Transmitter Release

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An Overall View

Some of the brain's most remarkable feats, such as learning and memory, are thought to emerge from the elementary properties of chemical synapses. The distinctive feature of these synapses is that action potentials in the presynaptic terminals lead to the release of chemical transmitters. In the past three chapters we saw how postsynaptic receptors for these transmitters control the ion channels that generate the postsynaptic potential. Now we return to the presynaptic cell and consider how electrical events in the terminal are coupled to the secretion of neurotransmitters. In the next chapter we shall examine the chemistry of the neurotransmitters themselves.

Transmitter Release Is Regulated by Depolarization of the Presynaptic Terminal

How does an action potential in the presynaptic cell lead to the release of transmitter? The importance of depolarization of the presynaptic membrane was demonstrated by Bernard Katz and Ricardo Miledi using the giant synapse of the squid. This synapse is large enough to permit the insertion of two electrodes into the presynaptic terminal (one for stimulating and one for recording) and an electrode into the postsynaptic cell for recording the synaptic potential, which provides an index of transmitter release.

The presynaptic cell typically produces an action potential with an amplitude of 110 mV, which leads to transmitter release and the generation of a large synaptic potential in the postsynaptic cell. The action potential is produced by voltage-gated Na\(^+\) influx and K\(^+\) efflux. Katz and Miledi found that when the voltage-gated Na\(^+\) channels are blocked upon application of tetrodotoxin, successive presynaptic action potentials become progressively smaller, owing to the progressive blockade of Na\(^+\) channels during the onset of tetrodotoxin's effect. The postsynaptic potential is reduced accordingly. When the Na\(^+\) channel blockade becomes so profound as to reduce the amplitude of the presynaptic spike below 40 mV, the synaptic potential disappears altogether (Figure 14-1B). Thus, transmitter release (as measured by the size of the postsynaptic potential) shows a steep dependence on presynaptic depolarization.
Part III / Elementary Interactions Between Neurons: Synaptic Transmission

Figure 14-1  The contribution of voltage-gated Na\(^+\) channels to transmitter release is tested by blocking the channels and measuring the amplitude of the presynaptic action potential and the resulting postsynaptic potential. (Adapted from Katz and Miledi 1967a.)

A. Recording electrodes are inserted in both the pre- and postsynaptic fibers of the giant synapse in the stellate ganglion of a squid.

B. Tetrodotoxin (TTX) is added to the solution bathing the cell in order to block the voltage-gated Na\(^+\) channels. The amplitudes of both the presynaptic action potential and the postsynaptic potential gradually decrease. After 7 min the presynaptic action potential can still produce a suprathreshold synaptic potential that triggers an action potential in the postsynaptic cell (1). After 14 and 15 min the presynaptic spike gradually becomes smaller and produces smaller synaptic potentials (2 and 3). When the presynaptic spike is reduced to 40 mV or less, it fails to produce a synaptic potential (4).

C. An input-output curve for transmitter release can be inferred from the dependence of the amplitude of the synaptic potential on the amplitude of the presynaptic action potential. This is obtained by stimulating the presynaptic nerve as the Na\(^+\) channels for the presynaptic action potential are progressively blocked. 1. A 40 mV presynaptic depolarization is required to produce a synaptic potential. Beyond this threshold there is a steep increase in amplitude of the synaptic potential in response to small changes in the amplitude of the presynaptic potential. 2. The semilogarithmic plot of the data in the input-output curve illustrates that the relationship between the presynaptic spike and the postsynaptic potential is logarithmic. A 10 mV increase in the presynaptic spike produces a 10-fold increase in the synaptic potential.

transmitter is released (40–70 mV above the resting level), a 10 mV increase in depolarization produces a 10-fold increase in transmitter release. Thus, the presynaptic terminal is able to release transmitter without an influx of Na\(^+\). The Na\(^+\) influx is important only insofar as it depolarizes the membrane enough to generate the action potential necessary for transmitter release.

Might the voltage-gated K\(^+\) efflux triggered by the action potential be responsible for release of transmitter? 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\(^+\) chan-
A. Experimental setup

B. Potentials when Na\(^+\) channels are blocked

C. Potentials when K\(^+\) channels are blocked

D. Input-output curve of transmitter release

Figure 14-2 Blocking the voltage-sensitive Na\(^+\) channels and K\(^+\) channels in the presynaptic terminals affects the amplitude and duration of the presynaptic action potential and the resulting postsynaptic potential, but does not block the release of transmitter. (Adapted from Katz and Miledi 1967a.)

A. The experimental arrangement is the same as in Figure 14-1, except that a current-passing electrode has been inserted into the presynaptic cell. (TEA = tetraethylammonium.)

B. The voltage-gated Na\(^+\) channels are completely blocked by adding tetrodotoxin (TTX) to the cell-bathing solution. Each set of three traces represents (from bottom to top) the depolarizing current pulse injected into the presynaptic terminal (I), the resulting potential in the presynaptic terminal (Pre), and the postsynaptic potential generated as a result of transmitter release onto the postsynaptic cell (Post). Progressively stronger current pulses are applied to produce correspondingly greater depolarizations of the presynaptic terminal (2-4). These presynaptic depolarizations cause postsynaptic potentials even in the absence of Na\(^+\) flux. The greater the presynaptic depolarization, the larger the postsynaptic potential, indicating that membrane potential exerts a direct control over transmitter release. The presynaptic depolarizations are not maintained throughout the duration of the depolarizing current pulse because of the delayed activation of the voltage-gated K\(^+\) channels, which causes repolarization.

C. After the voltage-gated Na\(^+\) channels of the action potential have been blocked, tetraethylammonium (TEA) is injected into the presynaptic terminal to block the voltage-gated K\(^+\) channels as well. Each set of three traces represents current pulse, presynaptic potential, and postsynaptic potential as in part B. Because the presynaptic K\(^+\) channels are blocked, the presynaptic depolarization is maintained throughout the current pulse. The large sustained presynaptic depolarizations produce large sustained postsynaptic potentials (2-4). This indicates that neither Na\(^+\) nor K\(^+\) channels are required for effective transmitter release.

D. Blocking both the Na\(^+\) and K\(^+\) channels permits the measurement of a more complete input-output curve than that in Figure 14-1. In addition to the steep part of the curve, there is now a plateau. Thus, beyond a certain level of presynaptic depolarization, further depolarization does not cause any additional release of transmitter. The initial level of the presynaptic membrane potential was about \(-70\) mV.

Transmitter Release Is Triggered by Calcium Influx

Katz and Miledi then turned their attention to Ca\(^{2+}\) ions. Earlier, José del Castillo and Katz had found that
increasing the extracellular Ca\(^{2+}\) concentration enhanced transmitter release; lowering the extracellular Ca\(^{2+}\) concentration reduced and ultimately blocked synaptic transmission. However, since 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 had identified a class of voltage-gated Ca\(^{2+}\) channels. As there is a very large inward electrochemical driving force on Ca\(^{2+}\)—the extracellular Ca\(^{2+}\) concentration is normally four orders of magnitude greater than the intracellular concentration—opening of voltage-gated Ca\(^{2+}\) channels would result in a large Ca\(^{2+}\) influx. These Ca\(^{2+}\) channels are, however, sparsely distributed along the main axon. Katz and Miledi proposed that the Ca\(^{2+}\) channels might be much more abundant at the presynaptic terminal and that Ca\(^{2+}\) might serve dual functions: as a carrier of depolarizing charge during the action potential (like Na\(^{+}\)) and as a special signal conveying information about changes in membrane potential to the intracellular machinery responsible for transmitter release.

Direct evidence for the presence of a voltage-gated Ca\(^{2+}\) current at the squid presynaptic terminal was provided by Rodolfo Llinás and his colleagues. Using a microelectrode voltage clamp, Llinás depolarized the terminal while blocking the voltage-gated Na\(^{+}\) and K\(^{+}\) channels with tetrodotoxin and tetraethylammonium, respectively. He found that graded depolarizations activated a graded inward Ca\(^{2+}\) current, which in turn resulted in graded release of transmitter (Figure 14-3). The Ca\(^{2+}\) current is graded because the Ca\(^{2+}\) channels possess voltage-dependent activation gates, like the voltage-gated Na\(^{+}\) and K\(^{+}\) channels. The Ca\(^{2+}\) channels in the squid terminals differ from Na\(^{+}\) channels, however, in that they do not inactivate quickly but stay open as long as the presynaptic depolarization lasts. One striking feature of transmitter release at all synapses is its steep and nonlinear dependence on Ca\(^{2+}\) influx—a two-fold increase in Ca\(^{2+}\) influx can increase transmitter release up to 16-fold. This relationship indicates that at some site—called the calcium sensor—the binding of up to four Ca\(^{2+}\) ions is required to trigger release.

Even in the axon terminal Ca\(^{2+}\) currents are small and are normally masked by Na\(^{+}\) and K\(^{+}\) currents, which are 10–20 times larger. However, in the region of the active zone (the site of transmitter release) Ca\(^{2+}\) influx is 10 times greater than elsewhere in the terminal. This localization is consistent with the distribution of intramembranous particles seen in freeze-fracture electron micrographs and thought to be the Ca\(^{2+}\) channels (see Figure 14-7 in Box 14-2).

The localization of Ca\(^{2+}\) channels at active zones provides a high, local rise in Ca\(^{2+}\) concentration at the site of transmitter release during the action potential. Indeed, during an action potential the Ca\(^{2+}\) concentration at the active zone can rise more than a thousandfold (to ~100 \(\mu\)M) within a few hundred microseconds. This large and rapid increase is required for the rapid synchronous release of transmitter. The calcium sensor responsible for fast transmitter release is thought to have a low affinity for Ca\(^{2+}\). On the order of 50–100 \(\mu\)M intracellular Ca\(^{2+}\) is required to trigger release, whereas only 1 \(\mu\)M of Ca\(^{2+}\) is required for many enzymatic reactions. Because of the low-affinity calcium sensor, release only takes place in a narrow region surrounding the intracellular mouth of a Ca\(^{2+}\) channel, the only location where the Ca\(^{2+}\) concentration is sufficient to trigger release. The requirement for a high concentration of Ca\(^{2+}\) also ensures that release will be rapidly terminated upon repolarization. Once the Ca\(^{2+}\) channels close, the
high local Ca$^{2+}$ concentration dissipates rapidly (within 1 ms) because of diffusion.

Calcium channels open somewhat more slowly than the Na$^+$ channels and therefore Ca$^{2+}$ influx does not occur until the action potential in the presynaptic cell has begun to repolarize (Figure 14-4). The delay that is characteristic of chemical synaptic transmission—the time from the onset of the action potential in the presynaptic terminals to the onset of the postsynaptic potential—is due in large part to the time required for Ca$^{2+}$ channels to open in response to depolarization. However, because the voltage-dependent Ca$^{2+}$ channels are located very close to the transmitter release sites, Ca$^{2+}$ needs to diffuse only a short distance, permitting transmitter release to occur within 0.2 ms of Ca$^{2+}$ entry!

As we shall see later in this chapter, the duration of the action potential is an important determinant of the amount of Ca$^{2+}$ that flows into the terminal. If the action potential is prolonged, more Ca$^{2+}$ flows into the cell and therefore more transmitter is released, causing a greater postsynaptic potential.

Calcium channels are found in all nerve cells as well as in cells outside the nervous system, such as skeletal and cardiac muscle cells, where the channels are important for excitation-contraction coupling, and endocrine cells, where they mediate release of hormones. There are many types of Ca$^{2+}$ channels—called L, P/Q, N, R, and T—with specific biophysical and pharmacological properties and different physiological functions. The distinct properties of these channel types are determined by the identity of their pore-forming subunit (termed the α1-subunit), which is encoded by a family of related genes (Table 14-1). Calcium channels also have associated subunits (termed α2, β, γ, and δ) that modify the properties of the channel formed by the α1-subunits. All α1-subunits are homologous to the voltage-gated Na$^+$ channel α-subunits, consisting of four repeats of a basic domain containing six transmembrane segments (including an S4 voltage-sensor) and a pore-lining P region (see Figure 9-14).

Most nerve cells contain more than one type of Ca$^{2+}$ channel. Channels formed from the different α1-subunits can be distinguished by their different voltage-dependent gating properties, their distinctive sensitivity to pharmacological blockers, and their specific physiological function. The L-type channels are selectively blocked by the dihydropyridines, a class of clinically important drugs used to treat hypertension. The P/Q-type channels are selectively blocked by ω-agatoxin IVA, a component of the venom of the funnel web spider. The N-type channels are blocked selectively by a toxin obtained from the venom of the marine cone snail, the ω-conotoxin GVIA. The L-type, P/Q-type, N-type, and R-type channels all require fairly strong depolarizations for their activation (voltages positive to −40 to −20 mV are required), and are thus often referred to as high-voltage-activated Ca$^{2+}$ channels. In contrast, T-type Ca$^{2+}$ channels are low-voltage-activated Ca$^{2+}$ channels that open in response to small depolarizations around the threshold for generating an action potential (−60 to −40 mV). 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, both in the brain and the heart.

In neurons the rapid release of conventional transmitters associated with fast synaptic transmission is mediated by three main classes of Ca\(^{2+}\) channels: the P/Q-type, the N-type, and R-type channels. The L-type channels do not contribute to fast transmitter release but are important for the slower release of neuropeptides from neurons and of hormones from endocrine cells. The fact that Ca\(^{2+}\) influx through only certain types of Ca\(^{2+}\) channels, can control transmitter release is presumably due to the fact that these channels are concentrated at active zones. Localization of the N-type Ca\(^{2+}\) channels at the active zones has been visualized with fluorescently labeled ω-conotoxin at the frog neuromuscular junction (Figure 14-5). By contrast, L-type channels may be excluded from active zones, limiting their participation to slow synaptic transmission.

### Transmitter Is Released in Quantal Units

How and where does Ca\(^{2+}\) influx trigger release? To answer that question we must first consider how transmitter substances are released. Even though the release of synaptic transmitter appears smoothly graded, it is actually released in discrete packages 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 from an integral number of quanta! responses (Figure 14-6). Synaptic potentials seem smoothly graded in recordings only because each quantal (or unit) potential is small relative to the total potential.

Paul Fatt and Bernard Katz obtained the first clue as to the quantal nature of synaptic transmission when they made recordings from the nerve-muscle synapse of the frog without presynaptic stimulation and observed small spontaneous postsynaptic potentials of about 0.5 mV. Like the nerve-evoked end-plate potentials, these small depolarizing responses were largest at the site of nerve-muscle contact and decayed electronically with distance (see Figure 11-5). Similar results have since been obtained in mammalian muscle and in central neurons. Because the synaptic potentials at vertebrate nerve-muscle synapses are called end-plate potentials, Fatt and Katz called these spontaneous potentials **miniature end-plate potentials**.

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 evoked by nerve stimulation. Because acetylcholine (ACh) is the transmitter at the nerve-muscle synapse, the miniature end-plate potentials, like the end-plate potentials, are enhanced and prolonged by prostigmine, a drug that inhibits the hydrolysis of ACh by acetylcholinesterase. Likewise, the miniature end-plate potentials are reduced and finally abolished by agents that block the ACh receptor. In the absence of stimulation the miniature end-plate potentials occur at random intervals; their frequency can be increased by depolarizing the presynaptic terminal. They disappear if the presynaptic motor nerve degenerates but reappear when a new motor synapse is formed, indicating that these events represent small amounts of transmitter that are continuously released from the presynaptic nerve terminal.

What could account for the fixed size (around 0.5 mV) of the miniature end-plate potential? Del Castillo and Katz first tested the possibility that each quantum represented a fixed response due to the opening of a single ACh receptor-channel. By applying small amounts of
Figure 14-5 Calcium channels are concentrated at the neuromuscular junction in regions of the presynaptic nerve terminal opposite clusters of acetylcholine (ACH) receptors on the postsynaptic membrane. The fluorescent image shows the presynaptic Ca\(^{2+}\) channels in red, after labeling with a Texas red–coupled marine snail toxin that binds to Ca\(^{2+}\) channels. Postsynaptic ACh receptors are labeled in green with boron-dipyromethane difluoride-labeled 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. (From Robitaille et al. 1990.)

ACh to the frog muscle end-plate they were able to elicit depolarizing responses much smaller than 0.5 mV. From this it became clear that the miniature end-plate potential must reflect the opening of more than one ACh receptor-channel. In fact, Katz and Miledi were later able to estimate the elementary current through a single ACh receptor-channel as being only about 0.3 μV (see Chapter 6). This is about 1/2000 of the amplitude of a spontaneous miniature end-plate potential. Thus a miniature end-plate potential of 0.5 mV requires summation of the elementary currents of about 2000 channels. This estimate was later confirmed when the currents through single ACh-activated channels were measured directly using patch-clamp techniques (see Box 6-2).

Since the opening of a single channel requires the binding of two ACh molecules to the receptor (one molecule to each of the two α-subunits), and some of the released ACh never reaches the receptor molecules (either because it diffuses out of the synaptic cleft or is lost through hydrolysis), about 5000 molecules are needed to produce one miniature end-plate potential. This number has been confirmed by direct chemical measurement of the amount of ACh released with each quantal synaptic potential.

We can now ask some important questions. Is the normal postsynaptic potential evoked by nerve stimulation also composed of quantal responses that correspond to the quanta of spontaneously released transmitt-
Figure 14-6 Neurotransmitter is released in fixed increments, or quanta. Each quantum of transmitter produces a unit postsynaptic potential of fixed amplitude. The amplitude of the postsynaptic potential evoked by nerve stimulation is equal to the unit amplitude multiplied by the number of quanta of transmitter released.

A. Intracellular recordings from a muscle fiber at the endplate show the postsynaptic change in potential when eight consecutive stimuli of the same size are applied to the motor nerve. To reduce transmitter output and to keep the end-plate potentials small, the tissue was bathed in a Ca^{2+}-deficient (and Mg^{2+}-rich) solution. The postsynaptic responses to the stimulus vary. Two presynaptic impulses elicit no postsynaptic response (failures); two produce unit potentials; and the others produce responses that are approximately two to four times the amplitude of the unit potential. Note that the spontaneous miniature end-plate potentials (S) are the same size as the unit potential. (Adapted from Liley 1956.)

B. After many end-plate potentials were recorded, the number of responses at each amplitude was counted and then plotted 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. This unit response is the same amplitude as the spontaneous miniature end-plate potentials (inset). The other peaks in the histogram occur at amplitudes that are integral multiples of the amplitude of the unit potential. The red line shows a theoretical distribution composed of the sum of several Gaussian functions fitted to the data of the histogram. In this distribution each peak is slightly spread out, reflecting the fact that the amount of transmitter in each quantum, and hence the amplitude of the postsynaptic response, varies randomly about the peak. The number of events under each peak divided by the total number of events in the histogram is the probability that the presynaptic terminal releases the corresponding number of quanta. This probability follows a Poisson distribution (see Box 14-1). The distribution of amplitudes of the spontaneous miniature potentials, shown in the inset, is also fit by a Gaussian curve. (Adapted from Boyd and Martin 1956.)

Do you mean to ask: If so, what determines the number of quanta of transmitter released by a presynaptic action potential? Does Ca^{2+} alter the number of ACh molecules that make up each quantum or does it affect the number of quanta released by each action potential?

These questions were addressed by del Castillo and Katz in a study of synaptic signaling at the nerve-muscle synapse when the external concentration of Ca^{2+} is decreased. When the neuromuscular junction is bathed in a solution low in Ca^{2+}, the evoked end-plate potential (normally 70 mV in amplitude) is reduced markedly, to about 0.5-2.5 mV. Moreover, the amplitude of successively evoked end-plate potentials varies randomly from one stimulus to the next, and often no responses can be detected at all (termed failures). However, the minimum response above zero—the unit synaptic potential in response to a presynaptic potential—is identical in size (about 0.5 mV) and shape to the spontaneous miniature end-plate potentials. All end-plate potentials larger than the quantal synaptic potential are integral multiples of the unit potential (Figure 14-6).

Del Castillo and Katz could now ask: How does the rise of intracellular Ca^{2+} that accompanies each action potential affect the release of transmitter? They found
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number of quanta that are released in response to an action potential has only two possible outcomes—a quantum is or is not released. This event resembles a binomial or Bernoulli trial (similar to tossing a coin in the air to determine whether it comes up heads or tails). The probability of a quantum being released by an action potential is independent of the probability of other quanta being released by that action potential. Therefore, for a population of releasable quanta, each action potential represents a series of independent binomial trials (comparable to tossing a handful of coins to see how many coins come up heads).

In a binomial distribution, \( p \) stands for the average probability of success (i.e., the probability that any given quantum will be released) and \( q \) (or \( 1 - p \)) stands for the mean probability of failure. Both the average probability \( (p) \) that an individual quantum will be released and the store \( (n) \) of readily releasable quanta are assumed to be constant. (Any reduction in the store is assumed to be quickly replenished after each stimulus.) The product of \( n \) and \( p \) yields an estimate, \( m \), of the mean number of quanta that are released to make up the end-plate potential. This mean is called the **quantal content** or quantal output.

Calculation of the probability of transmitter release can be illustrated with the following example. Let us consider a terminal that has a releasable store of five quanta \((n = 5)\). If we assume that \( p = 0.1 \), then \( q \) (the probability that an individual quantum is not released from the terminals) is \( 1 - p \), or 0.9. We can now determine the probability that a stimulus will release no quanta (failure), a single quantum, two quanta, three quanta, or any number of quanta (up to \( n \)). The probability that none of the five available quanta will be released by a given stimulus is the product of the individual probabilities that each quantum will not be released: \( q^5 = 0.9^5 \), or 0.59. We would thus expect to see 59 failures in 100 stimuli. The probabilities of observing zero, one, two, three, four, or five quanta are represented by the successive terms of the binomial expansion:

\[
(q + p)^5 = q^5(\text{failures}) + 5q^4p(1 \text{ quantum}) + 10q^3p^2(2 \text{ quanta}) + 10q^2p^3(3 \text{ quanta}) + 5qp^4(4 \text{ quanta}) + p^5(5 \text{ quanta}).
\]

Thus, in 100 stimuli the binomial expansion would predict 33 unit responses, 7 double responses, 1 triple response, and 0 quadruple and quintuple responses.

Values for \( n \) vary from about 100-300 at the vertebrate nerve-muscle synapse, the squid giant synapse, and Aplysia central synapses, to as few as 1-4 in the synapses of the sympathetic ganglion and spinal cord of vertebrates. The probability of release \( p \) 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 1000 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 known. Although the parameter \( n \) is assumed to refer to the number of readily releasable (or available) quanta of transmitter, it may actually represent the number of release sites or active zones in the presynaptic terminals that are loaded with vesicles. 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 probability that a vesicle has 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 docked active zone. The parameter \( p \) is thought to depend on the presynaptic \( Ca^{2+} \) influx during an action potential.

The quantal size \((q)\) is the response of the postsynaptic membrane to 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 sensitivity 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.
potential has the same mean amplitude as that of the spontaneous miniature end-plate potentials led del Castillo and Katz to conclude that transmitter is released in fixed packets or quanta. When the external \( \text{Ca}^{2+} \) concentration is normal, an action potential in the presynaptic terminal releases about 150 quanta, each about 0.5 mV in amplitude, resulting in a large end-plate potential. In the absence of an action potential, the rate of quantal release is very low—only one quantum per second is released spontaneously at the end-plate. The rate of quantal release increases 100,000-fold when \( \text{Ca}^{2+} \) enters the presynaptic terminal with an action potential, bringing about the synchronous release of about 150 quanta in one or two milliseconds.

Transmitter Is Stored and Released by Synaptic Vesicles

What morphological features of the cell might account for the quantum of transmitter? The physiological observations indicating that transmitter is released in fixed quanta coincided with the discovery, through electron microscopy, of accumulations of small vesicles in the presynaptic terminal. The electron micrographs suggested to del Castillo and Katz that the vesicles were organelles for the storage of transmitter. They also argued that each vesicle stored one quantum of transmitter (amounting to several thousand molecules) and that each vesicle releases its entire contents into the synaptic cleft when the vesicle fuses with the inner surface of the presynaptic terminal at specific release sites.

At these sites, the active zones, a band of synaptic vesicles cluster above a fuzzy electron-dense material attached to the internal face of the presynaptic membrane, directly above the junctional folds in the muscle (see Figure 11-1). As we saw in Chapter 11, the neuromuscular junction in frogs contains about 300 active zones with a total of about \( 10^6 \) vesicles. Here, and at central synapses, the vesicles are typically clear, small, and ovoid, with a diameter of about 50 nm.

Neuropeptides and certain transmitters released from neuroendocrine cells are packaged in larger vesicles that contain an electron-dense material. These large dense-core vesicles are not localized at active zones. They can be released from anywhere within a neuron, including the cell body. Release of transmitter from large dense-core vesicles is associated with slow modulatory synaptic actions (see Chapter 13).

Quantal transmission has been demonstrated at all chemical synapses so far examined, with one exception, in the retina, which we shall examine in Chapter 26. At most synapses in the central nervous system each action potential releases only between 1 and 10 quanta, many fewer than the 150 quanta released at the nerve-muscle synapse. Whereas the surface area of a presynaptic motor terminal ending on a muscle fiber is large (about 2000–6000 \( \mu \)m\(^2\)) and contains about 300 active zones, a typical excitatory afferent fiber from a dorsal root ganglion cell forms only about four synapses on a motor neuron, each of which is about 2 \( \mu \)m\(^2\) and contains only one active zone.

Quantal analysis of transmitter release from these afferent neurons indicates that release from each active zone is all-or-none. That is, any given active zone releases either one quantum or none at all in response to a presynaptic action potential. The probability of release depends on the amount of \( \text{Ca}^{2+} \) influx during the action potential. Similar results have been obtained for other central synapses. Thus, variations in the response of a central neuron to a single presynaptic neuron result from the all-or-none release of one quantum from each of a few terminals, each usually with only one active zone.

Not all chemical signaling between neurons depends on vesicular storage and release. Some membrane-permeable substances, such as prostaglandins, the metabolites of arachidonic acid, and the gases CO and NO (see Chapter 13), can traverse the lipid bilayer of the membrane by diffusion. These substances may act at synapses either as chemical messengers or as retrograde signals that diffuse from the postsynaptic neuron back to the presynaptic neuron to regulate transmitter release. Other substances can be moved out of nerve endings by carrier proteins if their intracellular concentration is sufficiently high. In certain retinal glial cells, transporters for glutamate or GABA that normally take up transmitter into a cell from the extracellular space can reverse direction and release transmitter into the extracellular space. Still other substances simply leak out of nerve terminals at a low rate. For example, about 90% of the ACh that leaves the presynaptic terminal at the neuromuscular junction can be traced to continuous leakage. However, because this leakage is so diffuse and not targeted to receptors at the end-plate region, and because it is continuous and low level rather than synchronous and concentrated, it is ineffective functionally.

Synaptic Vesicles Discharge Transmitter by Exocytosis

Direct evidence that exocytosis of a single synaptic vesicle is responsible for the release of one quantum of transmitter was at first difficult to obtain, because the chance of finding a vesicle in the act of being discharged...
Box 14-2 Freeze-Fracture Technique

**Figure 14-7A.** Freeze-fracture exposes the intramembranous area to view. The path of membrane cleavage is along the hydrophobic interior of the lipid bilayer, resulting in two complementary fracture faces. The P face (corresponding to the cytoplasmic-facing leaflet of the bilayer) contains most of the integral membrane proteins (particles), because these are anchored to cytoskeletal structures. The E face (corresponding to the extracellular-facing leaflet of the bilayer) shows pits complementary to the integral protein particles. (Adapted from Fawcett 1981.)

**Figure 14-7B.** This idealized three-dimensional view of pre- and postsynaptic membranes shows the active zones with adjacent rows of synaptic vesicles, as well as places where the vesicles are undergoing exocytosis. The rows of particles on either side of the active zone are intramembranous proteins thought to be Ca²⁺ channels. (Adapted from Kuffler et al. 1984.)

Freeze fracture reveals the structural details of synaptic membranes. In this technique frozen tissue is broken open under a high vacuum and coated with platinum and carbon. Frozen membrane tends to break at the weakest plane, which is between the two molecular layers of lipids. Two complementary faces of the membrane are thus exposed: The leaflet nearest the cytoplasm (the interior half) is the protoplasmic (P) face, while the leaflet that borders the extracellular space is the external (E) face (Figure 14-7A).

Freeze-fracture exposes a large expanse of the presynaptic intramembranous area (Figure 14-7B). Deformations of the membrane that occur at the active zone, where vesicles are attached, are readily apparent. The advantage of the freeze-fracture technique is best appreciated by comparing a freeze-fracture electron micrograph with a conventional thin-section electron micrograph of the active zone (see Figure 14-8).
Exocytosis Involves the Formation of a Fusion Pore

Exactly how fusion of the synaptic vesicle membrane with the plasma membrane occurs and the role that Ca\textsuperscript{2+} plays in catalyzing this reaction is under intensive study. Morphological studies from mast cells using rapid freezing suggested that exocytosis depends on the temporary formation of a fusion pore that spans the membranes of the vesicle and plasma membrane. Subsequent studies of capacitance increases in mast cells showed that prior to complete fusion a channel-like fusion pore could be detected in the electrophysiological recordings (Figure 14-10). This fusion pore starts out with a single-channel conductance of around 200 pS, similar to that of gap-junction channels, which also bridge two membranes. During exocytosis the pore rapidly dilates, probably from around 1 nm to 50 nm, and the conductance increases dramatically (Figure 14-10A). In some instances the fusion pore flickers open and closed several times prior to complete fusion (Figure 14-10B).

Since transmitter release is so fast, fusion must occur within a fraction of a millisecond. Therefore, the proteins that fuse synaptic vesicles to the plasma membrane are most likely preassembled into a fusion pore that bridges the vesicle and plasma membranes before fusion occurs. Much like the gap-junction channels we learned about in Chapter 10, the fusion pore may consist of two hemichannels, one each in the vesicle membrane and the plasma membrane, which then join in the course of vesicle docking (Figure 14-10C). Calcium influx would then simply cause the preexisting pore to
The events of exocytosis at the presynaptic terminal are revealed by electron microscopy. The images on the left are freeze-fracture electron micrographs of the cytoplasmic half (P face) of the presynaptic membrane (compare Figure 14.7). Thin-section electron micrographs of the presynaptic membrane are shown on the right. (Adapted from Alberts et al. 1989.)

A. Parallel rows of intramembranous particles arrayed on either side of an active zone may be the voltage-gated Ca$^{2+}$ channels essential for transmitter release.

B. Synaptic vesicles begin fusing with the plasma membrane within 5 ms after the stimulus. Fusion is complete within another 2 ms. Each opening in the plasma membrane represents the fusion of one synaptic vesicle. In thin-section electron micrographs, vesicle fusion events are observed in cross section as Ω-shaped structures.

C. Membrane retrieval becomes apparent as coated pits form within about 10 s after fusion of the vesicles with the presynaptic membrane. After another 10 s the coated pits begin to pinch off by endocytosis to form coated vesicles. These vesicles include the original membrane proteins of the synaptic vesicle and also contain 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).
Figure 14-9 Capacitance measurements allow direct study of exocytosis and endocytosis.
A. Exocytosis from mast cells. Electron micrographs of a mast cell before (top) and after (bottom) inducing exocytosis. Mast cells are secretory cells of the immune system that contain large dense-core vesicles filled with the transmitter histamine. Exocytosis of mast cell 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 GTP in an intracellular recording electrode. (From Lawson et al., 1977.)

B. Stepwise increases in capacitance reflect the successive fusion of individual secretory vesicles with the cell membrane. The step increases are unequal because of a variability in the diameter (and thus 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 μm² of membrane area.) (Adapted from Fernandez et al. 1984.)

C. Exocytosis and membrane retrieval from a neuronal presynaptic terminal. Recordings were obtained from isolated synaptic terminals of bipolar neurons in the retina of the goldfish. Transmitter release was triggered by a depolarizing voltage-clamp step (applied at arrow), which elicited a large sustained Ca²⁺ current (inset). The Ca²⁺ influx causes a transient rise in the cytoplasmic Ca²⁺ concentration (bottom trace). This results in the exocytosis of several thousand small synaptic vesicles, leading to an increase in total capacitance (top trace). The increments in capacitance due to fusion of a single small synaptic vesicle are too small to resolve. As the internal Ca²⁺ concentration falls back to its resting level upon repolarization, the extra membrane area is rapidly retrieved and capacitance returns to its baseline value. (Adapted from von Gersdorff and Matthews 1994.)

Recent advances in chemical detection suggest that transmitter may be released through the fusion pore itself, prior to full dilation and vesicle fusion (Figure 14-10C). An electrochemical method termed voltammetry permits the detection of certain amine-containing transmitters, such as serotonin, using an extracellular carbon-fiber electrode (Figure 14-11). A large voltage is applied to the electrode, which leads to the oxidation of the released transmitter. This oxidation reaction releases free electrons, which can be detected as a transient electrical current that is proportional to the amount of transmitter released. In response to action potentials large transient increases in transmitter release are observed,
Transmitter is released from synaptic vesicles through the opening of a fusion pore that connects a secretory vesicle with the presynaptic membrane.

**A.** Patch-clamp recording setup for recording current through the fusion pore. As a vesicle fuses with the plasma membrane, the capacitance of the vesicle \((C_v)\) is initially connected to the capacitance of the rest of the cell \((C_m)\) through the high resistance \((r_p)\) of the fusion pore. (From Monck and Fernandez 1992.)

**B.** Electrical events associated with the opening of the fusion pore. Since the membrane potential of the vesicle (lumenal side negative) is normally much more negative than the membrane potential of the cell, there will be a transient flow of charge (current) from the vesicle to the cell membrane associated with fusion. This generates a transient current \((I)\) 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 \(\tau = C_d g_p = C_m g_p / r_p\). The fusion pore diameter can be calculated from the fusion 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. The fusion pore shows an initial conductance of around 200 pS, similar to the conductance of a gap-junction channel, corresponding to a pore diameter of around 2 nm. The conductance rapidly increases within a few milliseconds as the pore dilates to around 7–8 nm (dotted line). (From Spruce et al. 1990.)

**C.** Steps in exocytosis through a fusion pore. 1. A docked vesicle contains a preassembled fusion pore ready to open. 2. During the initial stages of exocytosis the fusion pore rapidly opens, allowing transmitter to leak out of the vesicle. 3. In most cases the fusion pore rapidly dilates as the vesicle undergoes complete fusion with the plasma membrane.

Synaptic Vesicles Are Recycled

If there were no process to compensate for the fusion of successive vesicles to the plasma membrane during continued nerve activity, the membrane of a synaptic terminal would enlarge and the number of synaptic vesicles would decline. This does not occur, however, because the vesicle membrane added to the terminal membrane is retrieved rapidly and recycled, generating new synaptic vesicles (Figure 14-12).
Figure 14-11 Transmitter release through the fusion pore can be measured using electrochemical detection methods. 
A. Setup for recording transmitter release by voltammetry. 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 electrode oxidizes certain amine-containing transmitters (such as serotonin or norepinephrine). This oxidation reaction generates one or more free electrons, which results in an electrical current that can be recorded through an amplifier ($A_T$) connected to the carbon electrode. The current is proportional to the amount of transmitter release. Membrane current and capacitance are recorded through the intracellular patch electrode amplifier ($A_m$).

B. Recordings of transmitter release and capacitance measurements from mast cell secretory vesicles indicate that the fusion pore may "flicker" (open and close several times) prior to complete membrane fusion. During these brief openings transmitter can diffuse out through the pore, producing a "foot" of low-level release that precedes a large spike of transmitter release upon a full fusion event. Sometimes the reversible fusion pore opening and closing is not followed by full fusion, resulting in "stand alone flicker" in which transmitter is released only by diffusion through the fusion pore. (From Neher 1993.)

C-D. Similar patterns of release of the transmitter serotonin are observed from Retzius neurons of the leech. The electron micrograph shows that these neurons package serotonin in both large, dense-core vesicles and small, clear synaptic vesicles (arrow). Amperometry measurements show that Ca$^{2+}$ elevation triggers both large spikes of serotonin release (top trace) and smaller release events (bottom trace) (note the difference in current scales). These correspond to fusion of the large dense-core vesicles and synaptic vesicles, respectively. The synaptic vesicles release their contents rapidly, in less than 1 ms. This rapid time course is consistent with the expected rate of diffusion of transmitter through a fusion pore of 300 pS. Each large vesicle contains around 15,000–300,000 molecules of serotonin. Each small vesicle contains approximately 5000 molecules of serotonin. (From Bruns and Jahn 1995.)
Figure 14-12 The cycling of synaptic vesicles at nerve terminals involves several distinct steps.

A. Free vesicles must be targeted to the active zone (1) and then dock at the active zone (2). The docked vesicles must become primed so that they can undergo exocytosis (3). In response to a rise in Ca\(^{2+}\), the vesicles undergo fusion and release their contents (4). The fused vesicle membrane is taken up into the interior of the cell by endocytosis (5). The endocytosed vesicles then fuse with the endosome, an internal membrane compartment. After processing, new synaptic vesicles bud off the endosome, completing the recycling process.

B. Retrieval of vesicles after exocytosis is thought to occur via three distinct mechanisms. In the first, classical pathway excess membrane is retrieved by means of clathrin-coated pits. These coated pits concentrate certain intramembranous particles into small packages. The pits are found throughout the terminal except at the active zones. As the plasma membrane enlarges during exocytosis, more membrane invaginations are coated on the cytoplasmic surface. (The path of the coated pits is shown by arrows after step 5.) This pathway may be important at normal to high rates of release. In the kiss-and-run pathway the vesicle does not completely integrate itself into the plasma membrane. This corresponds to release through the fusion pore. This pathway may predominate at lower to normal release rates. In the bulk endocytosis 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 reserved for retrieval after very high rates of release and may not be used during the usual functioning of the synapse. (Adapted from Schweizer et al. 1995.)

Although the number of vesicles in a nerve terminal does decrease transiently during release, the total amount of membrane in vesicles, cisternae, and plasma membrane remains constant, indicating that membrane is retrieved from the surface membrane into the internal organelles. How the synaptic vesicles are recycled has not yet been resolved, but the process is known to involve clathrin-coating of the vesicle and the protein dynamin (Chapter 4 and below) and is thought to be similar to known mechanisms in epithelial cells (Figure 14-12). According to this view, the excess membrane from synaptic vesicles that have undergone exocytosis is recycled through endocytosis into an intracellular organelle called the endosome. Endocytosis and recycling takes about 30 seconds to one minute to be completed.

More rapid components of membrane recovery have been detected with capacitance measurements. Importantly, the rate of membrane recovery appears to depend on the extent of stimulation and exocytosis. With relatively weak stimuli that release only a few vesicles, membrane retrieval is rapid and occurs within a few seconds (for example, see Figure 14-9B). Stronger stimuli that release more vesicles lead to a slowing of membrane recovery. The fastest form of vesicle cycling in-
volves the release of transmitter through the transient opening and closing of the fusion pore without full membrane fusion. The advantage of such "kiss-and-run" release is that it rapidly recycles the vesicle for subsequent release because it requires only closure of the fusion pore. Thus, different types of retrieval processes may operate under different conditions (Figure 14-12).

A Variety of Proteins Are Involved in the Vesicular Release of Transmitter

What is the nature of the molecular machinery that drives vesicles to cluster near synapses, to dock at active zones, to fuse with the membrane in response to Ca\(^{2+}\) influx, and then to recycle? Proteins have been identified that are thought to (1) restrain the vesicles so as to prevent their accidental mobilization, (2) target the freed vesicles to the active zone, (3) dock the targeted vesicles at the active zone and prime them for fusion, (4) allow fusion and exocytosis, and (5) retrieve the fused membrane by endocytosis (Figure 14-13).

We first consider proteins involved in restraint and mobilization. The vesicles outside the active zone represent a reserve pool of transmitter. They do not move about freely in the terminal but rather are restrained or anchored to a network of cytoskeletal filaments by the synapsins, a family of four proteins (Ia, Ib, IIa, and IIb). Of these four, synapsins Ia and Ib are the best studied. These two proteins are substrates for both the cAMP-dependent protein kinase and the Ca\(^{2+}\)/calmodulin-dependent kinase. When synapsin I is not phosphorylated, it is thought to immobilize synaptic vesicles by linking them to actin filaments and other components of the cytoskeleton. When the nerve terminal is depolarized and Ca\(^{2+}\) enters, synapsin I is thought to become phosphorylated by the Ca\(^{2+}\)/calmodulin-dependent protein kinase. Phosphorylation frees the vesicles from the cytoskeletal constraint, allowing them to move into the active zone (Figure 14-14).

The targeting of synaptic vesicles to docking sites for release may be carried out by Rab3A and Rab3C, two members of a class of small proteins, related to the ras proto-oncogene superfamily, that bind GTP and hydrolyze it to GDP and inorganic phosphate (Figure 14-14B). These Rab proteins bind to synaptic vesicles through a hydrophobic hydrocarbon group that is covalently attached to the carboxy terminus of the Rab protein. Hydrolysis of the GTP bound to Rab, converting it to GDP, may be important for the efficient targeting of synaptic vesicles to their appropriate sites of docking. During exocytosis the Rab proteins are released from the synaptic vesicles into the cytoplasm.

Following the targeting of a vesicle to its release site a complex set of interactions occurs between proteins in the synaptic vesicle membrane and proteins in the presynaptic membrane. Such interactions are thought to complete the docking of vesicles and to prime them so they are ready to undergo fusion in response to Ca\(^{2+}\) influx. Similar interactions are important for exocytosis in all cells, not only in the synaptic terminals of neurons.

As we have seen in Chapter 4, all secretory proteins are synthesized on ribosomes and injected into the lumen of the endoplasmic reticulum (ER). When these proteins leave the ER they are targeted to the Golgi apparatus in vesicles formed from the membrane of the ER. The vesicles then dock and fuse with the Golgi membrane, discharging their protein into the lumen of the Golgi, where the protein is modified. Other vesicles shuttle the secretory protein between the cis and the trans compartments (the different cisternae) of the Golgi apparatus until the protein becomes fully modified and mature. The mature protein is packaged in vesicles that bud off the Golgi and migrate to the cell surface, where the protein is released through exocytosis. This type of release is constitutive (that is the release is continuous and occurs independently of Ca\(^{2+}\)) in contrast to regulated release, which occurs at synapses in response to Ca\(^{2+}\) entry into the presynaptic terminal.

One prominent hypothesis for how membrane vesicles are docked and readied for exocytosis has been proposed by James Rothman, Richard Scheller, and Reinhard Jahn. According to this theory, specific integral proteins in the vesicle membrane (vesicle-SNAREs, or v-SNAREs) bind to specific receptor proteins in the target membrane (target membrane or t-SNARE) (Figure 14-15). In the brain two t-SNAREs have been identified: syntaxin, a nerve terminal integral membrane protein, and SNAP-25, a peripheral membrane protein of 25 kDa mass. In the synaptic vesicle the integral membrane protein VAMP (or synaptobrevin) has been identified as the v-SNARE.

The importance of the SNARE proteins in synaptic transmission is emphasized by the finding that all three proteins are targets of various clostridial neurotoxins. All of these toxins act by inhibiting synaptic transmission. One such toxin, tetanus toxin, a zinc endoprotease, specifically cleaves VAMP. Three other zinc endoproteases, botulinum toxins A, B, and C, specifically cleave SNAP-25, VAMP, and syntaxin, respectively. VAMP has the additional feature that it resembles a viral fusion peptide.

Reconstitution studies of purified proteins in lipid vesicles indicate that VAMP, syntaxin, and SNAP-25 may form the minimal functional unit that mediates membrane fusion. Moreover a detailed structural model
Figure 14-13 This diagram depicts characterized synaptic vesicle proteins and some of their postulated receptors and functions. Separate compartments are assumed for (1) storage (where vesicles are tethered to the cytoskeleton), (2) trafficking and targeting of vesicles to active zones, (3) the docking of vesicles at active zones and their priming for release, and (4) release. Some of these proteins represent the targets for neurotoxins that act by modifying transmitter release. VAMP (synaptobrevin), SNAP-25, and syntaxin are the targets for tetanus and botulinum toxins, two zinc-dependent metalloproteases, and are cleaved by these enzymes. α-Latrotoxin, a spider toxin that generates massive vesicle depletion and transmitter release, binds to the neurexins. 1. Synapsins are vesicle-associated proteins that are thought to mediate interactions between the synaptic vesicle and the cytoskeletal elements of the nerve terminal. 2. The Rab proteins (see Figure 14-14B) appear to be involved in vesicle trafficking within the cell and also in targeting of vesicles within the nerve terminal. 3. The docking, fusion, and release of vesicles appears to involve distinct interactions between vesicle proteins and proteins of the nerve terminal plasma membrane: VAMP (synaptobrevin) and synaptotagmin (p65) on the vesicle membrane, and syntaxins and neurexins on the nerve terminal membrane. Arrows indicate potential interactions suggested on the basis of in vitro studies. 4. The identity of the vesicle and plasma membrane proteins that comprise the fusion pore remains unclear. Synaptophysin, an integral membrane protein in synaptic vesicles, is phosphorylated by tyrosine kinases and may regulate release. Vesicle transporters are involved in accumulation of neurotransmitter within the synaptic vesicle (see Chapter 15).

has been proposed for how these proteins interact to promote membrane fusion (Figure 14-15B).

The ternary complex of VAMP, syntaxin, and SNAP-25 is extraordinarily stable. For efficient vesicle recycling to occur this complex must be disassembled by the binding of two soluble cytoplasmic proteins: the N-ethylmaleimide-sensitive fusion (NSF) protein and the soluble NSF attachment protein (SNAP—this protein is unrelated to SNAP-25; the similar names are coincidental). The v-SNARES and t-SNARES serve as receptors for SNAP (hence their name SNAP receptors), which then binds NSF. The NSF is an ATPase, utilizing the energy released upon hydrolysis of ATP to unravel the SNARE assembly.
Figure 14-14 The mobilization, docking, and function of synaptic vesicles are controlled by Ca\(^{2+}\) and low-molecular-weight GTP-binding proteins.

A. Synaptic vesicles in nerve terminals are sequestered in a storage compartment where they are tethered to the cytoskeleton, as well as in a releasable compartment where they are docked to the presynaptic membrane. Entry of Ca\(^{2+}\) into the nerve terminal leads to the opening of the fusion pore complex and neurotransmitter release. Calcium entry also frees vesicles from the storage compartment through phosphorylation of synapsins, thus increasing the availability of vesicles for docking at the presynaptic plasma membrane.

B. The Rab3A cycle targets vesicles to their release sites. Rab3A complexed to GTP binds to synaptic vesicles. During the targeting of synaptic vesicles to the active zone, Rab3A hydrolyzes its bound GTP to GDP. GTP hydrolysis may serve to make a reversible reaction irreversible, preventing vesicles from leaving the active zone once they arrive. During fusion and exocytosis, Rab3A-GDP dissociates from the vesicle. There is then an exchange of GTP for GDP. This is followed by the association of Rab3A-GTP with a new synaptic vesicle, thus completing the cycle.

One additional integral membrane protein of the synaptic vesicle, thought to be important for exocytosis, is synaptotagmin (or p65). Synaptotagmin contains two domains (the C2 domains) homologous to the regulatory region of protein kinase C. The C2 domains bind to phospholipids in a calcium-dependent manner. This property suggests that synaptotagmin might insert into the presynaptic phospholipid bilayer in response to Ca\(^{2+}\) influx, thus serving as the calcium sensor for exocytosis (see Figure 14-12). Synaptotagmin may also function as a v-SNARE since it binds syntaxin and a SNAP isoform.

Several mutant animals that lack synaptotagmin have been created to test this protein’s role in synaptic transmission. Based on these experiments two models have been proposed for the role of synaptotagmin. According to one view synaptotagmin acts as a fusion clamp or negative regulator of release (preventing exocytosis in the absence of Ca\(^{2+}\)). In this view, the influx of Ca\(^{2+}\) rapidly frees this clamp, allowing synchronous release. This hypothesis is attractive since the same machinery involved in synaptic vesicle fusion (the SNAP-SNARE complex) also functions in constitutive release that is independent of external Ca\(^{2+}\). This model is based on results from experiments with Drosophila and nematode mutants lacking synaptotagmin, which show greatly impaired synaptic transmission in response to an action potential in the presynaptic terminal. Moreover, in Drosophila the rate of spontaneous miniature end-plate potentials is increased, suggesting that synaptotagmin has an inhibitory role.

The second hypothesis is that synaptotagmin serves as a positive regulator of release, actively promoting vesicle fusion. This view is based on the observation that in mutant mice that lack a major isoform of synaptotagmin, fast synaptic transmission is blocked without an increase in spontaneous release. Since there are several isoforms of synaptotagmin in mammals, but only one isoform in invertebrates, it is possible that the different mammalian isoforms have different roles. One
may mediate regulated fast release and another may control constitutive release.

Synaptotagmin may also play an additional role in endocytosis. Following exocytosis the fused membrane is retrieved by endocytosis. Excess membrane anywhere in the terminal except at the active zone leads to the formation of a pit that is coated with clathrin. The binding of clathrin to the membrane is enhanced by certain adaptor proteins. Synaptotagmin serves as a receptor for the clathrin adaptor protein AP-2. The clathrin coat forms a regular lattice around the pit, which finally pinches off as a small coated vesicle. The pinching off of the vesicle depends on a cytoplasmic GTPase called dynamin, which forms a constricting helical ring around the neck of the vesicle during endocytosis. A Drosophila mutant defective in dynamin is impaired in synaptic transmission owing to an inhibition of vesicle recycling.

**Figure 14-15** The molecular machinery for fusion and exocytosis.

A. The SNARE hypothesis. Vesicle and target membrane compartments have distinct receptors—the v-SNARES (blue) and the t-SNARES (red)—that mediate docking and fusion (steps 1–4). Following fusion, two cytoplasmic proteins, NSF and SNAP, bind to the SNARE complex and disassemble it (steps 5 and 6).

B. Model of the minimal fusion apparatus. At presynaptic terminals the v-SNARE VAMP (blue) binds to the two t-SNAREs: syntaxin (red) and SNAP-25 (green). The ternary complex consists of a coil of four α-helices, one each from VAMP and syntaxin and two from one molecule of SNAP-25. This coiled-coil structure is oriented parallel to the plane of the membrane, bringing the vesicle and target membranes in close apposition and thus promoting fusion. The sites of cleavage by botulinum (BoNT) and tetanus toxin (TeNT) are indicated.
Part III / Elementary Interactions Between Neurons: Synaptic Transmission

The Amount of Transmitter Released Can Be Modulated by Regulating the Amount of Calcium Influx During the Action Potential

The effectiveness of chemical synapses can be modified for both short and long periods. This modifiability, or synaptic plasticity, is controlled by two types of processes: (1) processes within the neuron that result from changes in the resting potential or the firing of action potentials and (2) extrinsic processes, such as the synaptic input from other neurons.

Long-term changes in chemical synaptic action are crucial to development and learning, and we consider these changes in detail later in the book. Here we shall first discuss the short-term changes—changes in the amount of transmitter released due to either changes within the presynaptic terminal or extrinsic factors.

Intrinsic Cellular Mechanisms Regulate the Concentration of Free Calcium

As we saw at the beginning of this chapter, transmitter release depends strongly on the intracellular Ca\(^{2+}\) concentration. Thus, mechanisms within the presynaptic terminal that affect the concentration of free Ca\(^{2+}\) in the presynaptic terminal also affect the amount of transmitter released. In some cells there is a small steady influx of Ca\(^{2+}\) through the presynaptic terminal membrane, even at the resting membrane potential. This Ca\(^{2+}\) flows through the L-type voltage-gated Ca\(^{2+}\) channels, which inactivate little, if at all.

The steady state Ca\(^{2+}\) influx is enhanced by depolarization and decreased by hyperpolarization. A slight depolarization of the membrane can increase the steady state influx of Ca\(^{2+}\) and thus enhance the amount of transmitter released by subsequent action potentials. A slight hyperpolarization has the opposite effect (Figure 14-16). By altering the amount of Ca\(^{2+}\) that flows into the terminal, small changes in the resting membrane potential can make an effective synapse inoperative or a weak synapse highly effective. Such changes in membrane potential can also be produced by other neurons releasing transmitter at axo-axonic synapses that regulate presynaptic ion channels, as described later. They can also be produced experimentally by injecting current.

Synaptic effectiveness can also be altered in most nerve cells by intense activity. In these cells a high-frequency train of action potentials is followed by a period during which action potentials produce successively larger postsynaptic potentials. High-frequency stimulation of the presynaptic neuron (which in some cells can generate 500–1000 action potentials per second) is called tetanic stimulation. The increase in size of the postsynaptic potentials during tetanic stimulation is called potentiation; the increase that persists after tetanic stimulation is called posttetanic potentiation. This enhancement usually lasts several minutes, but it can persist for an hour or more (Figure 14-17).

Posttetanic potentiation is thought to result from a transient saturation of the various Ca\(^{2+}\) buffering systems in the presynaptic terminals, primarily the smooth endoplasmic reticulum and mitochondria. This leads to a temporary excess of Ca\(^{2+}\), called residual Ca\(^{2+}\), the result of the relatively large influx that accompanies the train of action potentials. The increase in the resting concentration of free Ca\(^{2+}\) enhances synaptic transmission for many minutes or longer by activating certain enzymes that are sensitive to the enhanced levels of resting Ca\(^{2+}\), for example, the Ca\(^{2+}\)/calmodulin-dependent protein kinase. Activation of such calcium-dependent enzymatic pathways is thought to increase the mobilization of synaptic vesicles in the terminals, for example through phosphorylation of the synapsins. Phosphorylation of synapsin allows synaptic vesicles to be freed from their cytoskeletal restraint and to be mobilized into

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**Figure 14-16** Changes in membrane potential of the presynaptic terminal affect the intracellular concentration of Ca\(^{2+}\) and thus the amount of transmitter released. When the presynaptic membrane is at its normal resting potential, an action potential (top trace) produces a postsynaptic potential of a given size (bottom). Hyperpolarizing the presynaptic terminal by 10 mV prior to an action potential decreases the steady state Ca\(^{2+}\) influx, so that the same-size action potential produces a smaller postsynaptic potential. In contrast, depolarizing the presynaptic neuron by 10 mV increases the steady state Ca\(^{2+}\) influx, so that the same-size action potential produces a postsynaptic potential large enough to trigger an action potential in the postsynaptic cell.
and docked at release sites. As a result, each action potential sweeping into the terminals of the presynaptic neuron will release more transmitter than before.

Here then is a simple kind of cellular memory! The presynaptic cell stores information about the history of its activity in the form of residual Ca²⁺ in its terminals. The storage of biochemical information in the nerve cell, after a brief period of activity, leads to a strengthening of the presynaptic connection that persists for many minutes. In Chapter 62 we shall see how posttetanic potentiation at certain synapses is followed by an even longer-lasting process (also initiated by Ca²⁺ influx), called long-term potentiation, which can last for many hours or even days.

Axo-axonic Synapses on Presynaptic Terminals Regulate Intracellular Free Calcium

Synapses are formed on axon terminals as well as the cell body and dendrites of neurons (see Chapter 12). Whereas 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 branches of the axon. One important action of axo-axonic synapses is to control Ca²⁺ influx into the presynaptic terminals of the postsynaptic cell, either depressing or enhancing transmitter release.

As we saw in Chapter 12, when one neuron hyperpolarizes the cell body (or dendrites) of another, it decreases the likelihood that the postsynaptic cell will fire; this action is called postsynaptic inhibition. In contrast, when a neuron contacts the axon terminal of another cell, it can reduce the amount of transmitter that will be released by the second cell onto a third cell; this action is called presynaptic inhibition (Figure 14-18A). Likewise, axo-axonic synaptic actions can increase the amount of transmitter released by the postsynaptic cell; this action is called presynaptic facilitation (Figure 14-18B). For reasons that are not well understood, presynaptic modulation usually occurs early in sensory pathways.

The best-analyzed mechanisms of presynaptic inhibition and facilitation are in the neurons of invertebrates and in the mechanoreceptor neurons (whose cell bodies lie in dorsal root ganglia) of vertebrates. Three mechanisms for presynaptic inhibition have been identified in these cells. One is mediated by activation of metabotropic receptors and in the simultaneous closure of Ca²⁺ channels and opening of voltage-gated K⁺ channels, which both decreases the influx of Ca²⁺ and enhances repolarization of the cell. The second mechanism is mediated by activation of ionotropic GABA-gated Cl⁻ channels, resulting in an increased conductance to Cl⁻, which decreases (or short-circuits) the amplitude of the action potential in the presynaptic terminal. As a result, less depolarization is produced and fewer Ca²⁺ channels are activated by the action potential. The third mechanism is also mediated by activation of metabotropic receptors and involves direct inhibition of the transmitter release machinery, independent of Ca²⁺ influx. This is thought to work by decreasing the Ca²⁺ sensitivity of one or more steps involved in the release process.

Presynaptic facilitation, in contrast, can be caused by an enhanced influx of Ca²⁺. In certain molluscan neurons serotonin acts through cAMP-dependent protein phosphorylation to close K⁺ channels, thereby broadening the action potential and allowing the Ca²⁺ influx to persist for a longer period (see Chapter 13).
addition, the cAMP-dependent protein kinase also acts directly on the machinery of exocytosis to enhance release in a manner that is independent of the amount of Ca\(^{2+}\) influx. In other cells activation of presynaptic ligand-gated channels, such as nicotinic ACh receptors or the kainate type of glutamate receptors, increases transmitter release, possibly by depolarizing the presynaptic terminals and enhancing Ca\(^{2+}\) influx.

Thus, regulation of the free Ca\(^{2+}\) concentration in the presynaptic terminal is an important factor in a variety of mechanisms that endow chemical synapses with plastic capabilities. Although we know a fair amount about short-term changes in synaptic effectiveness—changes that last minutes and hours—we are only beginning to learn about changes that persist days, weeks, and longer. These long-term changes often require alteration in gene expression and growth of synapses in addition to alteration in Ca\(^{2+}\) influx and enhancement of release from preexisting synapses.

**An Overall View**

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

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 ca-
pable of activating many cell functions. Calcium channels...serve as the only link to transduce depolarization into all the nonelectrical 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 neurotransmitters 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 and each vesicle contains approximately 5000 transmitter molecules. Release of transmitter from a single vesicle results in a quantal synaptic potential. Spontaneous miniature synaptic potentials result from the spontaneous fusion of single synaptic vesicles. Synaptic potentials evoked by nerve stimulation are composed of integral multiples of the quantal potential. 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, there is an increase in the number of vesicles released and a larger postsynaptic potential.

Rapid freezing experiments have shown that the vesicles fuse with the presynaptic plasma membrane in the vicinity of the active zone. Freeze-fracture studies have also revealed rows of large intramembranous particles along the active zone that are thought to be Ca\(^{2+}\) channels. These highly localized channels may be responsible for the rapid increase, as much as a thousand-fold, in the Ca\(^{2+}\) concentration of the axon terminal during an action potential. One hypothesis about how Ca\(^{2+}\) triggers vesicle fusion is that this ion permits the formation of a fusion pore that traverses both the vesicle and the plasma membrane. This pore allows the contents of the vesicle to be released into the extracellular space and may further dilate so that the entire vesicle fuses with the presynaptic plasma membrane.

Calcium also regulates the mobilization of the synaptic vesicles to the active zone. These vesicles appear to be bound to the cytoskeleton by synapsin, and Ca\(^{2+}\) is thought to free the vesicles by activating the Ca\(^{2+}\)/calmodulin-dependent protein kinase, which phosphorylates the synapsins.

Several molecular candidates have been identified that could account for the two other components of release: targeting and docking. Targeting is thought to be mediated by the small GTP-binding Rab3A and Rab3C proteins. Docking and fusion is thought to involve the synaptic vesicle v-SNARE VAMP (or synaptobrevin) and the plasma membrane t-SNARES, syntaxin and SNAP-25. Calcium binding to synaptotagmin may actively promote vesicle fusion or remove an inhibitory clamp that normally blocks fusion.

Finally, 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 (intrinsic) potentiation, which lasts a few minutes, is caused by Ca\(^{2+}\) left in the terminal after the large Ca\(^{2+}\) influx that occurs during the train of action potentials. Tonic depolarization or hyperpolarization of the presynaptic neuron can also modulate release by altering steady state Ca\(^{2+}\) influx. The extrinsic action of neurotransmitters on receptors in the axon terminal of another neuron can facilitate or inhibit transmitter release by altering the steady state level of resting Ca\(^{2+}\) or the Ca\(^{2+}\) influx during the action potential.

In the next chapter we shall carry our discussion of synaptic transmission further by examining the nature of the transmitter molecules that are used for chemical transmission.

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Selected Readings


Schweizer FE, Betz H, Augustine GJ. 1995. From vesicle

References


