency stimulation produces an increase in transmitter release called posttetanic potentiation. This potentiation, which lasts a few minutes, is caused by an action of the residual Ca^{2+} remaining in the terminal after the large Ca^{2+} signal that occurs during the train of action potentials

has been largely dissipated by diffusion, buffering, and extrusion. Neurotransmitters acting at axo-axonic synapses can facilitate or inhibit release of transmitter by altering the steady-state level of resting Ca^{2+}, the Ca^{2+} influx during the action potential, or the functioning of the release machinery.

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Eric R. Kandel
Thomas C. Südhof

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