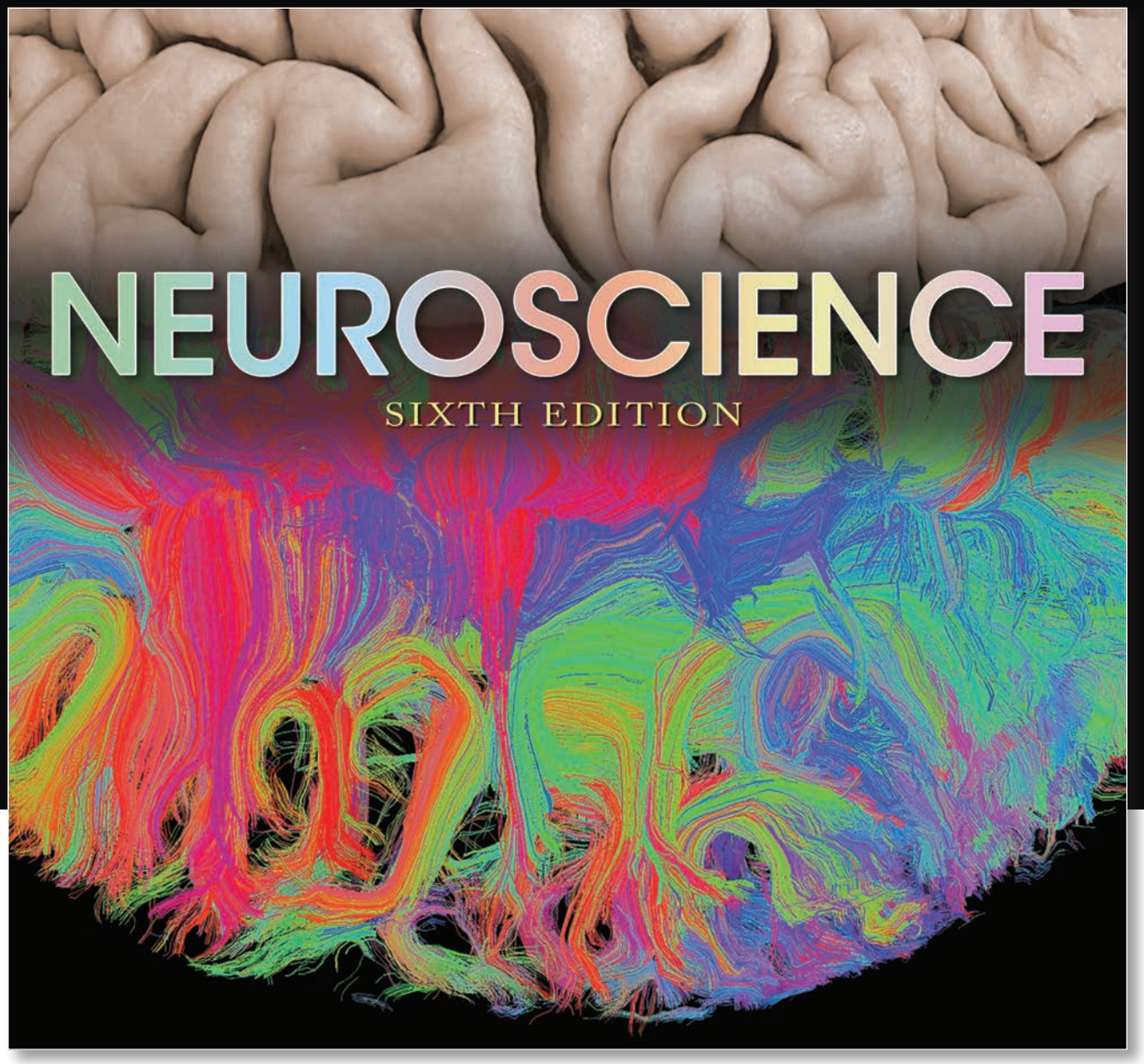

EDITORS

Dale Purves • George J. Augustine

David Fitzpatrick • William C. Hall • Anthony-Samuel LaMantia

Richard D. Mooney • Michael L. Platt • Leonard E. White

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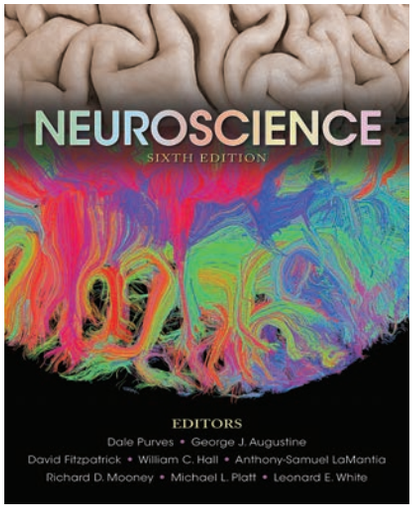

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Sinauer Associates
23 Plumtree Road
Sunderland, MA 01375 USA
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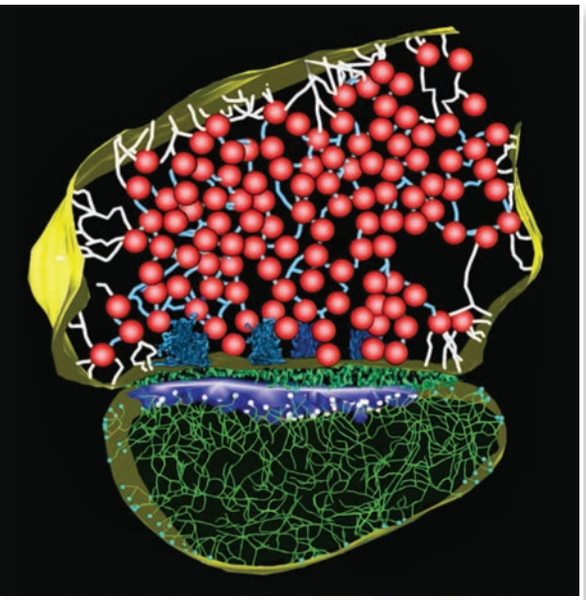
CHAPTER

5 Synaptic Transmission



Overview

THE HUMAN BRAIN CONTAINS 86 billion neurons, each with the ability to influence many other cells. Clearly, sophisticated and highly efficient mechanisms are needed to enable communication among this astronomical number of elements. Such communication is made possible by synapses, the functional contacts between neurons. Two different types of synapses—electrical and chemical—can be distinguished on the basis of their mechanism of transmission. At electrical synapses, current flows through connexons, which are specialized membrane channels that connect two cells at gap junctions. In contrast, chemical synapses enable cell-to-cell communication via the secretion of neurotransmitters; these chemical agents released by the presynaptic neurons produce secondary current flow in postsynaptic neurons by activating specific neurotransmitter receptors. The total number of neurotransmitters is well over 100. Virtually all neurotransmitters undergo a similar cycle of use: synthesis and packaging into synaptic vesicles; release from the presynaptic cell; binding to postsynaptic receptors; and finally, rapid removal or degradation. The influx of Ca^{2+} through voltage-gated channels triggers the secretion of neurotransmitters; this, in turn, gives rise to a transient increase in Ca^{2+} concentration in the presynaptic terminal. The rise in Ca^{2+} concentration causes synaptic vesicles to fuse with the presynaptic plasma membrane and release their contents into the space between the pre- and postsynaptic cells. Proteins on the surface of the synaptic vesicle and the presynaptic plasma membrane mediate the triggering of exocytosis by Ca^{2+}. Neurotransmitters evoke postsynaptic electrical responses by binding to members of a diverse group of neurotransmitter receptors. There are two major classes of receptors: those in which the receptor molecule is also an ion channel, and those in which the receptor and ion channel are separate entities. These receptors give rise to electrical signals by transmitter-induced opening or closing of the ion channels. Whether the postsynaptic actions of a particular neurotransmitter are excitatory or inhibitory is determined by the ion permeability of the ion channel affected by the transmitter, and by the electrochemical gradient for the permeant ions.

Two Classes of Synapses

The many kinds of synapses in the human brain fall into two general classes: electrical synapses and chemical synapses. These two classes of synapses can be distinguished based on their structures and the mechanisms they use to transmit signals from the "upstream" neuron, called the **presynaptic** element, and the "downstream" neuron, termed **postsynaptic**.

The structure of an electrical synapse is shown schematically in Figure 5.1A. Electrical synapses permit direct, passive flow of electrical current from one neuron to

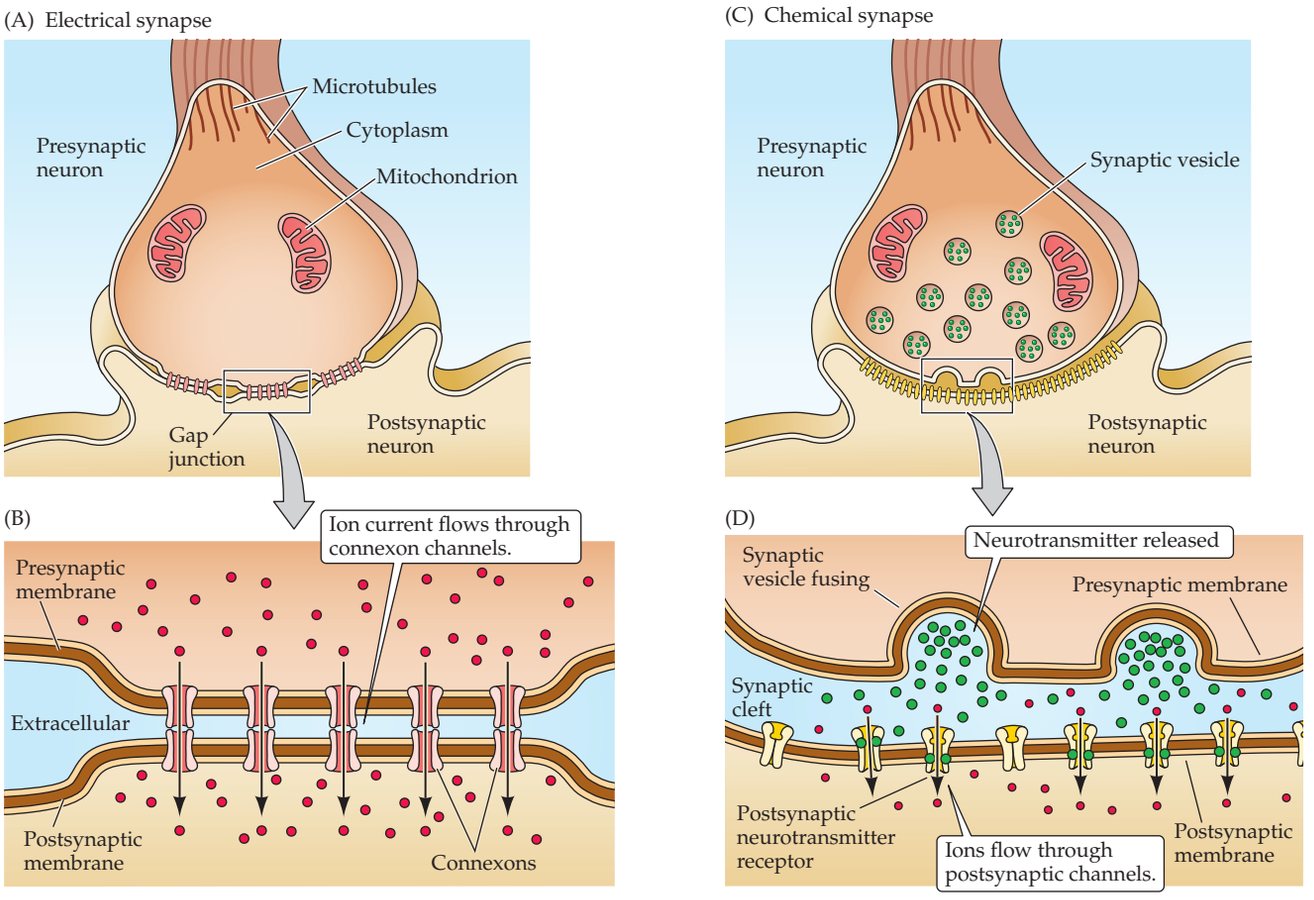


FIGURE 5.1 Electrical and chemical synapses differ fundamentally in their transmission mechanisms. (A) At electrical synapses, gap junctions occur between pre- and postsynaptic membranes. (B) Gap junctions contain connexon channels that permit current to flow passively from the presynaptic cell to the postsynaptic cell. (C) At chemical synapses, there is no intercellular continuity, and thus no direct flow of current from pre- to postsynaptic cell. (D) Synaptic current flows across the postsynaptic membrane only in response to the secretion of neurotransmitters, which open or close postsynaptic ion channels after binding to receptor molecules on the postsynaptic membrane.

another. The usual source of this current is the potential difference generated locally by the presynaptic action potential (see Chapter 3). Current flow at electrical synapses arises at an intercellular specialization called a **gap junction**, where membranes of the two communicating neurons come extremely close to one another and are linked together (Figure 5.1B). Gap junctions contain a unique type of channel, termed a **connexon**, which provides the path for electrical current to flow from one neuron to another (see Figure 5.2).

The general structure of a chemical synapse is shown schematically in Figure 5.1C. The space between the pre- and postsynaptic neurons is substantially greater at chemical synapses than at electrical synapses and is called the **synaptic cleft**. However, the most important structural feature of all chemical synapses is the presence of small, membrane-bounded organelles called **synaptic vesicles** within the presynaptic terminal. These spherical organelles are filled with one or more **neurotransmitters**, chemical signals that are secreted from the presynaptic neuron and detected by specialized receptors on the postsynaptic cell (Figure 5.1D). These chemical agents act as messengers between the communicating neurons and give this type of synapse its name.

Signaling Transmission at Electrical Synapses

Figure 5.2A shows an electron micrograph of an electrical synapse from a mammalian brain. As in the diagrams in Figure 5.1A and B, it can be seen that the processes of the presynaptic and postsynaptic neurons are connected via a gap junction (Figure 5.2B). The connexons contained within gap junctions are key to understanding how electrical

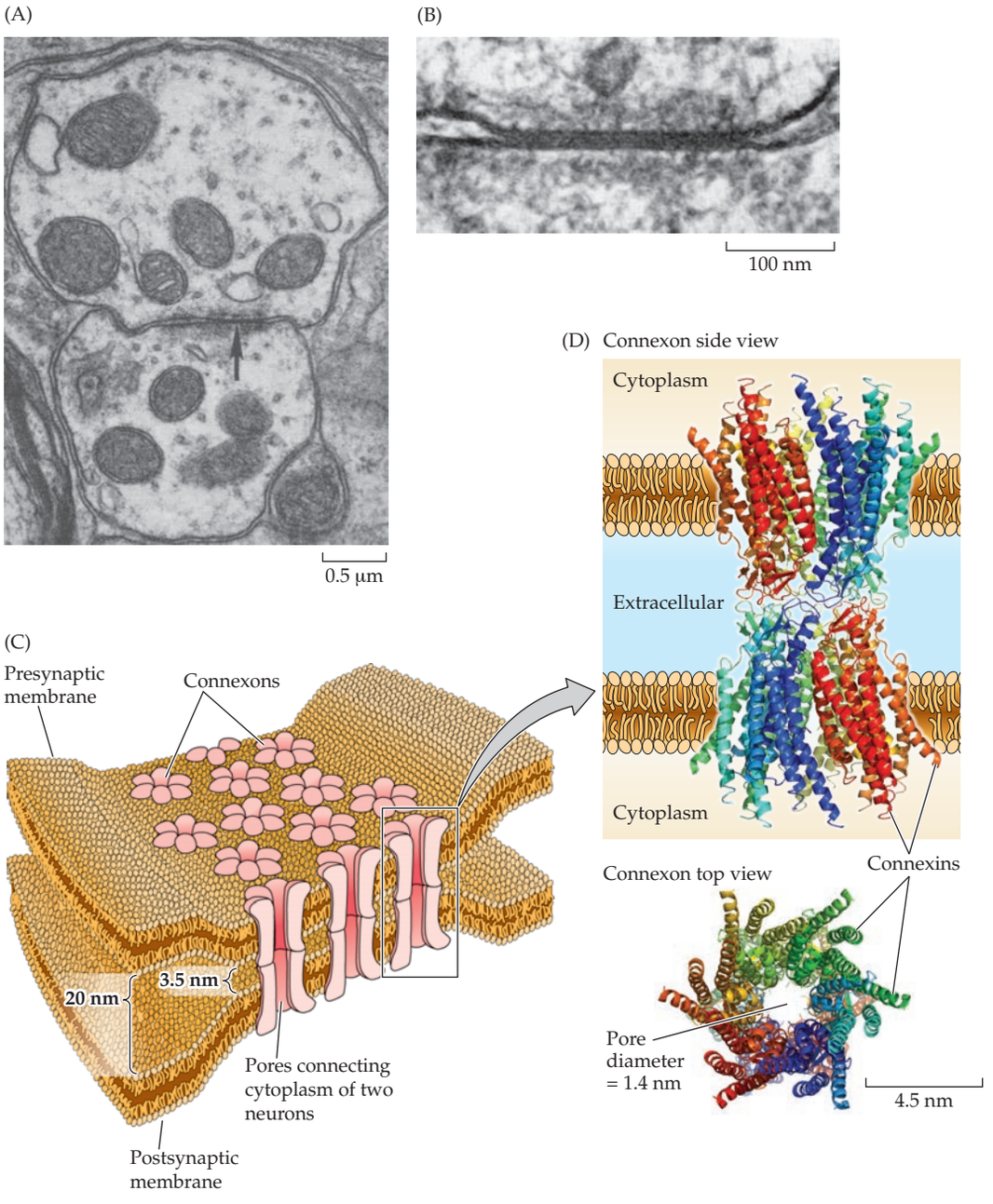


FIGURE 5.2 Structure of electrical synapses. (A) Electron micrograph of an electrical synapse (arrow) connecting two neurons within the inferior olive of a mammalian brain. (B) Higher-magnification electron micrograph of another electrical synapse, showing the gap junction structure characteristic of electrical synapses. (C) Gap junctions consist of connexons, hexameric complexes present in both the pre- and postsynaptic membranes. Channels assembled from connexons in these two membranes form pores that create electrical continuity between the two cells. (D) Crystallographic structure of connexons. Colors indicate individual connexins, integral membrane proteins that form the subunits of connexons. Side view shows the channels spanning the pre- and postsynaptic membranes; top view illustrates how six connexin subunits assemble in each membrane to form a channel with an exceptionally large pore. (A,B from Sotelo et al., 1974; D from Maeda et al., 2009.)

synapses work (Figure 5.2C). Connexons are composed of a unique family of ion channel proteins, the **connexins**, which serve as subunits to form connexon channels. There are twenty-one different types of human connexin genes (GJA–GJE) that are expressed in different cell types and yield connexons with diverse physiological properties. All connexins have four transmembrane domains, and all connexons consist of six connexins that come together to form a hemi-channel in both the pre- and postsynaptic neurons (Figure 5.2D). These hemi-channels are precisely aligned to form a pore that connects the two cells and permits electrical current to flow. The pore of a connexon channel is more than 1 nm in diameter, which is much larger than the pores of the voltage-gated ion channels described in Chapter 4. As a result, a variety of substances can simply diffuse between the cytoplasm of the pre- and postsynaptic neurons. In addition to ions, substances that diffuse through connexon pores include molecules with molecular weights as great as several hundred daltons. This permits important intracellular metabolites, such as ATP and second messengers (see Chapter 7), to be transferred between neurons.

Although they are a distinct minority, electrical synapses have several functional advantages. One is that transmission is extraordinarily fast: Because passive

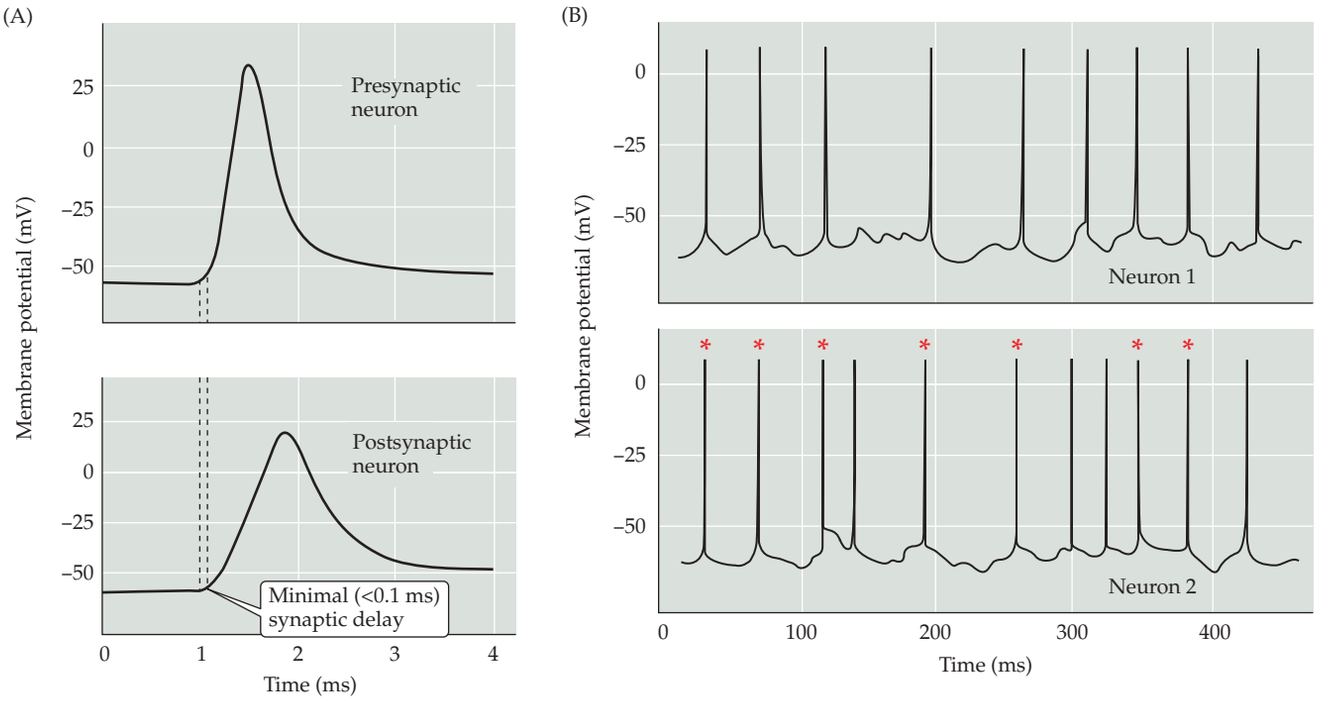


FIGURE 5.3 Function of gap junctions at electrical synapses. (A) Rapid transmission of signals at an electrical synapse in the crayfish. An action potential in the presynaptic neuron causes the postsynaptic neuron to be depolarized within a fraction of a millisecond. (B) Electrical synapses allow synchronization of electrical activity in hippocampal interneurons. In a pair of interneurons connected by electrical synapses, generation of an action potential in one neuron often results in the synchronized firing of an action potential in another neuron (asterisks). (A after Furshpan and Potter, 1959; B after Beierlein et al., 2000.)

current flow across connexons is virtually instantaneous, communication can occur without the delay that is characteristic of chemical synapses. The high speed of electrical synaptic transmission is apparent in the operation of the first electrical synapse to be discovered, which resides in the crayfish nervous system. A postsynaptic electrical signal is observed at this synapse within a fraction of a millisecond after the generation of a presynaptic action potential (Figure 5.3A). In fact, at least part of this brief synaptic delay is caused by propagation of the action potential into the presynaptic terminal, so there may be essentially no delay at all in the transmission of electrical signals across the synapse. Such synapses interconnect many of the neurons within the circuit that allows the crayfish to escape from its predators, thus minimizing the time between the presence of a threatening stimulus and a potentially life-saving motor response.

Another unique advantage of electrical synapses is that transmission can be bidirectional; although some connexons have special features for unidirectional transmission, in most cases current can flow in either direction, depending on which member of the coupled pair is invaded by an action potential. This allows electrical synapses to synchronize electrical activity among populations of neurons. For example, the brainstem neurons that generate rhythmic electrical activity underlying breathing are synchronized by electrical synapses, as are populations of interneurons in the cerebral cortex, thalamus, cerebellum, and other brain regions (Figure 5.3B). Electrical transmission between vasopressin- and oxytocin-secreting neurons in the hypothalamus ensures that all cells fire action potentials at about the same time, thus facilitating a synchronized burst of secretion of these hormones into the circulation (see Box 21A). The fact that connexon pores are large enough to allow second messengers to diffuse between cells also permits electrical synapses to synchronize the intracellular signaling of coupled cells. This feature may be particularly important for glial cells, which form large intracellular signaling networks via their gap junctions.

Signaling Transmission at Chemical Synapses

Figure 5.4A shows an electron micrograph of a chemical synapse in the cerebral cortex. This image illustrates the presynaptic terminal, with its abundance of synaptic vesicles, as well as the postsynaptic cell separated by a synaptic cleft. A three-dimensional rendering of this chemical

synapse, constructed from many images including the one in Figure 5.4A, reveals these features as well as many more structures, including filamentous elements in both pre- and postsynaptic processes, as well as structures in the synaptic cleft (Figure 5.4B). In the presynaptic terminal, dense projections (dark blue) are associated with the **active zone**, the place where synaptic vesicles discharge their neurotransmitters into the synaptic cleft, while the blue structure on the postsynaptic side represents the **postsynaptic density**, a structure important for postsynaptic signaling at excitatory synapses (see Box 7B).

Transmission at chemical synapses is based on the elaborate sequence of events depicted in Figure 5.4C. Prior to transmission, synaptic vesicles are formed and filled with neurotransmitter. Synaptic transmission is initiated when an action potential invades the terminal of the presynaptic neuron. The change in membrane potential caused by the arrival of the action potential leads to the opening of voltage-gated calcium channels in the presynaptic membrane. Because of the steep concentration gradient of Ca^{2+} across the presynaptic membrane (the external Ca^{2+} concentration is approximately 10^{-3} *M*, whereas the internal Ca^{2+} concentration is approximately 10^{-7} *M*), the opening of these channels causes a rapid influx of Ca^{2+} into the presynaptic terminal, with the result that the Ca^{2+} concentration of the cytoplasm in the terminal transiently rises to a much higher value. Elevation of the presynaptic Ca^{2+} concentration, in turn, allows synaptic vesicles to fuse with the plasma membrane of the presynaptic neuron. The Ca^{2+}-dependent fusion of synaptic vesicles with the terminal membrane causes their contents, most importantly neurotransmitters, to be released into the synaptic cleft, a process called **exocytosis**.

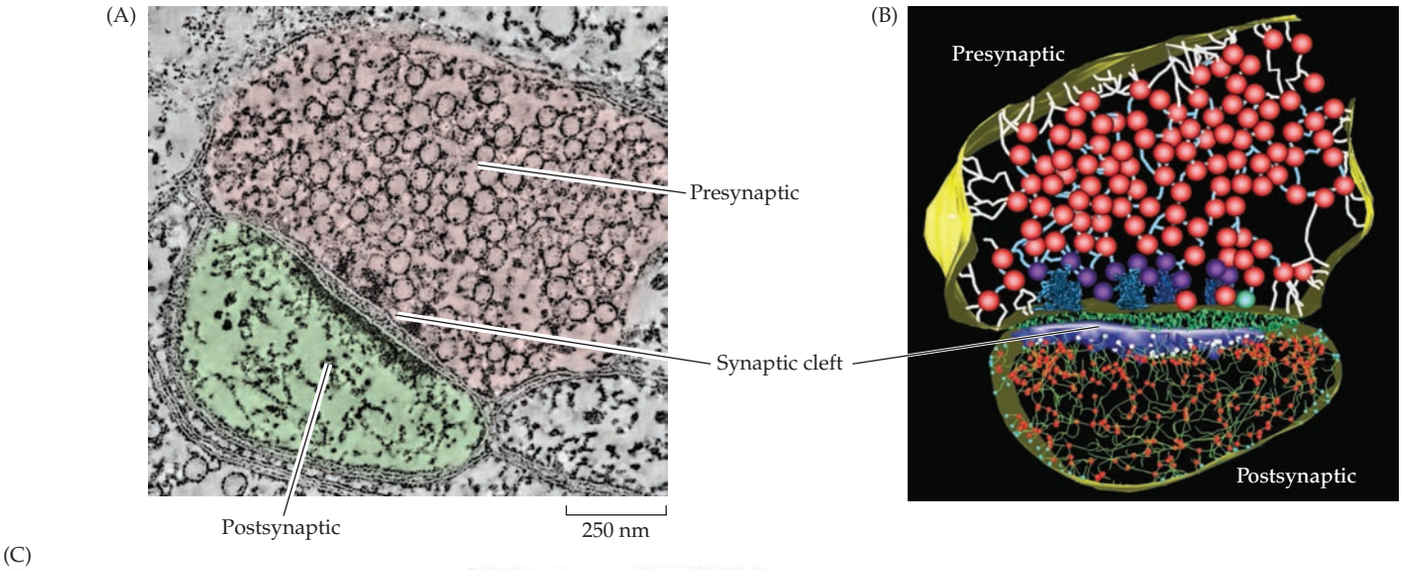
Following exocytosis, transmitters diffuse across the synaptic cleft and bind to specific receptors on the membrane of the postsynaptic neuron. The binding of neurotransmitter to the receptors causes channels in the postsynaptic membrane to open (or sometimes to close), thus changing the ability of ions to flow across the postsynaptic membrane. The resulting neurotransmitter-induced current flow alters the conductance and (usually) the membrane potential of the postsynaptic neuron, increasing or decreasing the probability that the neuron will fire an action potential. Subsequent removal of the neurotransmitter from the synaptic cleft, by uptake into glial cells or by enzymatic degradation, terminates the action of the neurotransmitter. In this way, information is transmitted transiently from one neuron to another.

Properties of Neurotransmitters

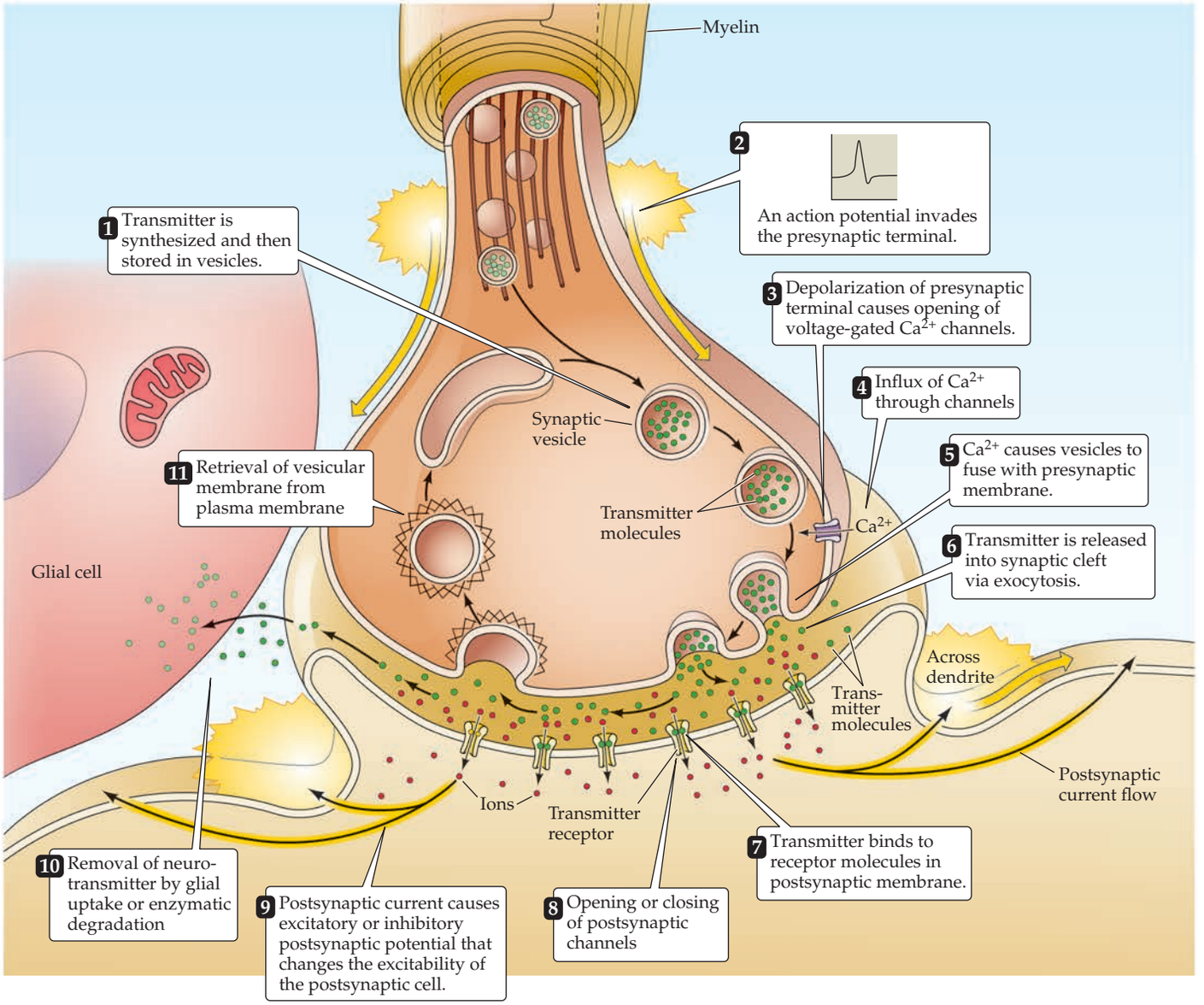
The notion that electrical information can be transferred from one neuron to the next by means of chemical signaling was the subject of intense debate through the first half of the twentieth century. In 1926, the German physiologist Otto Loewi performed a key experiment that supported this idea. Acting on an idea that allegedly came to him in the middle of the night, Loewi proved that electrical stimulation of the vagus nerve slows the heartbeat by releasing a chemical signal that was later shown to be **acetylcholine** (**ACh**). ACh is now known to be a neurotransmitter that acts not only in the heart but also at a variety of postsynaptic targets in the central and peripheral nervous systems, preeminently at the neuromuscular junction of striated muscles and in the visceral motor system (see Chapters 6 and 21).

Formal criteria have been established to definitively identify a substance as a neurotransmitter. These criteria have led to the identification of more than 100 different neurotransmitters, which can be classified into two broad categories: small-molecule neurotransmitters, such as ACh, and neuropeptides (see Chapter 6). Having more than one transmitter diversifies the physiological repertoire of synapses. Multiple neurotransmitters can produce different types of responses on individual postsynaptic cells. For example, a neuron can be excited by one type of neurotransmitter and inhibited by another type of neurotransmitter. The speed of postsynaptic responses produced by different transmitters also differs, allowing control of electrical signaling over different timescales. In general, small-molecule neurotransmitters mediate rapid synaptic actions, whereas neuropeptides tend to modulate slower, ongoing neuronal functions. In some cases, neurons synthesize and release two or more different neurotransmitters; in this case, the molecules are called **co-transmitters**. Co-transmitters can be differentially released according to the pattern of synaptic activity, so that the signaling properties of such synapses change dynamically according to the rate of activity.

Effective synaptic transmission requires close control of the concentration of neurotransmitters within the synaptic cleft. Neurons have therefore developed a sophisticated ability to regulate the synthesis, packaging, release, and degradation (or removal) of neurotransmitters to achieve the desired levels of transmitter molecules. The synthesis of small-molecule neurotransmitters occurs locally within presynaptic terminals. The precursor molecules required to make new molecules of neurotransmitter are usually taken into the nerve terminal by transporters found in the plasma membrane of the terminal (see Figure 4.13E). The enzymes that synthesize these neurotransmitters are present in the cytoplasm of the presynaptic terminal, and the newly synthesized transmitters are then loaded into synaptic vesicles via another type of transporter located in the vesicular membrane. Most small-molecule neurotransmitters are packaged in vesicles 40 to 60 nm in diameter, the centers of which appear clear in electron micrographs (see Figure 5.4A); accordingly, these vesicles are referred to as **small clear-core vesicles**. Neuropeptides are synthesized in the cell body of a neuron, and peptide-filled


(A)
Presynaptic
Synaptic cleft
Postsynaptic
250 nm
(B)
Presynaptic
Postsynaptic

(C)


Myelin
1 Transmitter is synthesized and then stored in vesicles.
2 An action potential invades the presynaptic terminal.
3 Depolarization of presynaptic terminal causes opening of voltage-gated Ca^{2+} channels.
4 Influx of Ca^{2+} through channels
5 Ca^{2+} causes vesicles to fuse with presynaptic membrane.
6 Transmitter is released into synaptic cleft via exocytosis.
7 Transmitter binds to receptor molecules in postsynaptic membrane.
8 Opening or closing of postsynaptic channels
9 Postsynaptic current causes excitatory or inhibitory postsynaptic potential that changes the excitability of the postsynaptic cell.
10 Removal of neuro-transmitter by glial uptake or enzymatic degradation
11 Retrieval of vesicular membrane from plasma membrane
Synaptic vesicle
Transmitter molecules
Ca^{2+}
Glial cell
Across dendrite
Trans-mitter molecules
Postsynaptic current flow
Ions
Transmitter receptor

◀ **FIGURE 5.4 Structure and function of chemical synapses.** (A) Structure of a chemical synapse in the cerebral cortex. A presynaptic terminal (pink) forms a synapse with a postsynaptic dendrite (green). (B) Three-dimensional reconstruction of the synapse shown in (A). Inside the presynaptic terminal, spheres indicate synaptic vesicles at various stages of their trafficking cycle, linear elements indicate intracellular filaments, and dark blue indicates dense projections associated with the active zone. Inside the postsynaptic neuron, the blue structure is the postsynaptic density, green structures represent filaments, red spheres indicate points where the filaments branch. Green material within the synaptic cleft indicates structures of unknown function. (C) Sequence of events involved in transmission at a typical chemical synapse. (A,B from Burette et al., 2012.)

vesicles are transported along an axon and down to the synaptic terminal via **axonal transport**. Neuropeptides are packaged into synaptic vesicles that range from 90 to 250 nm in diameter. Because the centers of these vesicles appear electron-dense in electron micrographs, they are referred to as **large dense-core vesicles**.

After a neurotransmitter has been secreted into the synaptic cleft, it must be removed to enable the postsynaptic cell to engage in another cycle of synaptic transmission. The removal of neurotransmitters involves diffusion away from the postsynaptic receptors, in combination with reuptake into nerve terminals or surrounding glial cells, degradation by specific enzymes, or a combination of these mechanisms. Specific transporter proteins remove most small-molecule neurotransmitters (or their metabolites) from the synaptic cleft, ultimately delivering them back to the presynaptic terminal for reuse (see Chapter 6).

Quantal Release of Neurotransmitters

Much of the evidence leading to the present understanding of chemical synaptic transmission was obtained from experiments examining the release of ACh at neuromuscular junctions. These synapses between spinal motor neurons and skeletal muscle cells are simple, large, and peripherally located, making them particularly amenable to experimental analysis. Such synapses occur at specializations called **end plates** because of the saucerlike appearance of the site on the muscle fiber where the presynaptic axon elaborates its terminals (Figure 5.5A). Most of the pioneering work on neuromuscular transmission was performed during the 1950s and 1960s by Bernard Katz and his collaborators at University College London. Although Katz worked primarily on the frog neuromuscular junction, numerous subsequent experiments have confirmed the applicability of his observations to transmission at all chemical synapses.

When an intracellular microelectrode is used to record the membrane potential of a muscle cell, an action potential in the presynaptic motor neuron can be seen to elicit a transient depolarization of the postsynaptic muscle fiber. This change in membrane potential, called an **end plate potential** (**EPP**), is normally large enough to bring the membrane potential of the muscle cell well above the threshold

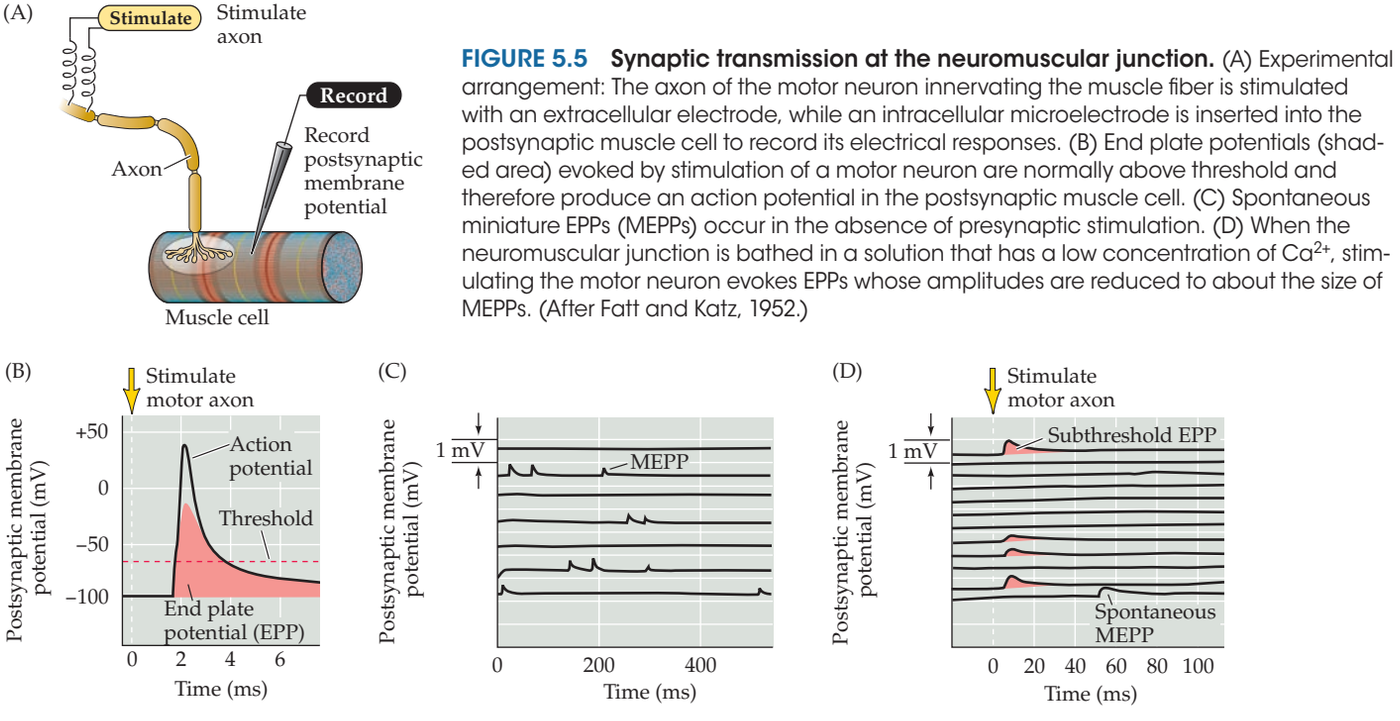


FIGURE 5.5 Synaptic transmission at the neuromuscular junction. (A) Experimental arrangement: The axon of the motor neuron innervating the muscle fiber is stimulated with an extracellular electrode, while an intracellular microelectrode is inserted into the postsynaptic muscle cell to record its electrical responses. (B) End plate potentials (shaded area) evoked by stimulation of a motor neuron are normally above threshold and therefore produce an action potential in the postsynaptic muscle cell. (C) Spontaneous miniature EPPs (MEPPs) occur in the absence of presynaptic stimulation. (D) When the neuromuscular junction is bathed in a solution that has a low concentration of Ca^{2+}, stimulating the motor neuron evokes EPPs whose amplitudes are reduced to about the size of MEPPs. (After Fatt and Katz, 1952.)

for producing a postsynaptic action potential (Figure 5.5B). The postsynaptic action potential triggered by the EPP causes the muscle fiber to contract. Unlike at electrical synapses, there is a pronounced delay between the time that the presynaptic motor neuron is stimulated and when the EPP occurs in the postsynaptic muscle cell. This synaptic delay is characteristic of all chemical synapses.

One of Katz's seminal findings, in studies carried out with Paul Fatt in 1951, was that spontaneous changes in muscle cell membrane potential occur even in the absence of stimulation of the presynaptic motor neuron (Figure 5.5C). These changes have the same shape as EPPs but are much smaller (typically less than 1 mV in amplitude, compared with an EPP of more than 50 mV). Both EPPs and these small, spontaneous events are sensitive to pharmacological agents that block postsynaptic acetylcholine receptors, such as curare (see Box 6A). These and other parallels between EPPs and the spontaneously occurring depolarizations led Katz and his colleagues to call these spontaneous events **miniature end plate potentials**, or **MEPPs**.

The relationship between the full-blown end plate potential and MEPPs was clarified by careful analysis of the EPPs. The magnitude of the EPP provides a convenient electrical assay of neurotransmitter secretion from a motor neuron terminal; however, measuring it is complicated by the need to prevent muscle contraction from dislodging the microelectrode. The usual means of eliminating muscle contractions is either to lower Ca^{2+} concentration in the extracellular medium or to partially block the postsynaptic ACh receptors with the drug curare. As expected from the scheme illustrated in Figure 5.4C, lowering the Ca^{2+} concentration reduces neurotransmitter secretion, thus reducing the magnitude of the EPP below the threshold for postsynaptic action potential production and allowing it to be measured more precisely. Under such conditions, stimulation of the motor neuron produces very small EPPs that fluctuate in amplitude from trial to trial (Figure 5.5D). These fluctuations give considerable insight into the mechanisms responsible for neurotransmitter release. In particular, the variable evoked response in low Ca^{2+} is now known to result from the release of unit amounts of ACh by the presynaptic nerve terminal. Indeed, the amplitude of the smallest evoked EPP response is strikingly similar to the size of single MEPPs (compare Figure 5.5C and D). Further supporting this similarity, increments in the EPP response (Figure 5.6A) occur in units about the size of single MEPPs (Figure 5.6B). These "quantal" fluctuations in the amplitude of EPPs indicated to Katz and his colleague Jose del Castillo that EPPs are made up of individual units, each equivalent to a MEPP.

The idea that EPPs represent the simultaneous release of many MEPP-like units can be tested statistically. A method of statistical analysis based on the independent occurrence of unitary events (called Poisson statistics) predicts what the distribution of EPP amplitudes would look like during a large number of trials of motor neuron stimulation, under the assumption that EPPs are built up from unitary events represented by MEPPs (see Figure 5.6B). The distribution of EPP amplitudes determined experimentally was found to be just that expected if transmitter release from the motor neuron is indeed quantal (the red curve in Figure 5.6A). Such analyses confirmed the idea that release of

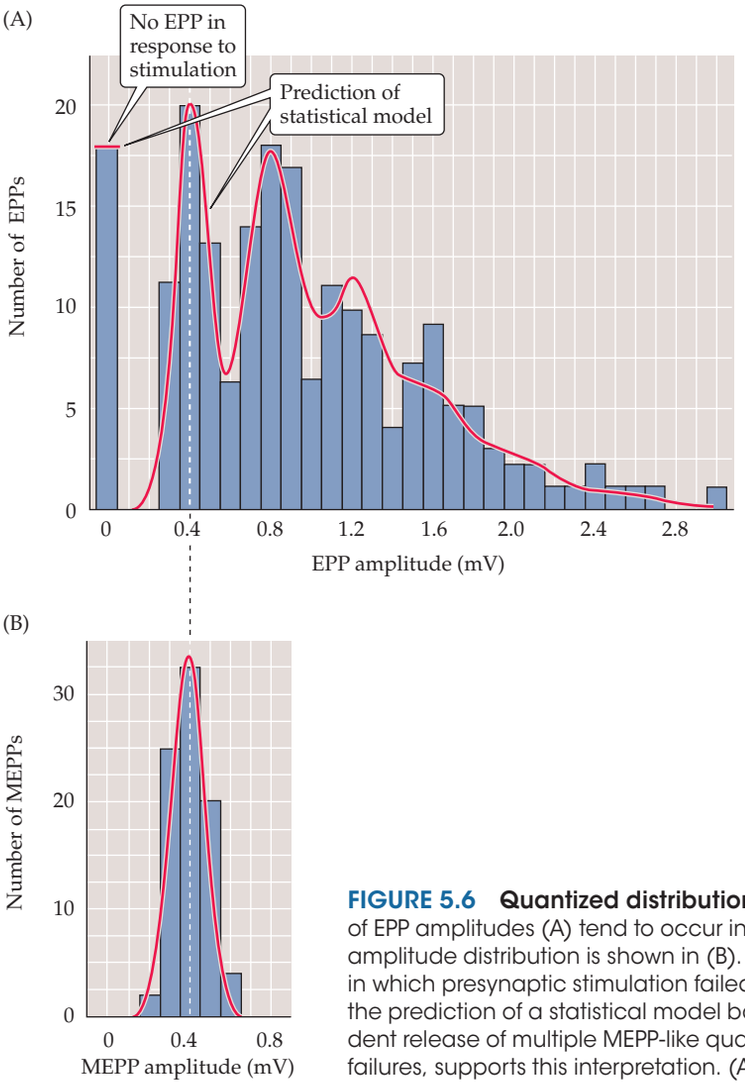


FIGURE 5.6 Quantized distribution of EPP amplitudes evoked in a low-Ca^{2+} solution. Peaks of EPP amplitudes (A) tend to occur in integer multiples of the mean amplitude of MEPPs, whose amplitude distribution is shown in (B). The leftmost bar in the EPP amplitude distribution shows trials in which presynaptic stimulation failed to elicit an EPP in the muscle cell. The red curve indicates the prediction of a statistical model based on the assumption that the EPPs result from the independent release of multiple MEPP-like quanta. The observed match, including the predicted number of failures, supports this interpretation. (After Boyd and Martin, 1955.)

acetylcholine does indeed occur in discrete packets, each equivalent to a MEPP. In short, a presynaptic action potential causes a postsynaptic EPP because it synchronizes the release of many transmitter quanta.

Release of Transmitters from Synaptic Vesicles

The discovery of the quantal release of packets of neurotransmitter immediately raised the question of how such quanta are formed and discharged into the synaptic cleft. At about the time Katz and his colleagues were using physiological methods to discover quantal release of neurotransmitter, electron microscopy revealed, for the first time, the presence of synaptic vesicles in presynaptic terminals. Putting these two discoveries together, Katz and others proposed that synaptic vesicles loaded with transmitter are the source of the quanta. Subsequent biochemical studies confirmed that synaptic vesicles are the repositories of transmitters. These studies have shown that ACh is highly concentrated in the synaptic vesicles of motor neurons, where it is present at a concentration of about 100 m*M*. Given the diameter of a small clear-core synaptic vesicle (~50 nm), approximately 10,000 molecules of neurotransmitter are contained in a single vesicle. This number corresponds quite nicely to the amount of ACh that must be applied to a neuromuscular junction to mimic a MEPP, providing further support for the idea that quanta arise from discharge of the contents of single synaptic vesicles.

To prove that quanta are caused by the fusion of individual synaptic vesicles with the plasma membrane, it is necessary to show that each fused vesicle produces a single quantal event in the postsynaptic cell. This challenge was met in the late 1970s, when John Heuser, Tom Reese, and colleagues correlated measurements of vesicle fusion with the quantal content of EPPs at the neuromuscular junction (Figure 5.7A). They used electron microscopy to determine the number of vesicles that fused with the presynaptic plasma membrane at the active zones of presynaptic terminals (Figure 5.7B). By treating terminals with different concentrations of a drug (4-aminopyridine, or 4-AP) that enhances the number of quanta released by single action potentials, it was possible to vary the amount of quantal release, determined from parallel electrical measurements of the quantal content of the EPPs. A comparison of the number of synaptic vesicle fusions observed with the electron microscope and

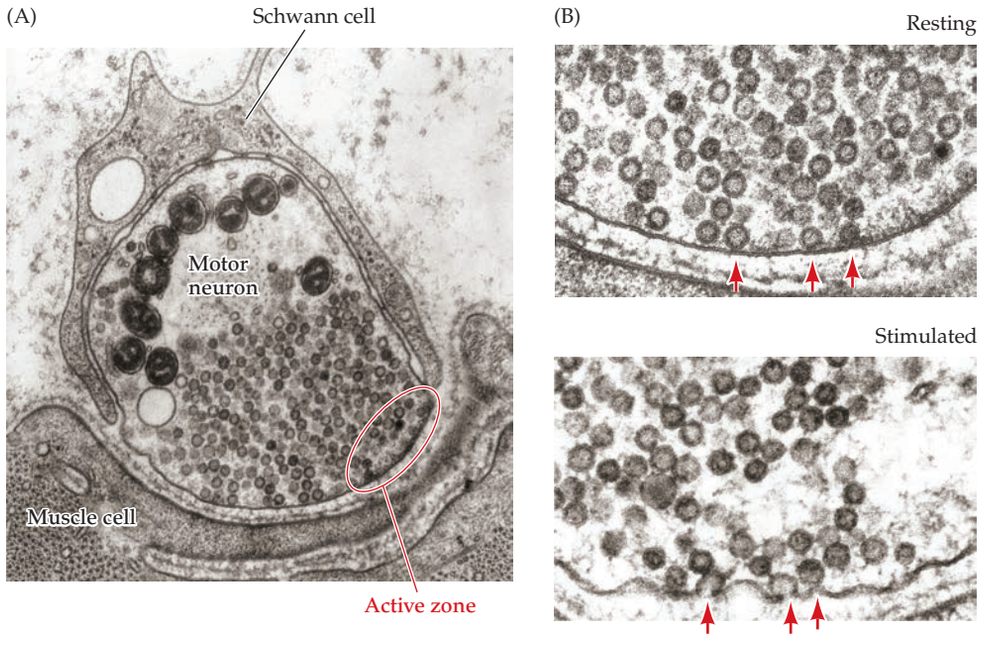


FIGURE 5.7 Relationship between synaptic vesicle exocytosis and quantal transmitter release. (A) Electron micrograph of a frog neuromuscular synapse. This synapse includes a presynaptic motor neuron that innervates a postsynaptic muscle cell and is covered by a type of glial cell called a Schwann cell. The active zone of the presynaptic terminal is the site of synaptic vesicle exocytosis. (B) Top: Active zone of an unstimulated presynaptic terminal. While many synaptic vesicles are present, including several that are docked at the active zone (arrows), none are fusing with the presynaptic plasma membrane. Bottom: Active zone of a terminal stimulated by an action potential; stimulation causes fusion (arrows) of synaptic vesicles with the presynaptic membrane. (C) Comparison of the number of observed vesicle fusion events with the number of quanta released by a presynaptic action potential. Transmitter release was varied by using different concentrations of a drug (4-AP) that affects the duration of the presynaptic action potential, thus changing the amount of calcium that enters during the action potential. The diagonal line is the 1:1 relationship expected if each vesicle that opened released a single quantum of transmitter. (C after Heuser et al., 1979; A and B courtesy of J. Heuser.).

the number of quanta released at the synapse showed a good correlation between these two measures (Figure 5.7C). These results remain one of the strongest lines of support for the idea that a quantum of transmitter release is due to fusion of a single synaptic vesicle with the presynaptic membrane. Subsequent evidence, based on other means of measuring vesicle fusion, has left no doubt about the validity of this interpretation, thereby establishing that chemical synaptic transmission results from the discharge of neurotransmitters from synaptic vesicles.

Local Recycling of Synaptic Vesicles

The fusion of synaptic vesicles causes new membrane to be added to the plasma membrane of the presynaptic terminal, but the addition is not permanent. Although a bout of exocytosis can dramatically increase the surface area of presynaptic terminals, this extra membrane is removed within a few minutes. Heuser and Reese performed another important set of experiments showing that the fused vesicle membrane is actually retrieved and taken back into the cytoplasm of the nerve terminal (a process called endocytosis). The experiments, again carried out at the frog neuromuscular junction, were based on filling the synaptic cleft with horseradish peroxidase (HRP), an enzyme that produces a dense reaction product that is visible in an electron microscope. Under appropriate experimental conditions, endocytosis could then be visualized by the uptake of HRP into the nerve terminal (Figure 5.8). To activate endocytosis, the presynaptic terminal was stimulated with a train of action potentials, and the subsequent fate of the HRP was followed by electron microscopy. Immediately following stimulation, the HRP was found in special endocytotic organelles called coated vesicles, which form from membrane budded off via coated pits (see Figure 5.8B). A few minutes later, however, the coated vesicles had disappeared, and the HRP was found in a different organelle, the endosome (see Figure 5.8C). Finally, within an hour after the terminal had been stimulated, the HRP reaction product appeared inside synaptic vesicles (see Figure 5.8D).

These observations indicate that synaptic vesicle membrane is recycled within the presynaptic terminal via the sequence summarized in Figure 5.8E. In this process, called the **synaptic vesicle cycle**, the retrieved vesicular

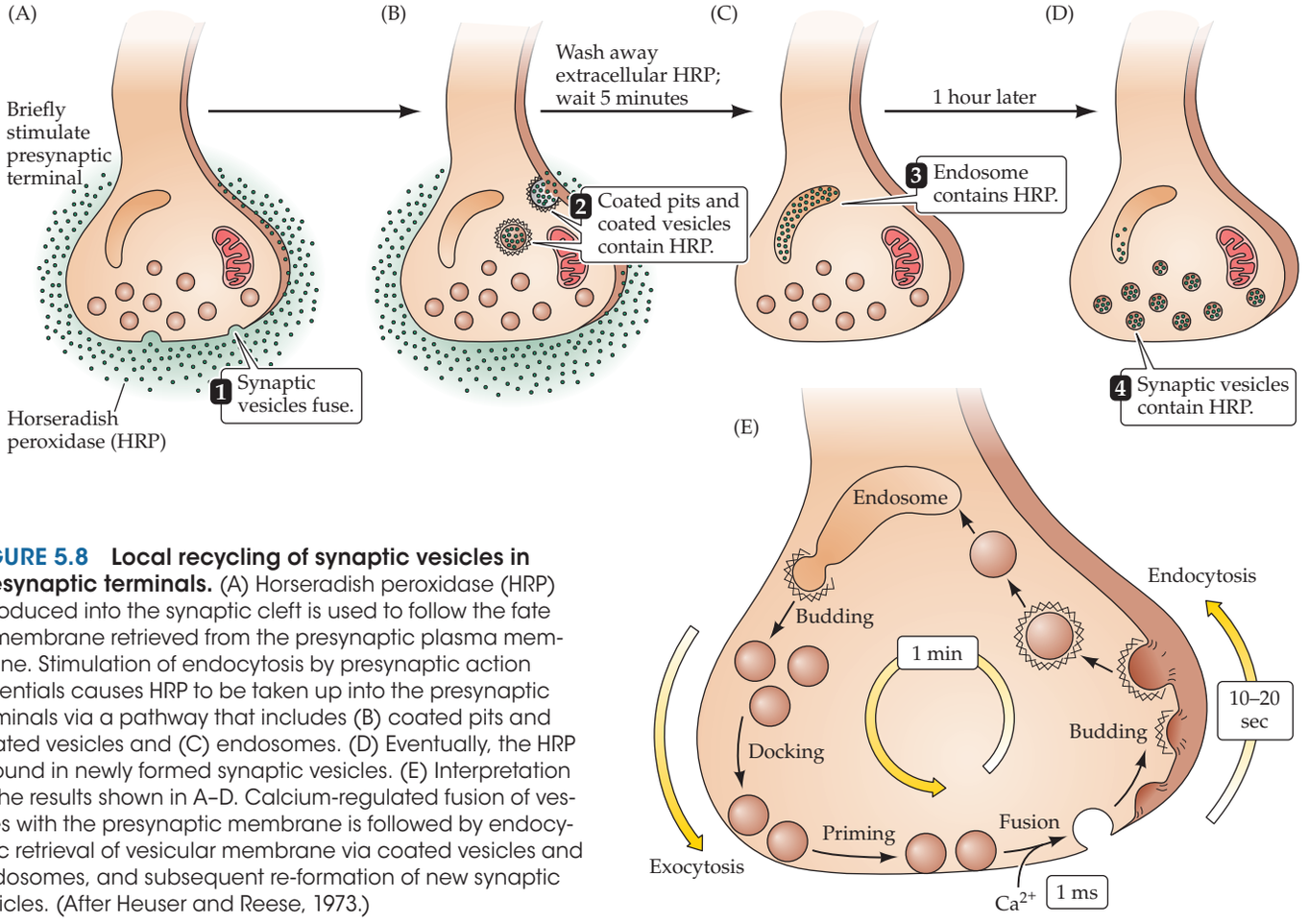


FIGURE 5.8 Local recycling of synaptic vesicles in presynaptic terminals. (A) Horseradish peroxidase (HRP) introduced into the synaptic cleft is used to follow the fate of membrane retrieved from the presynaptic plasma membrane. Stimulation of endocytosis by presynaptic action potentials causes HRP to be taken up into the presynaptic terminals via a pathway that includes (B) coated pits and coated vesicles and (C) endosomes. (D) Eventually, the HRP is found in newly formed synaptic vesicles. (E) Interpretation of the results shown in A–D. Calcium-regulated fusion of vesicles with the presynaptic membrane is followed by endocytotic retrieval of vesicular membrane via coated vesicles and endosomes, and subsequent re-formation of new synaptic vesicles. (After Heuser and Reese, 1973.)

membrane passes through several intracellular compartments—such as coated vesicles and endosomes—and is eventually used to make new synaptic vesicles. After synaptic vesicles are re-formed, they are stored in a reserve pool within the cytoplasm until they need to participate again in neurotransmitter release. These vesicles are mobilized from the reserve pool, docked at the presynaptic plasma membrane, and primed to participate in exocytosis once again. More recent experiments, employing a fluorescent label rather than HRP, have determined the time course of synaptic vesicle recycling. These studies indicate that the entire vesicle cycle requires approximately 1 minute, with membrane budding during endocytosis requiring 10 to 20 seconds of this time. As can be seen from the 1-millisecond delay in transmission following excitation of the presynaptic terminal (see Figure 5.5B), membrane fusion during exocytosis is much more rapid than budding during endocytosis. Thus, all of the recycling steps interspersed between membrane fusion and subsequent regeneration of a new vesicle are completed in less than a minute.

The precursors to synaptic vesicles *originally* are produced in the endoplasmic reticulum and Golgi apparatus in the neuronal cell body. Because of the long distance between the cell body and the presynaptic terminal in most neurons, transport of vesicles from the soma would not permit rapid replenishment of synaptic vesicles during continuous neural activity. Thus, local recycling is well suited to the peculiar anatomy of neurons, giving nerve terminals the means to provide a continual supply of synaptic vesicles.

The Role of Calcium in Transmitter Secretion

As was apparent in the experiments of Katz and others described in the preceding sections, lowering the concentration of Ca^{2+} outside a presynaptic motor nerve terminal reduces the size of the EPP (compare Figure 5.5B and D). Moreover, measurement of the number of transmitter quanta released under such conditions shows that the reason the EPP gets smaller is that lowering Ca^{2+} concentration decreases the number of vesicles that fuse with the plasma membrane of the terminal. An important insight into *how* Ca^{2+} regulates the fusion of synaptic vesicles was the discovery that presynaptic terminals have voltage-gated Ca^{2+} channels in their plasma membranes (see Chapter 4).

The first indication of presynaptic Ca^{2+} channels was provided by Katz and Ricardo Miledi. They observed that presynaptic terminals treated with tetrodotoxin (which blocks voltage-gated Na^+ channels; see Chapter 3) could still produce a peculiarly prolonged type of action potential. The explanation for this surprising finding was that current was still flowing through Ca^{2+} channels, substituting for the current ordinarily carried by the blocked Na^+ channels. Subsequent voltage clamp experiments, performed by Rodolfo Llinás and others at a giant presynaptic terminal of the squid (Figure 5.9A), confirmed the presence of voltage-gated Ca^{2+} channels in the presynaptic terminal (Figure 5.9B). Such experiments showed that the amount of neurotransmitter released is very sensitive to the exact amount of Ca^{2+} that enters. Furthermore, blockade of these Ca^{2+} channels with drugs also inhibits transmitter release (see Figure 5.9B, right). These observations establish that the voltage-gated Ca^{2+} channels are

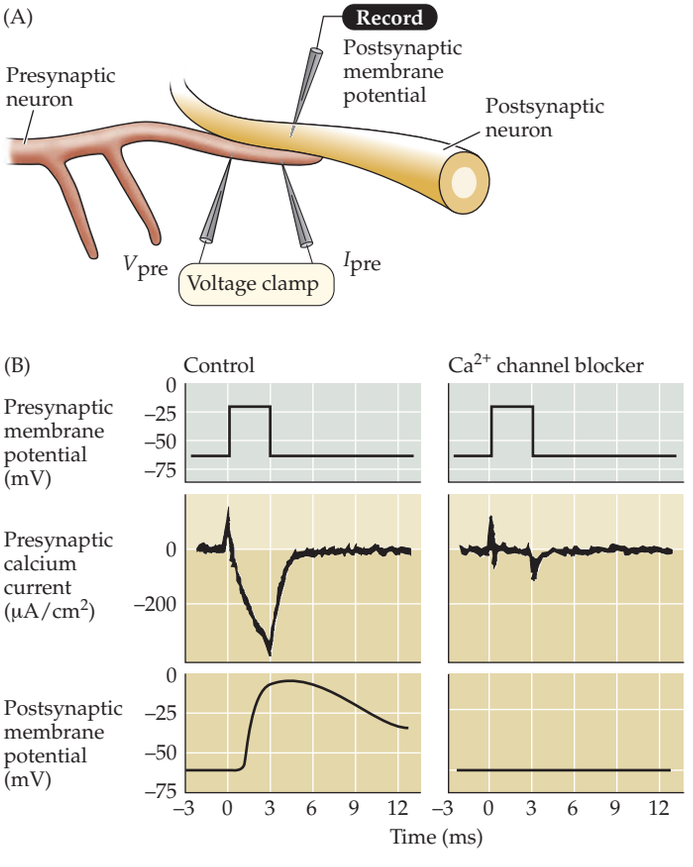
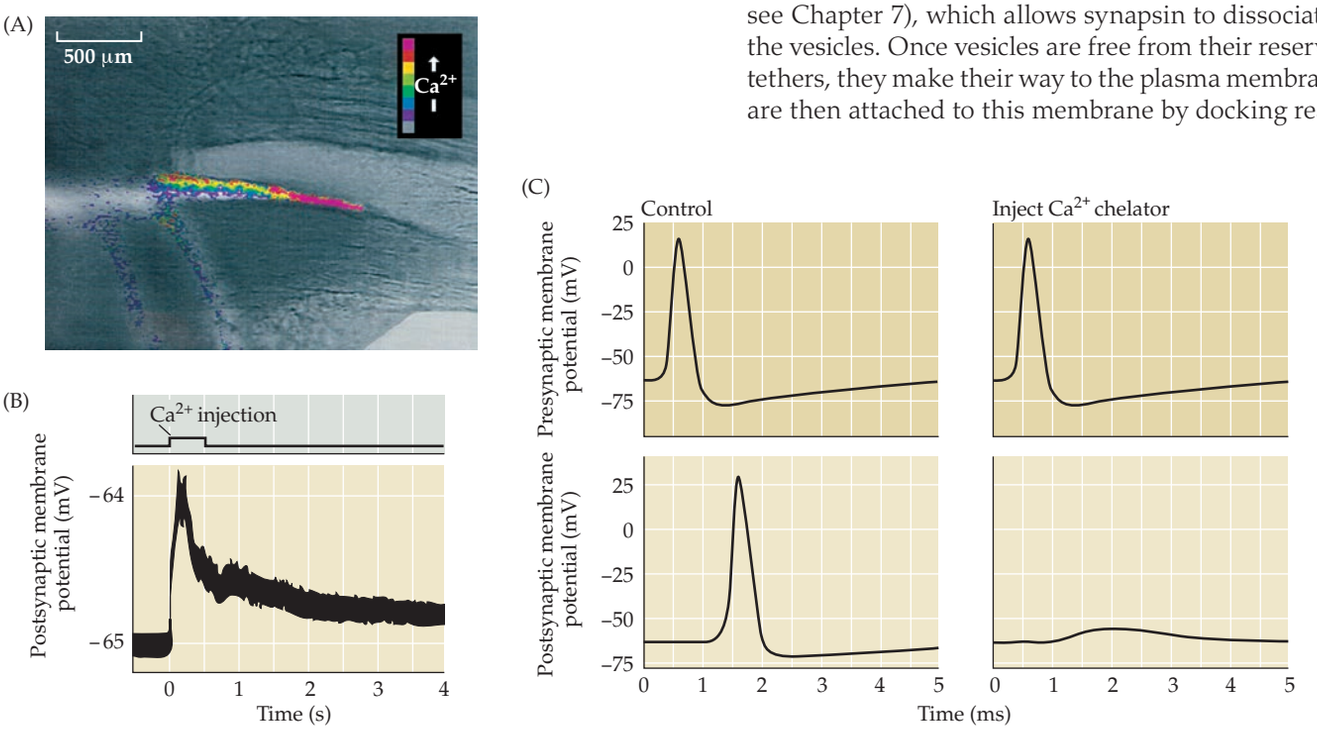


FIGURE 5.9 Entry of Ca^{2+} through presynaptic voltage-gated calcium channels causes transmitter release. (A) Experimental setup using an extraordinarily large synapse in the squid. The voltage clamp method detects currents flowing across the presynaptic membrane when the membrane potential is depolarized. (B) Pharmacological agents that block currents flowing through Na^+ and K^+ channels reveal a remaining inward current flowing through Ca^{2+} channels. This influx of calcium triggers transmitter secretion, as indicated by a change in the postsynaptic membrane potential. Treatment of the same presynaptic terminal with cadmium, a calcium channel blocker, eliminates both the presynaptic calcium current and the postsynaptic response. (After Augustine and Eckert, 1984.)

directly involved in synaptic transmission: Presynaptic action potentials open these Ca^{2+} channels, yielding an influx of Ca^{2+} into the presynaptic terminal.

As is the case for many other forms of neuronal signaling (see Chapter 7), Ca^{2+} serves as a second messenger during transmitter release. Ca^{2+} entering into presynaptic terminals accumulates within the terminal, as can be seen with microscopic imaging of terminals filled with Ca^{2+}-sensitive dyes (Figure 5.10A). The presynaptic second messenger function of Ca^{2+} has been directly shown in two complementary ways. First, microinjection of Ca^{2+} into presynaptic terminals triggers transmitter release even in the absence of presynaptic action potentials (Figure 5.10B). Second, presynaptic microinjection of calcium chelators (chemicals that bind Ca^{2+} and keep its concentration buffered at low levels) prevents presynaptic action potentials from causing transmitter secretion (Figure 5.10C). These results prove beyond any doubt that a rise in presynaptic Ca^{2+} concentration is both necessary and sufficient for neurotransmitter release. While Ca^{2+} is a universal trigger for transmitter release, not all transmitters are released with the same speed. For example, while secretion of ACh from motor neurons requires only a fraction of a millisecond (see Figure 5.5), release of neuropeptides requires high-frequency bursts of action potentials for many seconds. These differences in the rate of release probably arise from differences in the spatial arrangement of vesicles relative to presynaptic Ca^{2+} channels, yielding differences in the time course of local Ca^{2+} signaling.

FIGURE 5.10 Evidence that a rise in presynaptic Ca^{2+} concentration triggers transmitter release from presynaptic terminals. (A) Fluorescence microscopy measurements of presynaptic Ca^{2+} concentration at the squid giant synapse (see Figure 5.9A). A train of presynaptic action potentials causes a rise in Ca^{2+} concentration, as revealed by a dye (called fura-2) that fluoresces more strongly when the Ca^{2+} concentration increases (colors). (B) Microinjection of Ca^{2+} into a squid giant presynaptic terminal triggers transmitter release, measured as a depolarization of the postsynaptic membrane potential. (C) Microinjection of BAPTA, a Ca^{2+} chelator, into a squid giant presynaptic terminal prevents transmitter release. (A from Smith et al., 1993; B after Miledi, 1973; C after Adler et al., 1991.)



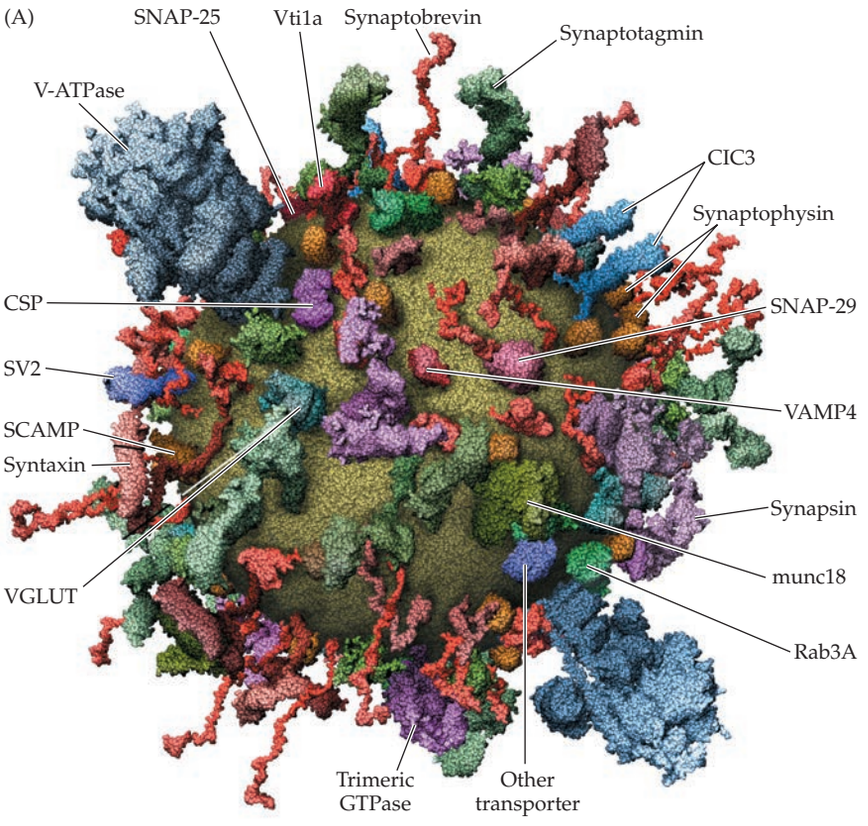
Molecular Mechanisms of Synaptic Vesicle Cycling

Precisely how an increase in presynaptic Ca^{2+} concentration goes on to trigger vesicle fusion and neurotransmitter release is not fully understood. Molecular studies have identified and characterized the proteins found on synaptic vesicles (Figure 5.11A) and their binding partners on the presynaptic plasma membrane and cytoplasm. Most, if not all, of these proteins act at one or more steps in the synaptic vesicle cycle (Figure 5.11B).

Several lines of evidence indicate that the protein **synapsin**, which reversibly binds to synaptic vesicles, may keep these vesicles tethered within the reserve pool by cross-linking vesicles to each other. Mobilization of these reserve pool vesicles is caused by phosphorylation of synapsin by proteins kinases, most notably the **Ca^{2+}/calmodulin-dependent protein kinase, type II** (CaMKII; see Chapter 7), which allows synapsin to dissociate from the vesicles. Once vesicles are free from their reserve pool tethers, they make their way to the plasma membrane and are then attached to this membrane by docking reactions

that involve SNARE proteins (see below). A series of priming reactions then prepares the vesicular and plasma membranes for fusion. A large number of proteins are involved in priming, including some proteins that are also involved in other types of membrane fusion events common to all cells (see Figure 5.11B). For example, two proteins originally found to be important for the fusion of vesicles with membranes of the Golgi apparatus, the ATPase **NSF** (*N*EM-sensitive *f*usion protein) and **SNAPs** (*s*oluble *N*SF-*a*ttachment *p*roteins), are also involved in priming synaptic vesicles for fusion. These two proteins work by regulating the assembly of other proteins that are called **SNAREs** (*SNAP re*ceptors). Many of the other proteins involved in priming—such as munc13, munc18, complexin, snapin, syntaphilin, and tomosyn—also interact with the SNAREs.

One of the main purposes of priming is to organize SNARE proteins into the correct conformation for membrane fusion. One of the SNARE proteins, **synaptobrevin**, is in the membrane of synaptic vesicles, while two other SNARE proteins called **syntaxin** and **SNAP-25** are found primarily on the plasma



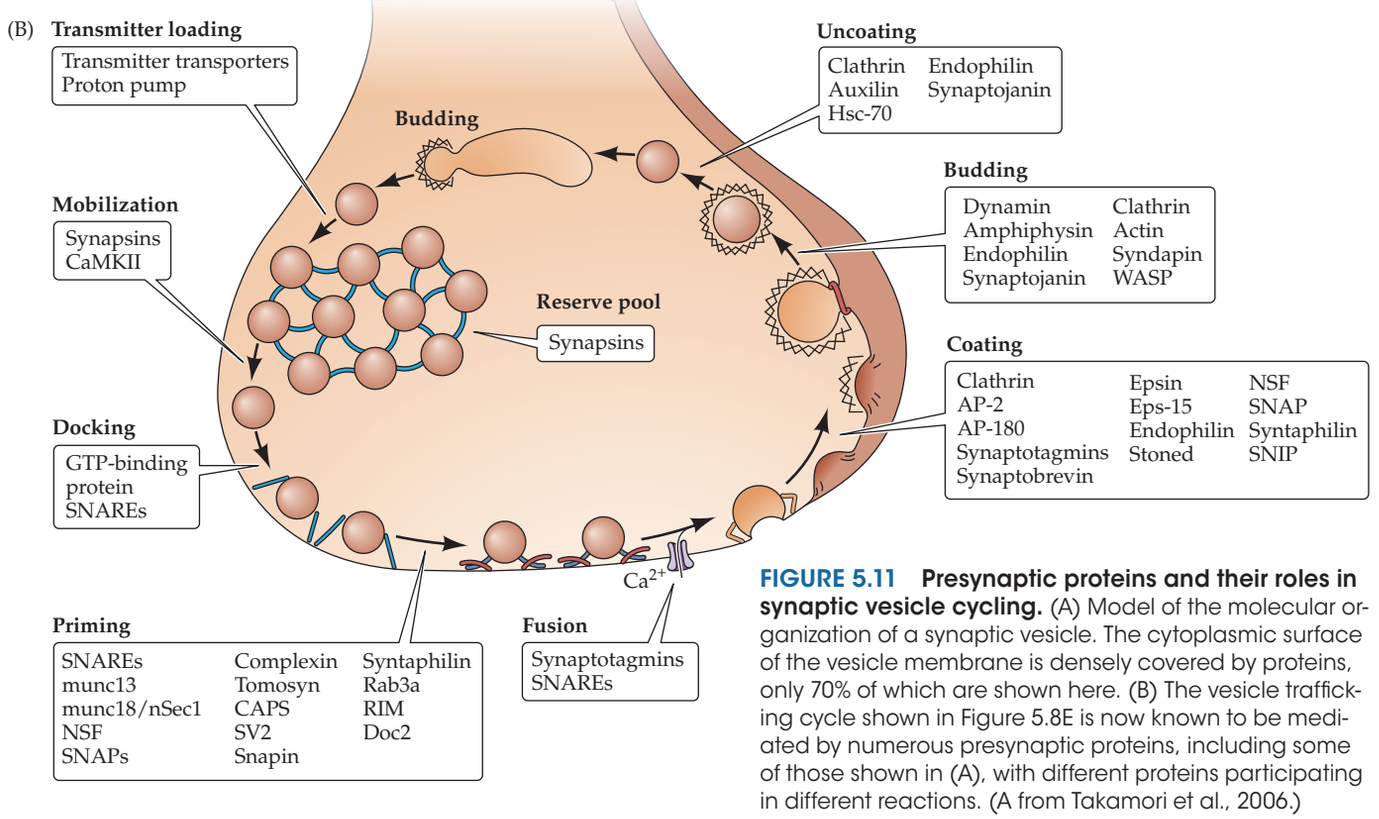


FIGURE 5.11 Presynaptic proteins and their roles in synaptic vesicle cycling. (A) Model of the molecular organization of a synaptic vesicle. The cytoplasmic surface of the vesicle membrane is densely covered by proteins, only 70% of which are shown here. (B) The vesicle trafficking cycle shown in Figure 5.8E is now known to be mediated by numerous presynaptic proteins, including some of those shown in (A), with different proteins participating in different reactions. (A from Takamori et al., 2006.)

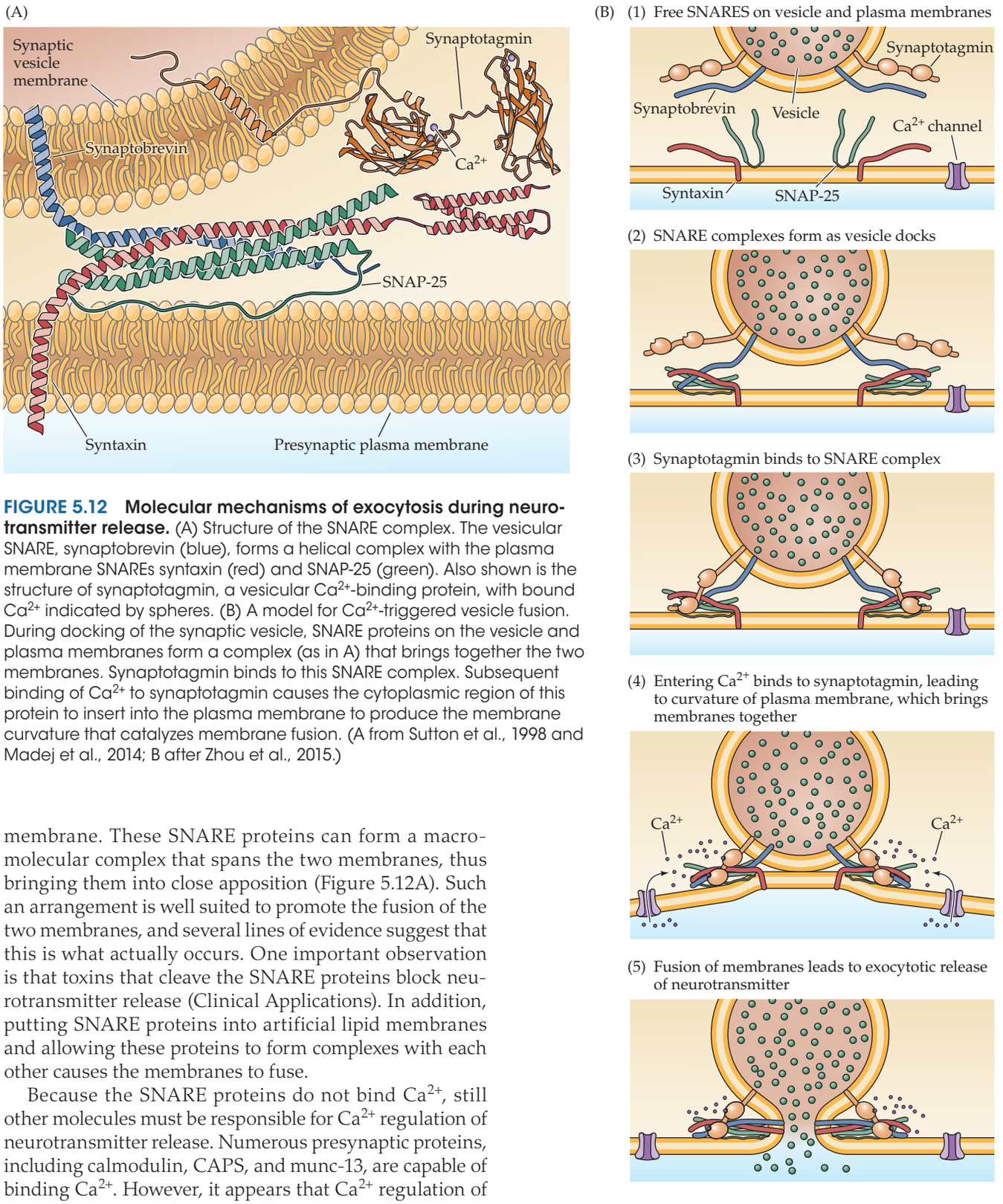


FIGURE 5.12 Molecular mechanisms of exocytosis during neurotransmitter release. (A) Structure of the SNARE complex. The vesicular SNARE, synaptobrevin (blue), forms a helical complex with the plasma membrane SNAREs syntaxin (red) and SNAP-25 (green). Also shown is the structure of synaptotagmin, a vesicular Ca^{2+}-binding protein, with bound Ca^{2+} indicated by spheres. (B) A model for Ca^{2+}-triggered vesicle fusion. During docking of the synaptic vesicle, SNARE proteins on the vesicle and plasma membranes form a complex (as in A) that brings together the two membranes. Synaptotagmin binds to this SNARE complex. Subsequent binding of Ca^{2+} to synaptotagmin causes the cytoplasmic region of this protein to insert into the plasma membrane to produce the membrane curvature that catalyzes membrane fusion. (A from Sutton et al., 1998 and Madej et al., 2014; B after Zhou et al., 2015.)

membrane. These SNARE proteins can form a macromolecular complex that spans the two membranes, thus bringing them into close apposition (Figure 5.12A). Such an arrangement is well suited to promote the fusion of the two membranes, and several lines of evidence suggest that this is what actually occurs. One important observation is that toxins that cleave the SNARE proteins block neurotransmitter release (Clinical Applications). In addition, putting SNARE proteins into artificial lipid membranes and allowing these proteins to form complexes with each other causes the membranes to fuse.

Because the SNARE proteins do not bind Ca^{2+}, still other molecules must be responsible for Ca^{2+} regulation of neurotransmitter release. Numerous presynaptic proteins, including calmodulin, CAPS, and munc-13, are capable of binding Ca^{2+}. However, it appears that Ca^{2+} regulation of neurotransmitter release usually is conferred by **synaptotagmins**, a family of proteins found in the membrane of synaptic vesicles (see Figure 5.12A). Synaptotagmin

CLINICAL APPLICATIONS

Disorders That Affect the Presynaptic Terminal

Defects in various steps in the exocytosis and endocytosis of synaptic vesicles have been shown to be at the root of several rare but debilitating neurological diseases.

Myasthenic syndromes

In myasthenic syndromes, abnormal transmission at neuromuscular synapses leads to weakness and fatigability of skeletal muscles. Some of these syndromes affect the acetylcholinesterase that degrades acetylcholine in the synaptic cleft; others arise from autoimmune attack of acetylcholine receptors (see Clinical Applications, Chapter 6); and yet others affect acetylcholine release from motor neurons. One of the best understood myasthenic syndromes is *Lambert-Eaton myasthenic syndrome*, or LEMS, an occasional complication in patients with certain kinds of cancers. Biopsies of muscle tissue removed from LEMS patients allow intracellular recordings identical to those in Figure 5.5. These recordings from affected tissues show that when a motor neuron is stimulated, the number of quanta contained in EPPs is greatly reduced while the size of individual quanta is unaffected. Several lines of evidence indicate that this reduction in neurotransmitter release is due to a loss of voltage-gated Ca^{2+} channels in the presynaptic terminal of motor neurons (see Figure A); perhaps most compelling are anatomical studies that reveal a lowered density of Ca^{2+} channels in the presynaptic plasma membrane.

The loss of presynaptic Ca^{2+} channels in LEMS patients apparently arises from an autoimmune disorder. Their blood has a very high concentration of antibodies that bind to Ca^{2+} channels, and it seems likely that these antibodies are the primary cause of LEMS. For example, removal of Ca^{2+} channel antibodies from the blood of LEMS patients by plasma exchange reduces muscle weakness. Similarly, immunosuppressant drugs also can alleviate LEMS symptoms. Perhaps most telling, injecting these antibodies into experimental animals elicits muscle weakness and abnormal neuromuscular transmission.

Why the immune system generates antibodies against Ca^{2+} channels is not clear. Most LEMS patients have small-cell carcinoma, a form of lung cancer that may somehow initiate the immune response to Ca^{2+} channels. Whatever the origin, the binding of antibodies to Ca^{2+} channels reduces the ability of these channels to carry Ca^{2+} current. It is this antibody-induced defect in presynaptic Ca^{2+} entry that accounts for the muscle weakness associated with LEMS.

Congenital myasthenic syndromes are genetic disorders that, like LEMS, cause muscle weakness by affecting neuromuscular transmission. Several congenital myasthenic syndromes also arise from defects in acetylcholine release from the motor neuron terminal. Neuromuscular synapses in some of these patients have EPPs with reduced quantal content, a deficit that is especially prominent when the synapse is activated repeatedly. Electron microscopy shows that presynaptic motor nerve terminals have a greatly reduced number of synaptic vesicles. The defect in neurotransmitter release evidently results from an inadequate number of synaptic vesicles available for release during sustained presynaptic activity. The origins of this shortage of synaptic vesicles is not clear, but could result either from an impairment in endocytosis in the nerve terminal (see Figure A) or from a reduced supply of vesicle components from the motor neuron cell body.

Still other patients suffering from *familial infantile myasthenia* appear to have neuromuscular weakness that arises from reductions in the size of individual quanta rather than the number of quanta released. Motor nerve terminals from these patients have synaptic vesicles that are typical in number but smaller than usual in diameter. This finding suggests a different type of genetic lesion that somehow alters formation of new synaptic vesicles following endocytosis, leading to less acetylcholine in each vesicle.

Botulism and tetanus

Impairment of synaptic transmitter release also results from poisoning by anaerobic *Clostridium* bacteria. This genus of microorganisms produces some of the most potent toxins known, including several botulinum toxins and tetanus toxin. Both botulism and tetanus are potentially deadly disorders.

Botulism can occur by consuming food containing *Clostridium* bacteria, or by infection of wounds with the spores of these ubiquitous organisms. In either case, the presence of the toxin can cause paralysis of peripheral neuromuscular synapses due to impaired neurotransmitter release. This interference with neuromuscular transmission causes skeletal muscle weakness; in extreme cases respiratory failure results from paralysis of the diaphragm and other muscles required for breathing. Botulinum toxins also block synapses innervating the smooth muscles of several organs, giving rise to visceral motor dysfunction. This paralysis of neuromuscular transmission also serves as the basis for clinical use of botulinum toxin in cosmetic surgery

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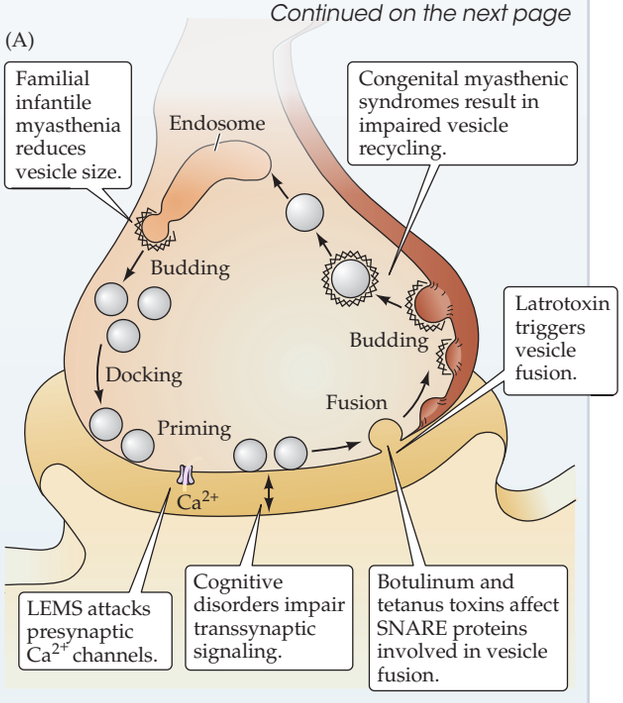


(A) Presynaptic targets of several neurological pathologies.

CLINICAL APPLICATIONS (*continued*)

and other applications where highly local relaxation of muscle contraction is of therapeutic benefit to the patient.

Tetanus typically results from the contamination of puncture wounds by *Clostridium* bacteria that produce tetanus toxin. In contrast to botulism, tetanus poisoning blocks the release of inhibitory transmitters from interneurons in the spinal cord. This effect causes a loss of synaptic inhibition on spinal motor neurons (see Chapter 16), producing hyperexcitation of skeletal muscle and tetanic contractions in affected muscles (hence the name of the disease).

Although the clinical consequences of tetanus toxin are dramatically different from those of botulinum toxins, clever and patient biochemical work has shown that these toxins have a common mechanism of action: they are highly specific proteases that inhibit neurotransmitter release by cleaving the SNARE proteins involved in fusion of synaptic vesicles with the presynaptic plasma membrane (see Figure B). Tetanus toxin and botulinum toxin types B, D, F, and G specifically cleave the vesicle SNARE protein synaptobrevin. Other botulinum toxins cleave syntaxin (type C) and SNAP-25 (types A and E), SNARE proteins found on the presynaptic plasma membrane. Destruction of these presynaptic proteins is the basis for the inhibitory actions of clostridial toxins on neurotransmitter release.

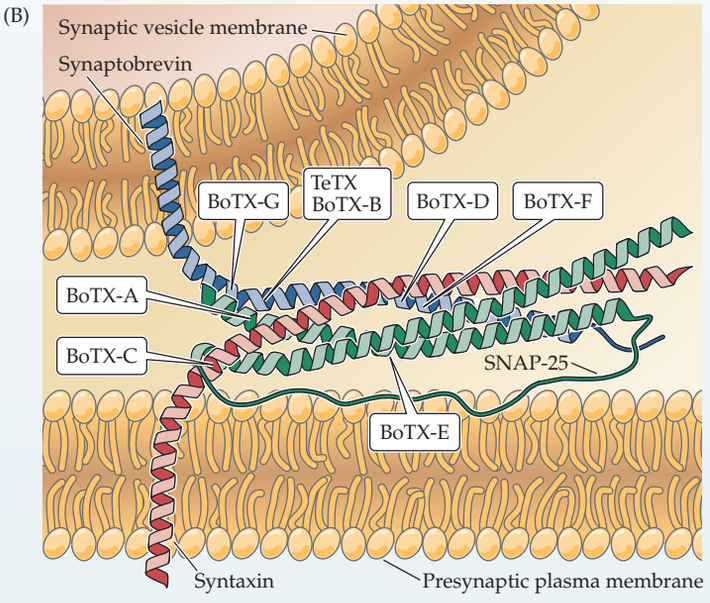
The different actions of these toxins on synaptic transmission at excitatory motor versus inhibitory synapses apparently result from the fact that these toxins are taken up by different types of neurons: Whereas the botulinum toxins are taken up by motor neurons, tetanus toxin is preferentially targeted to interneurons. The differential uptake of toxins presumably arises from the presence of different types of toxin receptors on the two types of neurons.

Insights from α-latrotoxin

Latrodectism refers to the severe muscle pain and cramping experienced by patients bitten by the black widow spider, *Latrodectus*. These symptoms arise from the presynaptic neurotoxin α-latrotoxin, present in black widow spider venom; this toxin causes a massive discharge of neurotransmitter from presynaptic terminals. Although neurotransmitter release ordinarily requires Ca^{2+}, α-latrotoxin is capable of releasing neurotransmitter even when Ca^{2+} is absent from the extracellular medium. Although it is not yet clear how the toxin triggers Ca^{2+}-independent exocytosis, we know that α-latrotoxin binds to two different types of presynaptic proteins that may mediate its actions. One group of binding partners for α-latrotoxin is the neurexins, a group of integral membrane proteins found in presynaptic terminals. Neurexins bind to synaptotagmin, the presynaptic Ca^{2+} sensor, and this interaction may allow α-latrotoxin to bypass the usual Ca^{2+} requirement for triggering vesicle fusion. Another type of presynaptic protein that can bind to α-latrotoxin is called CL1 (based on its previous names, *C*a^{2+}-independent receptor for latrotoxin and *l*atrophilin-*1*). CL1 is a relative of the G-protein-coupled receptors that mediate the actions of neurotransmitters and other extracellular chemical signals (see Figure 5.14B). Thus, the binding of α-latrotoxin to CL1 could activate an intracellular signal transduction cascade involved in the Ca^{2+}-independent actions of α-latrotoxin. While more work is needed to establish the definitive roles of neurexins and CL1 in α-latrotoxin action, these two proteins are probably the basis for the toxin's potent presynaptic activity.

In addition to their possible role in latrodectism, neurexins have been linked to a variety of cognitive disorders. Mutations in the neurexin gene have been identified in several patients suffering from schizophrenia, a psychiatric disease causing delusions, hallucinations, and loss of emotional expression (see Box 18B). Neurexins are known to play an important role in signaling across the synaptic cleft by binding to a family of postsynaptic membrane proteins called neuroligins. Remarkably, mutations in neuroligins also have been associated with autism, a spectrum of psychiatric disorders characterized by impaired social interaction, communication problems, and other behavioral disorders. While a direct connection between neurexins or neuroligins and these psychiatric disorders has not yet been established, it seems likely that defects in these transsynaptic signaling partners may serve as a central mechanism underlying several psychiatric disorders.

In summary, not only does research into synaptic vesicle trafficking illuminate the causes of numerous neurological and psychiatric disorders, but research into these diseases in turn has provided tools—such as clostridial toxins and α-latrotoxin—that have proven valuable in elucidating the basic mechanisms of synaptic vesicle trafficking.



(B) Cleavage of SNARE proteins by clostridial toxins. Indicated are the sites of proteolysis by tetanus toxin (TeTX) and various types of botulinum toxin (BoTX). (From Sutton et al., 1998.)

binds Ca^{2+} at concentrations similar to those required to trigger vesicle fusion within the presynaptic terminal, and this property allows synaptotagmin to act as a Ca^{2+} sensor that triggers vesicle fusion by signaling the elevation of Ca^{2+} within the terminal. In support of this idea, disruption of synaptotagmin in the presynaptic terminals of mice, fruit flies, squid, and other experimental animals impairs Ca^{2+}-dependent neurotransmitter release. In fact, deletion of only one of the 17 synaptotagmin genes (SYT) of mice is a lethal mutation, causing the mice to die soon after birth. It is thought that Ca^{2+} binding to synaptotagmin leads to exocytosis by changing the chemical properties of synaptotagmin, thereby allowing it to insert into the plasma membrane. This causes the plasma membrane to locally curve and leads to fusion of the two membranes. Thus, SNARE proteins bring the two membranes close together, while Ca^{2+}-induced changes in synaptotagmin then produce the final curvature that enables rapid fusion of these membranes (Figure 5.12B).

Still other proteins appear to be involved at the endocytosis steps of the synaptic vesicle cycle (Figure 5.13). The most important protein involved in endocytotic budding of vesicles from the plasma membrane is **clathrin**. Clathrin has a unique structure that is called a triskelion because of its three-legged appearance; these triskelia can assemble to form a cagelike coating around the vesicle membrane (see Figure 5.13A). Several adaptor proteins, such as AP-2 and AP-180, connect clathrin to the proteins and lipids of this membrane. These adaptor proteins, as well as other proteins such as amphiphysin, epsin, and Eps-15, help assemble individual triskelia into structures that resemble geodesic domes (see Figure 5.13A, bottom). Such domelike structures form coated pits that initiate membrane budding, increasing the curvature of the budding membrane until it forms a coated vesicle-like structure that remains connected to the plasma membrane via a narrow lipid stalk (Figure 5.13C). Another protein, called **dynamin**, forms a ringlike coil that surrounds the lipid stalk (see Figure 5.13B). This coil causes the final pinching-off of membrane that severs the stalk and completes the production of coated vesicles. Coated vesicles then

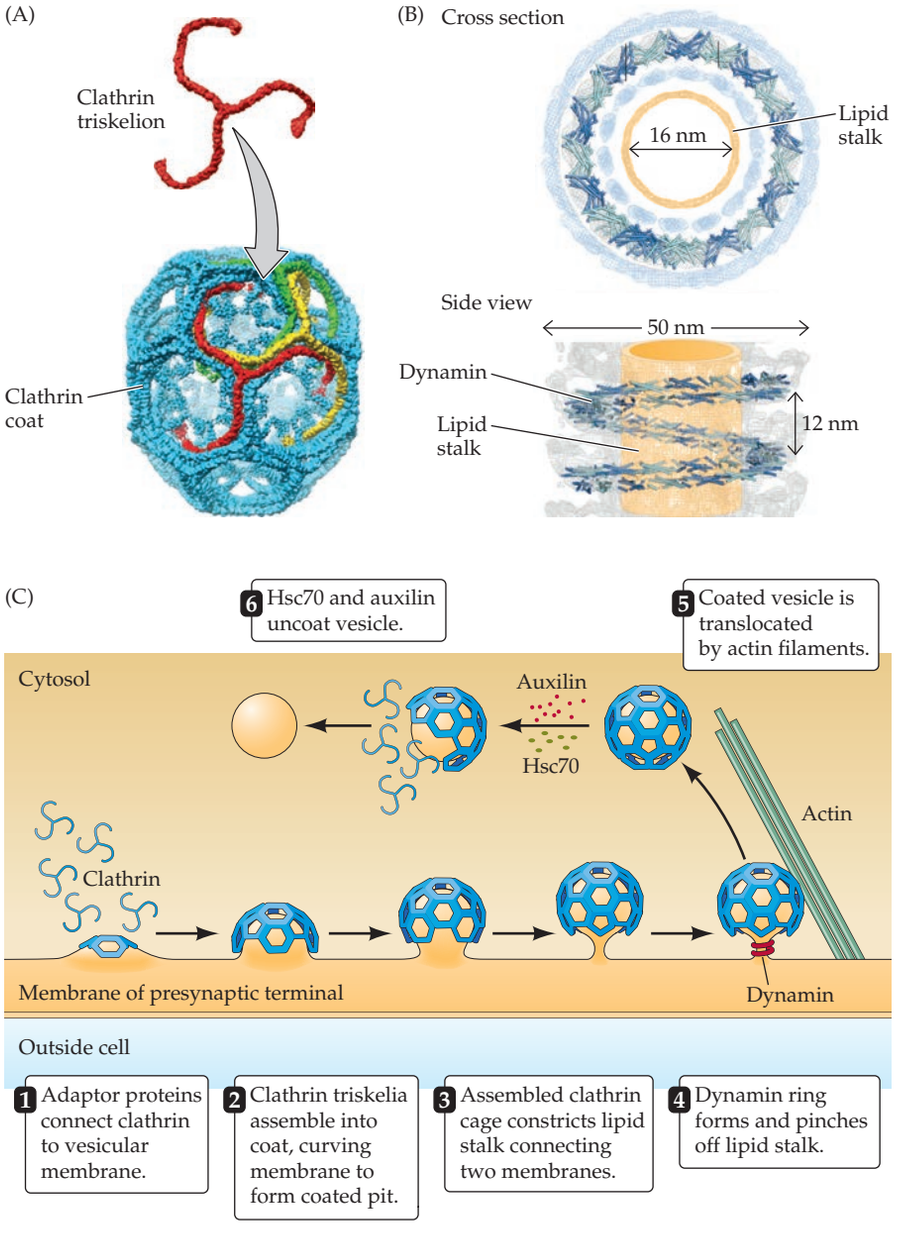


FIGURE 5.13 Molecular mechanisms of endocytosis following neurotransmitter release. (A) Individual clathrin triskelia assemble together to form membrane coats involved in membrane budding during endocytosis. (B) Dynamin forms ringlike coils around the lipid stalks of budding membranes; these rings disconnect vesicle membrane from plasma membrane during endocytosis. (C) A model for membrane budding during endocytosis. Following addition of synaptic vesicle membrane during exocytosis, clathrin triskelia attach to the vesicular membrane. Adaptor proteins, such as AP-2 and AP-180, aid their attachment. Polymerization of clathrin causes the membrane to curve and constrict, allowing dynamin to pinch off the coated vesicle. Subsequent uncoating of the vesicle, by Hsc70 and auxilin, yields a recycled synaptic vesicle. (A from Fotin et al., 2004; B from Ruebold et al., 2015; C after Shupliakov et al., 2010.)

are transported away from the plasma membrane by the cytoskeletal protein **actin**. This allows the clathrin coats to be removed by an ATPase, **Hsc70**, with another protein, **auxilin**, serving as a co-factor that recruits Hsc70 to the coated vesicle. Other proteins, such as **synaptojanin**, are also important for vesicle uncoating. Uncoated vesicles can then continue their journey through the recycling process, eventually becoming refilled with neurotransmitter due to the actions of neurotransmitter transporters in the vesicle membrane. These transporters exchange protons within the vesicle for neurotransmitter; the acidic interior of the vesicle is produced by a proton pump that also is located in the vesicle membrane.

In summary, a complex cascade of proteins, acting in a defined temporal and spatial order, allows neurons to secrete transmitters. This molecular cascade underlies the powerful ability of the brain to use its synapses to process and store information.

Neurotransmitter Receptors

The generation of postsynaptic electrical signals is also understood in considerable depth. Such studies began in 1907, when the British physiologist John N. Langley introduced the concept of **receptor molecules** to explain the specific and potent actions of certain chemicals on muscle and nerve cells. We now know that neurotransmitter receptors are proteins that are embedded in the plasma membrane of postsynaptic cells and have an extracellular neurotransmitter binding site that detects the presence of neurotransmitters in the synaptic cleft.

There are two broad families of receptor proteins that differ in their mechanism of transducing transmitter binding into postsynaptic responses. The receptors in one family contain a membrane-spanning domain that forms an ion channel (Figure 5.14A). These receptors combine transmitter-binding and channel functions into a single molecular entity and thus are called **ionotropic receptors** (Greek *tropos*, "to move in response to a stimulus") or ligand-gated ion channels.

The second family of neurotransmitter receptor is **metabotropic receptors**, so called because the eventual movement of ions through a channel depends on intervening metabolic steps. These receptors do not have ion channels as part of their structure; instead, they have an intracellular domain that indirectly affects channels though the activation of intermediate molecules called **G-proteins** (Figure 5.14B). Neurotransmitter binding to these receptors activates G-proteins, which then dissociate from the receptor and interact directly with ion channels or bind to other effector proteins, such as enzymes, that make intracellular messengers that open or close ion channels. Thus, G-proteins can be thought of as transducers that couple neurotransmitter binding to a receptor with regulation of

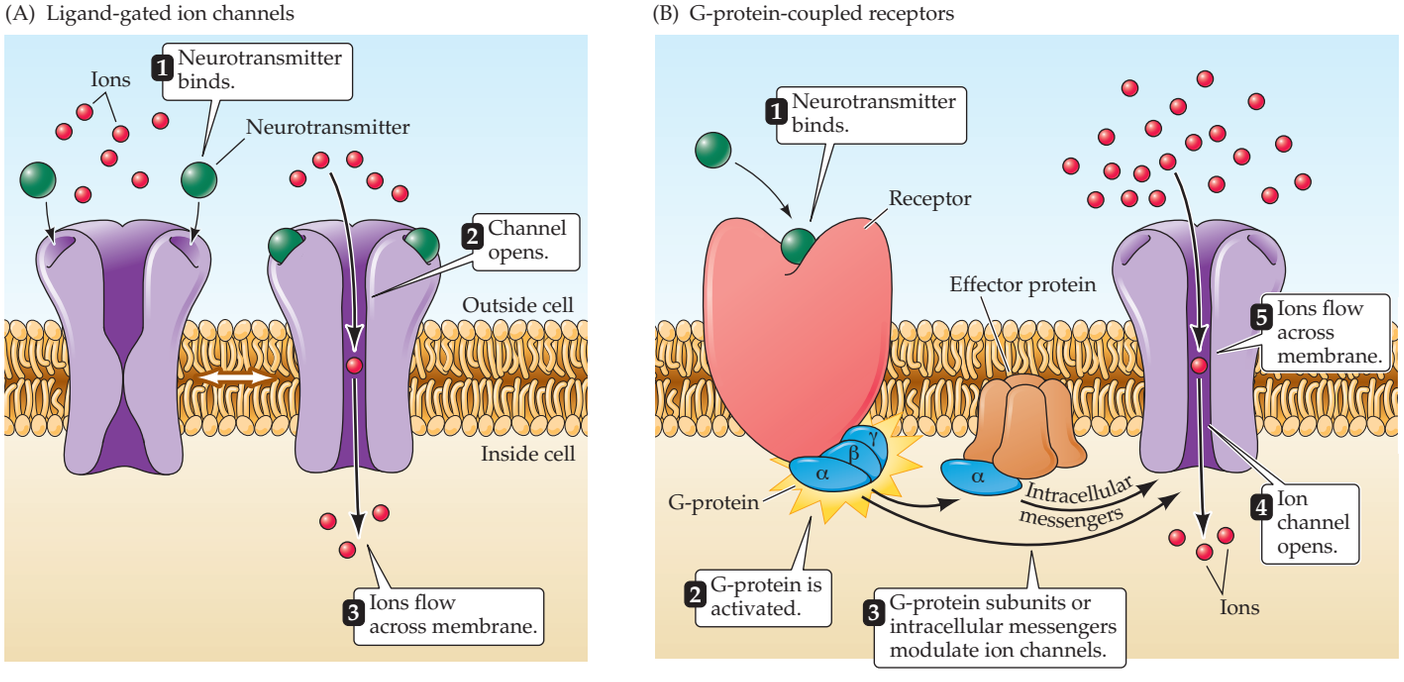


FIGURE 5.14 Two different types of neurotransmitter receptors. (A) Ligand-gated ion channels combine receptor and channel functions in a single protein complex. (B) Metabotropic receptors usually activate G-proteins, which modulate ion channels directly or indirectly through intracellular effector enzymes and second messengers.

postsynaptic ion channels. For this reason, metabotropic receptors are also called **G-protein-coupled receptors**. The postsynaptic signaling events initiated by metabotropic receptors are described in Chapter 7.

These two families of postsynaptic receptors give rise to postsynaptic actions that range from less than a millisecond to minutes, hours, or even days. Ionotropic receptors generally mediate rapid postsynaptic effects. Examples are the EPP produced at neuromuscular synapses by ACh (see Figure 5.5B), as well as the postsynaptic responses produced at certain glutamatergic synapses and GABAergic synapses (see Figure 5.19B). In these cases, the postsynaptic potentials arise within a millisecond or two of an action potential invading the presynaptic terminal and last for only a few tens of milliseconds or less. In contrast, the activation of metabotropic receptors typically produces much slower responses, ranging from hundreds of milliseconds to minutes or even longer. The comparative slowness of metabotropic receptor actions reflects the fact that multiple proteins need to bind to each other sequentially in order to produce the final physiological response. Importantly, many transmitters can activate both ionotropic and metabotropic receptors to produce both fast and slow postsynaptic potentials (sometimes even at the same synapse).

Postsynaptic Membrane Permeability Changes during Synaptic Transmission

Just as studies of the neuromuscular synapse paved the way for understanding neurotransmitter release mechanisms, this peripheral synapse has been equally valuable for understanding the mechanisms that allow neurotransmitter receptors to generate postsynaptic signals. The binding of ACh to postsynaptic receptors opens ion channels in the muscle fiber membrane. This effect can be demonstrated directly by using the patch clamp method (see Box 4A) to measure the minute postsynaptic currents that flow when two molecules of individual ACh bind to receptors, as Erwin Neher and Bert Sakmann first did in 1976. Exposure of the extracellular surface of a patch of postsynaptic membrane to ACh causes single-channel currents to flow for a few milliseconds (Figure 5.15A). This shows that ACh binding to its receptors opens ligand-gated ion channels,

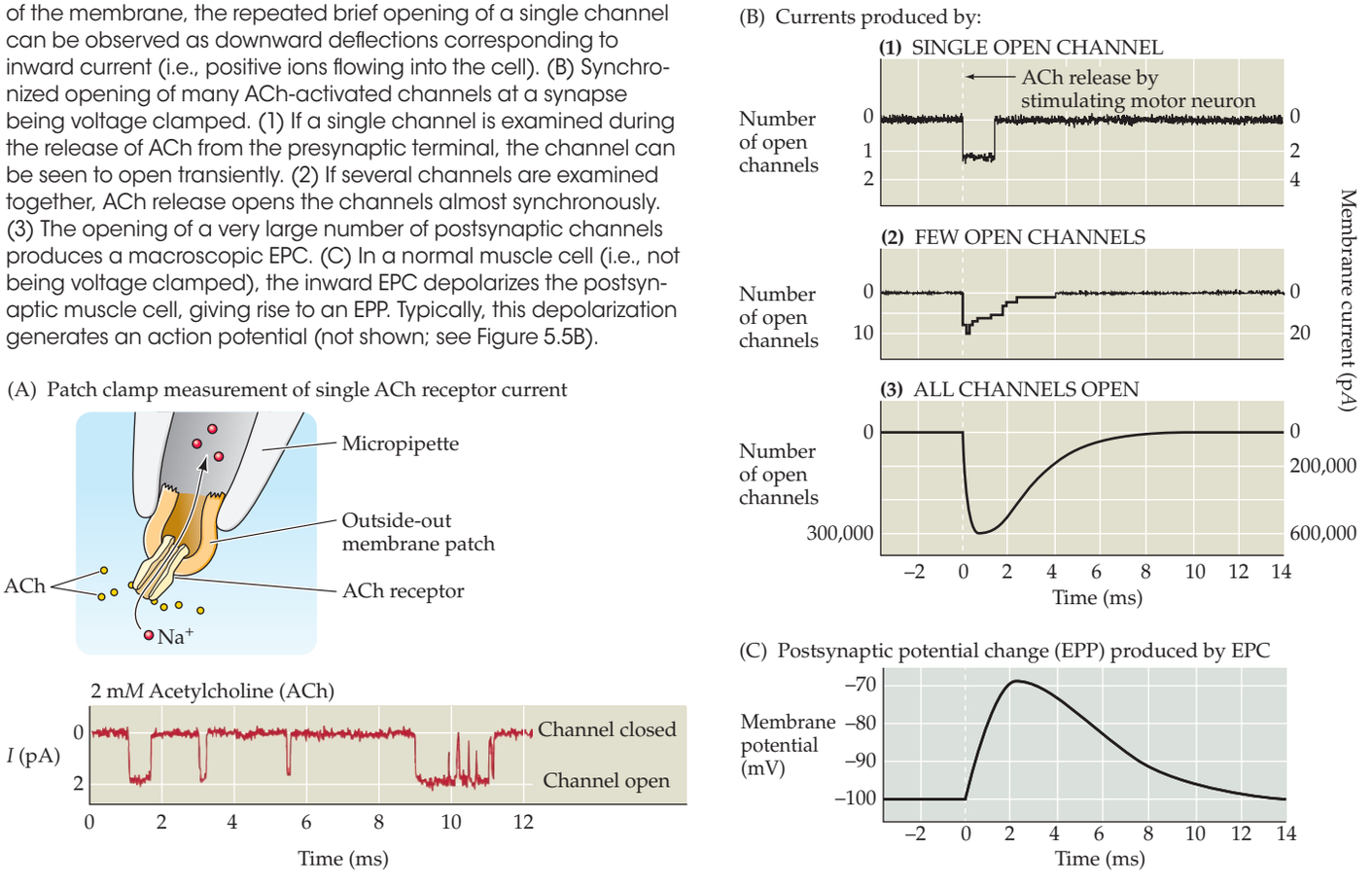


FIGURE 5.15 Activation of ACh receptors at neuromuscular synapses. (A) Patch clamp measurement of single ACh receptor currents from a patch of membrane removed from the postsynaptic muscle cell. When ACh is applied to the extracellular surface of the membrane, the repeated brief opening of a single channel can be observed as downward deflections corresponding to inward current (i.e., positive ions flowing into the cell). (B) Synchronized opening of many ACh-activated channels at a synapse being voltage clamped. (1) If a single channel is examined during the release of ACh from the presynaptic terminal, the channel can be seen to open transiently. (2) If several channels are examined together, ACh release opens the channels almost synchronously. (3) The opening of a very large number of postsynaptic channels produces a macroscopic EPC. (C) In a normal muscle cell (i.e., not being voltage clamped), the inward EPC depolarizes the postsynaptic muscle cell, giving rise to an EPP. Typically, this depolarization generates an action potential (not shown; see Figure 5.5B).

much in the way that changes in membrane potential open voltage-gated ion channels (see Chapter 4).

The electrical actions of ACh are greatly multiplied when an action potential in a presynaptic motor neuron causes the release of millions of molecules of ACh into the synaptic cleft. In this more physiological case, the transmitter molecules bind to many thousands of ACh receptors packed in a dense array on the postsynaptic membrane, transiently opening a very large number of postsynaptic ion channels. Although individual ACh receptors generate a microscopic current of only a few picoamperes (Figure 5.15B1), the opening of a large number of channels are opened synchronously when ACh is secreted from presynaptic terminals (Figure 5.15B2,3). The macroscopic current resulting from the summed opening of many ion channels is called the **end plate current**, or **EPC**. Because the EPC normally is inward, it causes the postsynaptic membrane potential to depolarize. This depolarizing change in

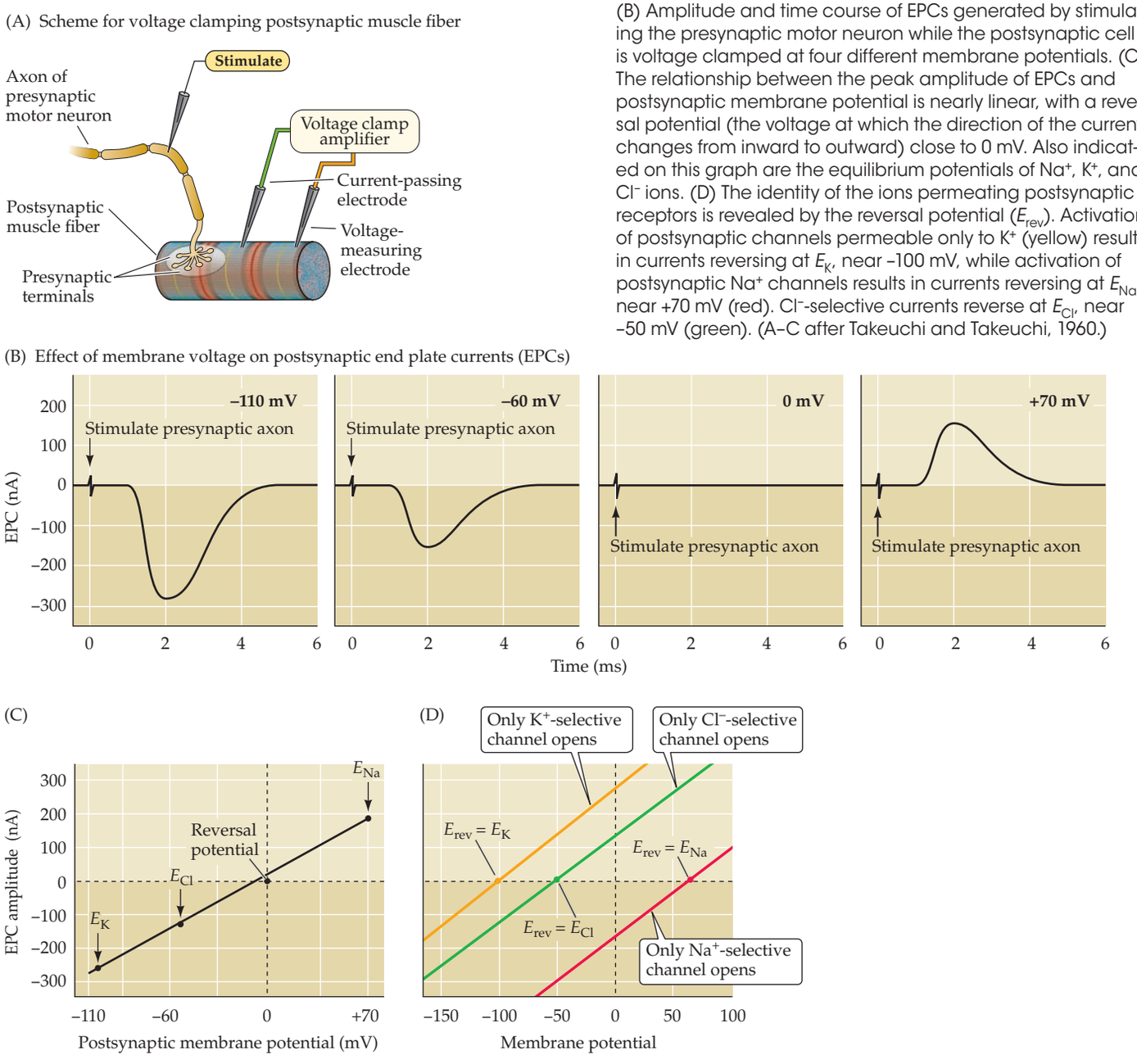


FIGURE 5.16 Influence of the postsynaptic membrane potential on end plate currents. (A) A postsynaptic muscle fiber is voltage clamped using two electrodes, while the presynaptic neuron is electrically stimulated to cause the release of ACh from presynaptic terminals. This experimental arrangement allows the recording of EPCs produced by ACh. (B) Amplitude and time course of EPCs generated by stimulating the presynaptic motor neuron while the postsynaptic cell is voltage clamped at four different membrane potentials. (C) The relationship between the peak amplitude of EPCs and postsynaptic membrane potential is nearly linear, with a reversal potential (the voltage at which the direction of the current changes from inward to outward) close to 0 mV. Also indicated on this graph are the equilibrium potentials of Na^+, K^+, and Cl^- ions. (D) The identity of the ions permeating postsynaptic receptors is revealed by the reversal potential (E_{rev}). Activation of postsynaptic channels permeable only to K^+ (yellow) results in currents reversing at E_K, near –100 mV, while activation of postsynaptic Na^+ channels results in currents reversing at E_{Na}, near +70 mV (red). Cl^--selective currents reverse at E_{Cl}, near –50 mV (green). (A–C after Takeuchi and Takeuchi, 1960.)

potential is the EPP (Figure 5.15C), which typically triggers a postsynaptic action potential by opening voltage-gated Na^+ and K^+ channels (see Figure 5.5B).

The identity of the ions that flow during the EPC can be determined via the same approaches used to identify the roles of Na^+ and K^+ fluxes in the currents underlying action potentials (see Chapter 3). Key to such an analysis is identifying the membrane potential at which no current flows during transmitter action. When the potential of the postsynaptic muscle cell is controlled by the voltage clamp method (Figure 5.16A), the magnitude of the membrane potential clearly affects the amplitude and polarity of EPCs (Figure 5.16B). Thus, when the postsynaptic membrane potential is made more negative than the resting potential, the amplitude of the EPC becomes larger, whereas this current is reduced when the membrane potential is made more positive. At approximately 0 mV, no EPC is detected, and at even more positive potentials, the current reverses its polarity, becoming outward rather than inward (Figure 5.16C). The potential where the EPC reverses, about 0 mV in the case of the neuromuscular junction, is called the **reversal potential**.

As was the case for currents flowing through voltage-gated ion channels (see Chapter 4), the magnitude of the EPC at any membrane potential is given by the product of the ion conductance activated by ACh (g_{ACh}) and the electrochemical driving force on the ions flowing through ligand-gated channels. Thus, the value of the EPC is given by the relationship

$$EPC = g_{ACh}(V_m - E_{rev})$$

where E_{rev} is the reversal potential for the EPC. This relationship predicts that the EPC will be an inward current at potentials more negative than E_{rev} because the electrochemical driving force, $V_m - E_{rev}$, is a negative number. Furthermore, the EPC will become smaller at potentials approaching E_{rev} because the driving force is reduced. At potentials more positive than E_{rev}, the EPC is outward because the driving force is reversed in direction (that is, positive). Because the channels opened by ACh are largely insensitive to membrane voltage, g_{ACh} will depend only on the number of channels opened by ACh, which depends in turn on the concentration of ACh in the synaptic cleft. Thus, the magnitude and polarity of the postsynaptic membrane potential determine the direction and amplitude of the EPC solely by altering the driving force on ions flowing through the receptor channels opened by ACh.

When V_m is at the reversal potential, $V_m - E_{rev}$ is equal to 0 and there is no net driving force on the ions that can permeate the receptor-activated channel. The identity of the ions that flow during the EPC can be deduced by observing how the reversal potential of the EPC compares with the equilibrium potential for various ion species (Figure 5.16D). For example, if ACh were to open an ion channel permeable only to K^+, then the reversal potential of the EPC would be at the equilibrium potential for K^+, which for a muscle cell is close to –100 mV. If the ACh-activated channels were permeable only to Na^+, then the reversal potential of the current would be approximately +70 mV, the Na^+ equilibrium potential of muscle cells; if these channels were permeable only to Cl^-, then the reversal potential would be approximately –50 mV. By this reasoning, ACh-activated channels cannot be permeable to only one of these ions, because the reversal potential of the EPC is not near the equilibrium potential for any of them (see Figure 5.16C). However, if these channels were permeable to both Na^+ and K^+, then the reversal potential of the EPC would be between +70 mV and –100 mV.

The fact that EPCs reverse at approximately 0 mV is therefore consistent with the idea that ACh-activated ion channels are almost equally permeable to both Na^+ and K^+. This hypothesis was tested in 1960, by the Japanese husband and wife team of Akira and Noriko Takeuchi, by altering the extracellular concentration of these two ions. As predicted, the magnitude and reversal potential of the EPC were changed by altering the concentration gradient of each ion. Lowering the external Na^+ concentration, which makes E_{Na} more negative, produces a negative shift in E_{rev} (Figure 5.17A), whereas elevating external K^+

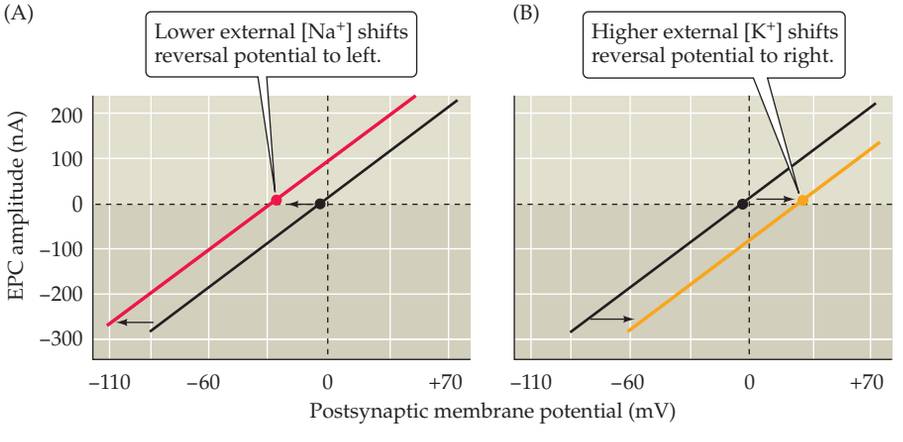


FIGURE 5.17 Reversal potential of the end plate current changes when ion gradients change. (A) Lowering the external Na^+ concentration causes EPCs to reverse at more negative potentials. (B) Raising the external K^+ concentration makes the reversal potential more positive. (After Takeuchi and Takeuchi, 1960.)

concentration, which makes E_K more positive, causes E_{rev} to shift to a more positive potential (Figure 5.17B). Such experiments establish that the ACh-activated ion channels are in fact permeable to both Na^+ and K^+.

Relationship between Ion Fluxes and Postsynaptic Potential Changes

Defining the ion fluxes occurring during the EPC permits understanding of how these ion fluxes generate the EPP. If the membrane potential of the muscle fiber is kept at E_K (approximately –100 mV), the EPC will arise entirely from an influx of Na^+ because at this potential there is no driving force on K^+. In the absence of a voltage clamp to prevent postsynaptic membrane potential changes, such an influx of Na^+ would cause a large depolarization and yield a large depolarizing EPP (Figure 5.18A). At the usual resting membrane potential of –90 mV, there is a small driving force on K^+, but a much greater one on Na^+. This means that much more Na^+ flows into the muscle cell than K^+ flows out (Figure 5.18B, left); the net influx of cations causes an EPC somewhat smaller than that measured at –100 mV and yields a depolarizing EPP that is also somewhat smaller than the EPP measured at –100 mV (Figure 5.18B, right). Thus, at the resting membrane potential, the EPP is generated primarily by Na^+ influx, along with a small efflux of K^+. At the reversal potential of 0 mV, Na^+ influx and K^+ efflux are exactly balanced, so no net current flows during the opening of channels by ACh binding (Figure 5.18C). This yields neither an EPC nor an EPP. At potentials more positive than E_{rev} the balance reverses; for example, at E_{Na} there

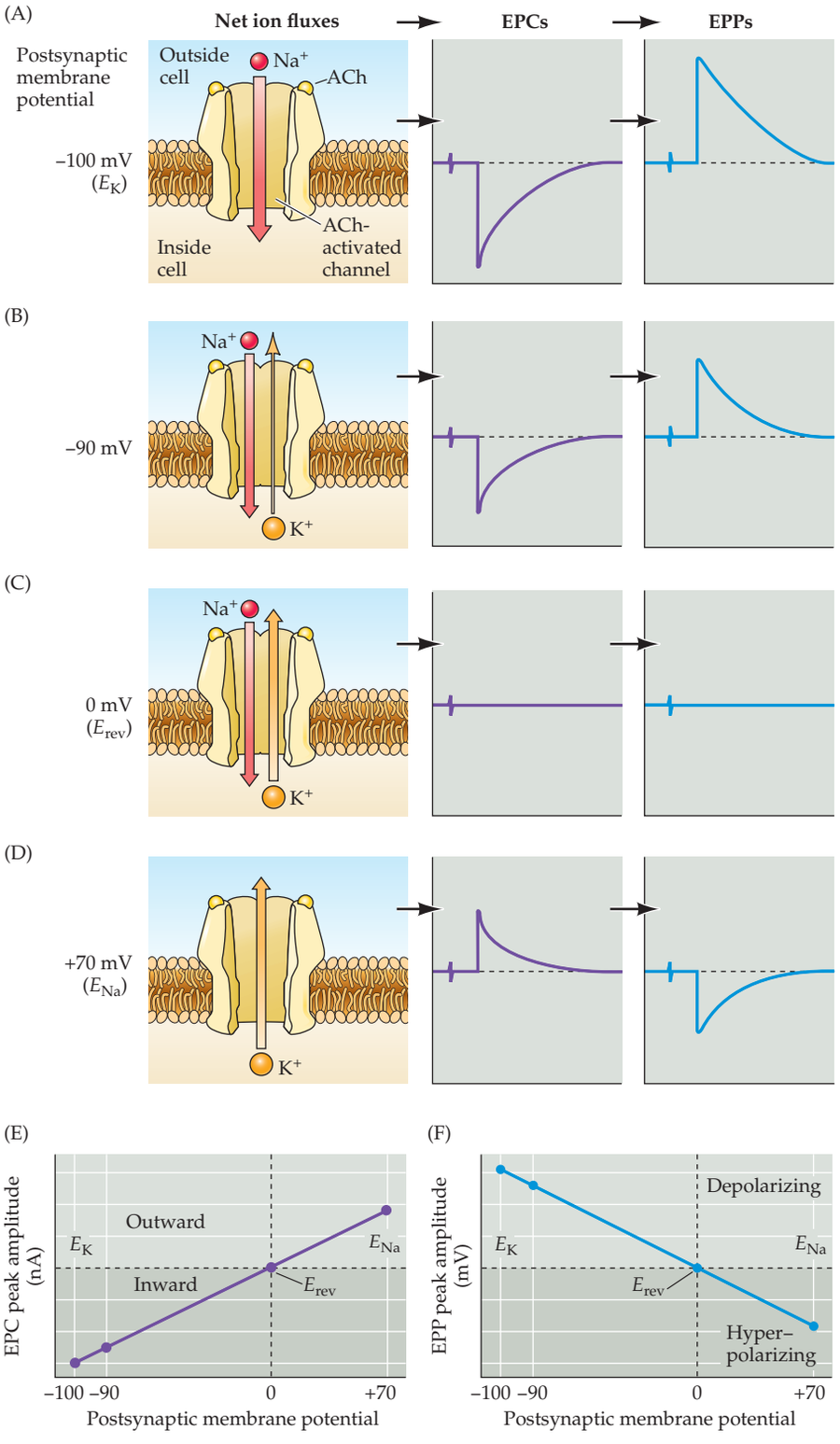


FIGURE 5.18 Na^+ and K^+ movements during EPCs and EPPs. (A–D) Each of the postsynaptic potentials indicated at the left results in different relative fluxes of Na^+ and K^+ (net ion fluxes). These ion fluxes determine the amplitude and polarity of the EPCs, which in turn determine the EPPs. Note that at about 0 mV the Na^+ flux is exactly balanced by an opposite K^+ flux, resulting in no net current flow, and hence no change in the membrane potential. (E) EPCs are inward at potentials more negative than E_{rev} and outward at potentials more positive than E_{rev}. (F) EPPs depolarize the postsynaptic cell at potentials more negative than E_{rev}. At potentials more positive than E_{rev}, EPPs hyperpolarize the cell.

is no influx of Na^+ and a large efflux of K^+ because of the large driving force on K^+ (Figure 5.18D). This produces an outward EPC and a hyperpolarizing EPP. In summary, the polarity and magnitude of the EPC (Figure 5.18E) depend on the electrochemical driving force on the permeant ions, which in turn determines the polarity and magnitude of the EPP (Figure 5.18F). EPPs will depolarize when the membrane potential is more negative than E_{rev}, and hyperpolarize when the membrane potential is more positive than E_{rev}. The general rule, then, is that *the action of a transmitter drives the postsynaptic membrane potential toward E_{rev} for the particular ion channels being activated*.

Although this discussion has focused on the neuromuscular junction, similar mechanisms generate postsynaptic responses at all chemical synapses: Transmitter binding to postsynaptic receptors produces a postsynaptic conductance change as ion channels are opened (or sometimes closed). The postsynaptic conductance is increased if—as at the neuromuscular junction—channels are opened, and is decreased if channels are closed. This conductance change typically generates an electrical current, the **postsynaptic current** (**PSC**), which in turn changes the postsynaptic membrane potential to produce a **postsynaptic potential** (**PSP**). As in the specific case of the EPP at the neuromuscular junction, PSPs are depolarizing if their reversal potential is more positive than the resting membrane potential and hyperpolarizing if their reversal potential is more negative.

The conductance changes and the PSPs that typically accompany them are the ultimate outcome of most chemical synaptic transmission, concluding a sequence of electrical and chemical events that begins with the invasion of an action potential into the terminals of a presynaptic neuron. In many ways, the events that produce PSPs at synapses are similar to those that generate action potentials in axons; in both cases, conductance changes produced by ion channels lead to ion current flow that changes the membrane potential.

Excitatory and Inhibitory Postsynaptic Potentials

PSPs ultimately alter the probability that an action potential will be produced in the postsynaptic cell. At the neuromuscular junction, synaptic action increases the probability that an action potential will occur in the postsynaptic muscle cell; indeed, the large amplitude of the EPP ensures that an action potential always is triggered. At many other synapses, PSPs similarly increase the probability of firing a postsynaptic action potential. However, still other synapses actually *decrease* the probability that the postsynaptic cell will generate an action potential. PSPs are called **excitatory** (or **EPSPs**) if they increase the likelihood of a postsynaptic action potential occurring, and **inhibitory** (or **IPSPs**) if they decrease this likelihood. Given that most neurons receive inputs from both excitatory and inhibitory synapses, it is important to understand more precisely the mechanisms that determine whether a particular synapse excites or inhibits its postsynaptic partner.

The principles of excitation just described for the neuromuscular junction are pertinent to all excitatory synapses. The principles of postsynaptic inhibition are much the same as for excitation, and they are also quite general. In both cases, neurotransmitters binding to receptors open or close ion channels in the postsynaptic cell. Whether a postsynaptic response is an EPSP or an IPSP depends on the type of channel that is coupled to the receptor, and on the concentration of permeant ions inside and outside the cell. In fact, the only distinction between postsynaptic excitation and inhibition is the reversal potential of the PSP in relation to the threshold voltage for generating action potentials in the postsynaptic cell.

Consider, for example, a neuronal synapse that uses glutamate as the transmitter. Many such synapses have receptors that, like the ACh receptors at neuromuscular synapses, open ion channels that are nonselectively permeable to cations (see Chapter 6). When these glutamate receptors are activated, both Na^+ and K^+ flow across the postsynaptic membrane, yielding an E_{rev} of approximately 0 mV for the resulting postsynaptic current. If the resting potential of the postsynaptic neuron is –60 mV, the resulting EPSP will depolarize by bringing the postsynaptic membrane potential toward 0 mV. For the hypothetical neuron shown in Figure 5.19A, the action potential threshold voltage is –40 mV. Thus, a glutamate-induced EPSP will increase the probability that this neuron produces an action potential, defining the synapse as excitatory.

As an example of inhibitory postsynaptic action, consider a neuronal synapse that uses GABA as its transmitter. At such synapses, the GABA receptors typically open channels that are selectively permeable to Cl^-, and the action of GABA causes Cl^- to flow across the postsynaptic membrane. Consider a case where E_{Cl} is –70 mV, as is the case for some neurons, so that the postsynaptic resting potential of –60 mV is less negative than E_{Cl}. The resulting positive electrochemical driving force ($V_m - E_{rev}$) will cause negatively charged Cl^- to flow into the cell and produce a hyperpolarizing IPSP (Figure 5.19B). This hyperpolarizing IPSP will take the postsynaptic membrane away from the action potential threshold of –40 mV, clearly inhibiting the postsynaptic cell.

Surprisingly, inhibitory synapses need not produce hyperpolarizing IPSPs. For instance, if E_{Cl} were –50 mV instead of –70 mV, then the negative electrochemical driving force would cause Cl^- to flow out of the cell and produce a depolarizing IPSP (Figure 5.19C). However, the synapse would still be inhibitory: Given that the reversal potential of the IPSP still is more negative than the action potential threshold (–40 mV), the depolarizing IPSP would inhibit

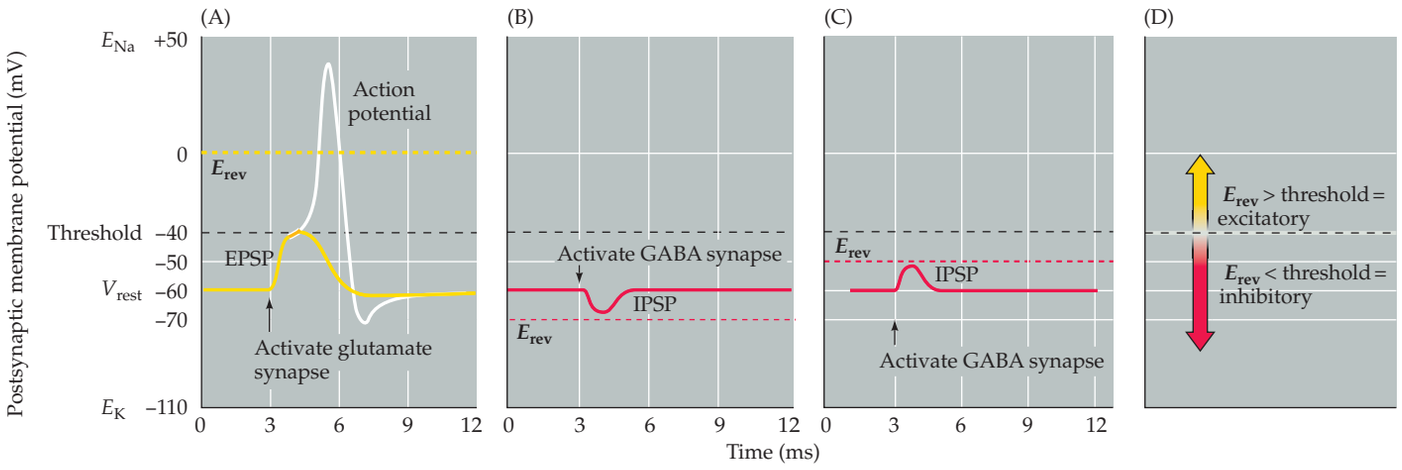


FIGURE 5.19 Reversal potentials and threshold potentials determine postsynaptic excitation and inhibition. (A) If the reversal potential for a PSP (0 mV) is more positive than the action potential threshold (–40 mV), the effect of a transmitter is excitatory, and it generates EPSPs. (B) If the reversal potential for a PSP is more negative than the action potential threshold, the transmitter is inhibitory and generates IPSPs. (C) IPSPs can nonetheless depolarize the postsynaptic cell if their reversal potential is between the resting potential and the action potential threshold. (D) The general rule of postsynaptic action is: If the reversal potential is more positive than threshold, excitation results; inhibition occurs if the reversal potential is more negative than threshold.

because the postsynaptic membrane potential would be kept more negative than the threshold for action potential initiation. Another way to think about this peculiar situation is that if another excitatory input onto this neuron brought the cell's membrane potential to –41 mV—just below the threshold for firing an action potential—the IPSP would then hyperpolarize the membrane potential toward –50 mV, bringing the potential away from the action potential threshold. Thus, while EPSPs depolarize the postsynaptic cell, IPSPs can either hyperpolarize or depolarize; indeed, an inhibitory conductance change may produce no potential change at all and still exert an inhibitory effect by making it more difficult for an EPSP to evoke an action potential in the postsynaptic cell.

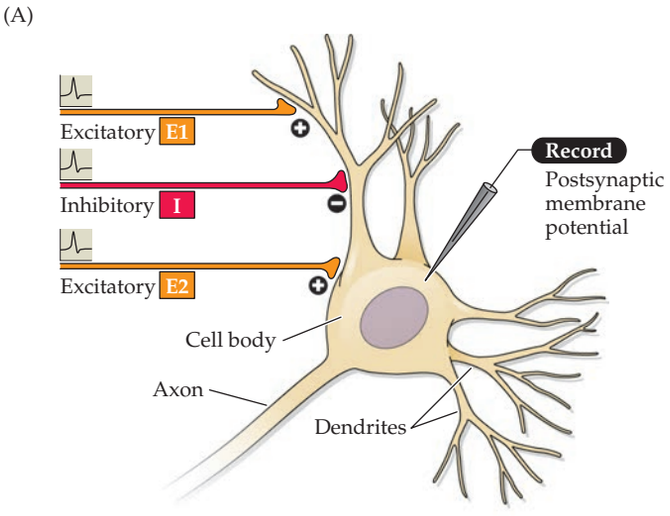
Although the particulars of postsynaptic action can be complex, a simple rule distinguishes postsynaptic excitation from inhibition: An EPSP has a reversal potential more positive than the action potential threshold, whereas an IPSP has a reversal potential more negative than threshold (Figure 5.19D). Intuitively, this rule can be understood by realizing that an EPSP will tend to depolarize the membrane potential so that it exceeds threshold, whereas an IPSP will always act to keep the membrane potential more negative than the threshold potential.

Summation of Synaptic Potentials

The PSPs produced at most synapses in the brain are much smaller than those at the neuromuscular junction; indeed, EPSPs produced by individual excitatory synapses may be only a fraction of a millivolt and are usually well below the threshold for generating postsynaptic action potentials. How, then, can such synapses transmit information if their PSPs are subthreshold? The answer is that neurons in the central nervous system are typically innervated by thousands of synapses, and the PSPs produced by each active synapse can *sum together*—in space and in time—to determine the behavior of the postsynaptic neuron.

Consider the highly simplified case of a neuron that is innervated by two excitatory synapses, each generating a subthreshold EPSP, and an inhibitory synapse that produces an IPSP (Figure 5.20A). While activation of either one of the excitatory synapses alone (E1 or E2 in Figure 5.20B) produces a subthreshold EPSP, activation of both excitatory synapses at about the same time causes the two EPSPs to sum together. If the sum of the two EPSPs (E1 + E2) depolarizes the postsynaptic neuron sufficiently to reach the threshold potential, a postsynaptic action potential results. **Summation** thus allows subthreshold EPSPs to influence action potential production. Likewise, an IPSP generated by an inhibitory synapse (I) can sum (algebraically speaking) with a subthreshold EPSP to reduce its amplitude (E1 + I) or can sum with suprathreshold EPSPs to prevent the postsynaptic neuron from reaching threshold (E1 + I + E2).

In short, the summation of EPSPs and IPSPs by a postsynaptic neuron permits a neuron to integrate the electrical information provided by all the inhibitory and excitatory synapses acting on it at any moment. Whether the sum of active synaptic inputs results in the production of an action potential depends on the balance between excitation and inhibition. If the sum of all EPSPs and IPSPs results in a depolarization of sufficient amplitude to



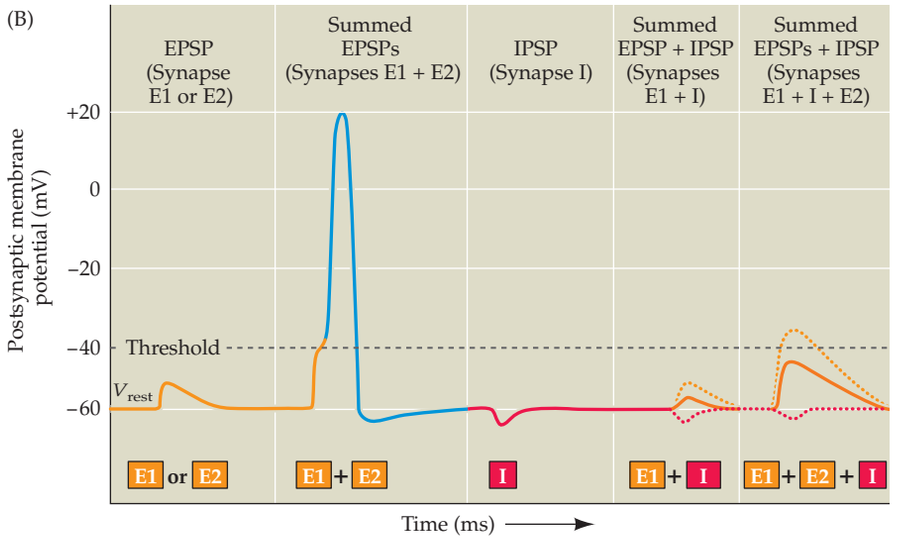


FIGURE 5.20 Summation of postsynaptic potentials. (A) A microelectrode records the postsynaptic potentials produced by the activity of two excitatory synapses (E1 and E2) and an inhibitory synapse (I). (B) Electrical responses to synaptic activation. Stimulating either excitatory synapse (E1 or E2) produces a subthreshold EPSP, whereas stimulating both synapses at the same time (E1 + E2) produces a suprathreshold EPSP that evokes a postsynaptic action potential (shown in blue). Activation of the inhibitory synapse alone (I) results in a hyperpolarizing IPSP. Summing this IPSP (dashed red line) with the EPSP (dashed yellow line) produced by one excitatory synapse (E1 + I) reduces the amplitude of the EPSP (solid orange line), while summing it with the suprathreshold EPSP produced by activating synapses E1 and E2 keeps the postsynaptic neuron below threshold, so that no action potential is evoked.

raise the membrane potential above threshold, then the postsynaptic cell will produce an action potential. Conversely, if inhibition prevails, then the postsynaptic cell will remain silent. Typically, the balance between EPSPs and IPSPs changes continually over time, depending on the number of excitatory and inhibitory synapses active at a given moment and the magnitude of the current at each active synapse. Summation is therefore a tug-of-war between all excitatory and inhibitory postsynaptic currents; the outcome of the contest determines whether or not a postsynaptic neuron fires an action potential and thereby becomes an active element in the neural circuits to which it belongs.

In conclusion, at chemical synapses neurotransmitter release from presynaptic terminals initiates a series of postsynaptic events that culminate in a transient change in the probability of a postsynaptic action potential occurring (Figure 5.21). Such synaptic signaling allows neurons to form the intricate synaptic circuits that play fundamental roles in brain information processing. Recent work further suggests that glial cells also contribute to synaptic signaling, adding another dimension to information processing in the brain (Box 5A).

Summary

Synapses communicate the information carried by action potentials from one neuron to the next in neural circuits. The mechanisms underlying postsynaptic potentials generated during synaptic transmission are closely related to the mechanisms that generate other types of neuronal electrical signals, namely, ionic flow through membrane channels. In the case of electrical synapses, these channels are connexons; direct but passive flow of current through connexons is the basis for transmission. In the case of chemical synapses, channels with smaller and more selective pores are activated by the binding of neurotransmitters to postsynaptic receptors following release of the neurotransmitters from the presynaptic terminal. The large number of neurotransmitters in the nervous system can be divided into two broad classes: small-molecule transmitters and neuropeptides. Neurotransmitters are synthesized from defined precursors by regulated enzymatic pathways, packaged into one of several types of synaptic vesicles, and released into the synaptic cleft in a Ca^{2+}-dependent manner. Transmitter agents are released presynaptically in units, or quanta, reflecting their storage within synaptic vesicles. Vesicles discharge their contents into the synaptic cleft when the presynaptic depolarization generated by the invasion of an action potential opens voltage-gated calcium channels, allowing Ca^{2+} to enter the presynaptic terminal. Calcium triggers neurotransmitter release

BOX 5A ■ The Tripartite Synapse

Up to this point, the discussion has considered signaling between presynaptic neurons and their postsynaptic targets to be a private dialogue between these two cells. However, recent work suggests that this synaptic conversation may involve glial cells as well.

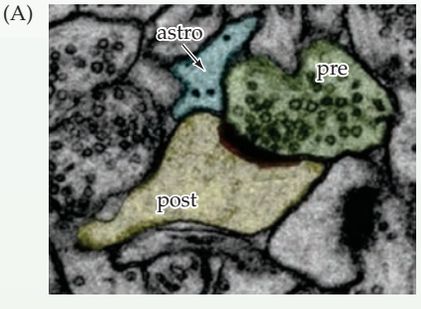
As mentioned in Chapter 1, glial cells support neurons in several ways. For example, it is well established that glia regulate the extracellular environment by removing K^+ that accumulates during action potential generation and by removing neurotransmitters at the conclusion of synaptic transmission. Consistent with such roles, glia seem to occupy virtually all of the non-neuronal volume of the brain. This means that glia are found in very close association with synapses (see Figure A); a given synapse typically is no more than a few hundred nanometers away from a glial cell. Glia form exceedingly fine processes that completely envelop synapses (see Figure B), an intimate association that raises the possibility of a signaling role for glia at synapses.

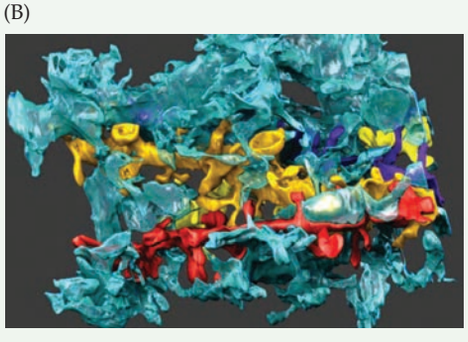
The first support for such a role came from the discovery that glia respond to application of neurotransmitters. The list of neurotransmitters that elicit responses in glia now includes acetylcholine, glutamate, GABA, and many others. These responses are mediated by the same sorts of neurotransmitter receptors that are employed in synaptic signaling, most often the metabotropic receptors that are coupled to intracellular signaling cascades (see Figure 5.14B). In some cases, neurotransmitters produce changes in the membrane potential of glia. More often, these neurotransmitters cause transient changes in intracellular calcium concentration within the glial cell. These intracellular calcium signals often are observed to trigger calcium waves that spread both within a single glial cell and also propagate between glia (see Figure C).

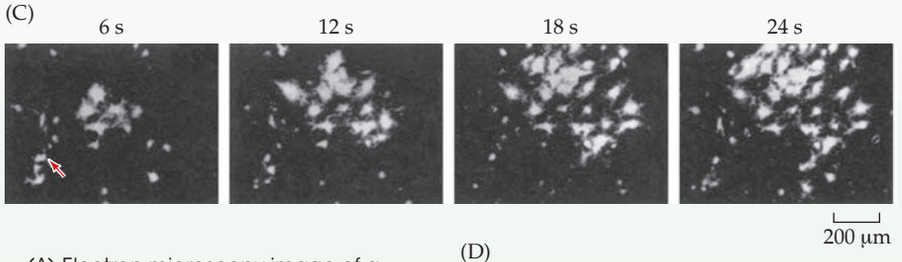
These transient rises in intracellular calcium serve as second messenger signals (see Chapter 7) that trigger a number of physiological responses in glia. The most remarkable response is the release of several molecules—such as glutamate, GABA, and ATP—that are traditionally considered to be neurotransmitters. Release of such "gliotransmitters" occurs both via the calcium-triggered exocytotic mechanisms employed in presynaptic terminals of neurons (see Figure 5.4C), as well as via unconventional release mechanisms such as permeation through certain ion channels.

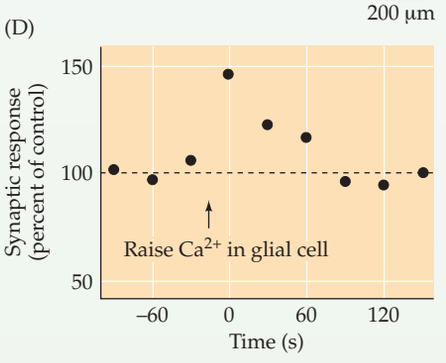
The ability both to respond to and to release neurotransmitters potentially makes glia participants in synaptic signaling. Indeed, release of neurotransmitters from a variety of presynaptic terminals has been found to elicit responses in glia. Furthermore, release of gliotransmitters has been found to regulate transmission at numerous synapses (see Figure D). In some cases, glia regulate release of transmitters from presynaptic terminals, while in other cases they alter postsynaptic responsiveness. Glia can also alter the ability of synapses to undergo activity-dependent, plastic changes in synaptic transmission (see Chapter 8).

This ability of glia to participate in synaptic signaling has led to the concept of the **tripartite synapse**, a three-way junction involving the presynaptic terminal, the postsynaptic process, and neighboring glia. While there is still debate about the physiological significance of such neuron–glia interactions, the ability of glia to release neurotransmitters, similar to presynaptic terminals, and to respond to neurotransmitters, similar to postsynaptic neurons, is dramatically changing our view of brain signaling mechanisms.









(A) Electron microscopy image of a synapse between a presynaptic terminal (pre) and postsynaptic neuron (post), with a glial cell (astrocyte; astro) immediately adjacent to the synapse. (B) Three-dimensional structure of the contact between a glial cell (blue) surrounding dendrites of four postsynaptic neurons (different colors). (C) Application of glutamate (at arrow) increases intracellular Ca^{2+} (white) in cultured glia. Examination of these cells at different times indicates that the calcium signals spread as a wave through neighboring glia. (D) Transient elevation of Ca^{2+} within a single glial cell (arrow) enhances transmission at an excitatory synapse in the hippocampus. (A,B from Witcher et al., 2007; C from Cornell-Bell et al., 1990; D after Perea and Araque, 2007.)

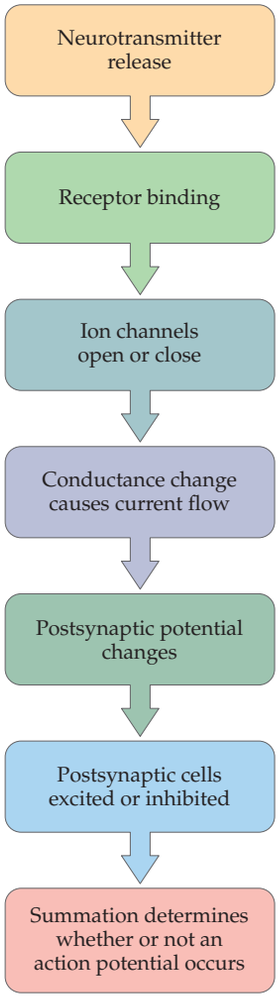


FIGURE 5.21 Overview of postsynaptic signaling. Neurotransmitter released from presynaptic terminals binds to its cognate postsynaptic receptor, which causes the opening or closing of specific postsynaptic ion channels. The resulting conductance change causes current to flow, which may change the membrane potential. The postsynaptic cell sums (or integrates) all of the EPSPs and IPSPs, resulting in moment-to-moment control of action potential generation.

by binding to the Ca^{2+} sensor protein, synaptotagmin, working in concert with SNARE proteins found on both vesicle and plasma membranes. Postsynaptic receptors are a diverse group of proteins that transduce binding of neurotransmitters into electrical signals by opening or closing postsynaptic ion channels. Two broadly different families of neurotransmitter receptors have evolved to carry out the postsynaptic signaling actions of neurotransmitters. The postsynaptic currents produced by the synchronous opening or closing of ion channels change the conductance of the postsynaptic cell, thus increasing or decreasing its excitability. Conductance changes that increase the probability of firing an action potential are excitatory, whereas those that decrease the probability of generating an action potential are inhibitory. Because postsynaptic neurons are usually innervated by many different inputs, the integrated effect of the conductance changes underlying all EPSPs and IPSPs produced in a postsynaptic cell at any moment determines whether or not the cell fires an action potential. The postsynaptic effects of neurotransmitters are terminated by the degradation of the transmitter in the synaptic cleft, by transport of the transmitter back into cells, or by diffusion out of the synaptic cleft. The response elicited at a given synapse depends on the type of neurotransmitter released and the postsynaptic complement of receptors and associated channels.

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