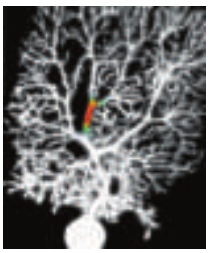
Chapter 5



Synaptic Transmission

Overview

The human brain contains at least 100 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 synapse—electrical and chemical—can be distinguished on the basis of their mechanism of transmission. At electrical synapses, current flows through gap junctions, which are specialized membrane channels that connect two cells. 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 receptor molecules. The total number of neurotransmitters is not known, but 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 and/or degradation. The secretion of neurotransmitters is triggered by the influx of Ca^{2+} through voltage-gated channels, which gives rise to a transient increase in Ca^{2+} concentration within 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. Although it is not yet understood exactly how Ca^{2+} triggers exocytosis, specific proteins on the surface of the synaptic vesicle and elsewhere in the presynaptic terminal mediate this process. 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 molecules. 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 ionic permeability of the ion channel affected by the transmitter, and by the concentration of permeant ions inside and outside the cell.

Electrical Synapses

Although there are many kinds of synapses within the human brain, they can be divided into two general classes: electrical synapses and chemical synapses. Although they are a distinct minority, electrical synapses are found in all nervous systems, permitting direct, passive flow of electrical current from one neuron to another.

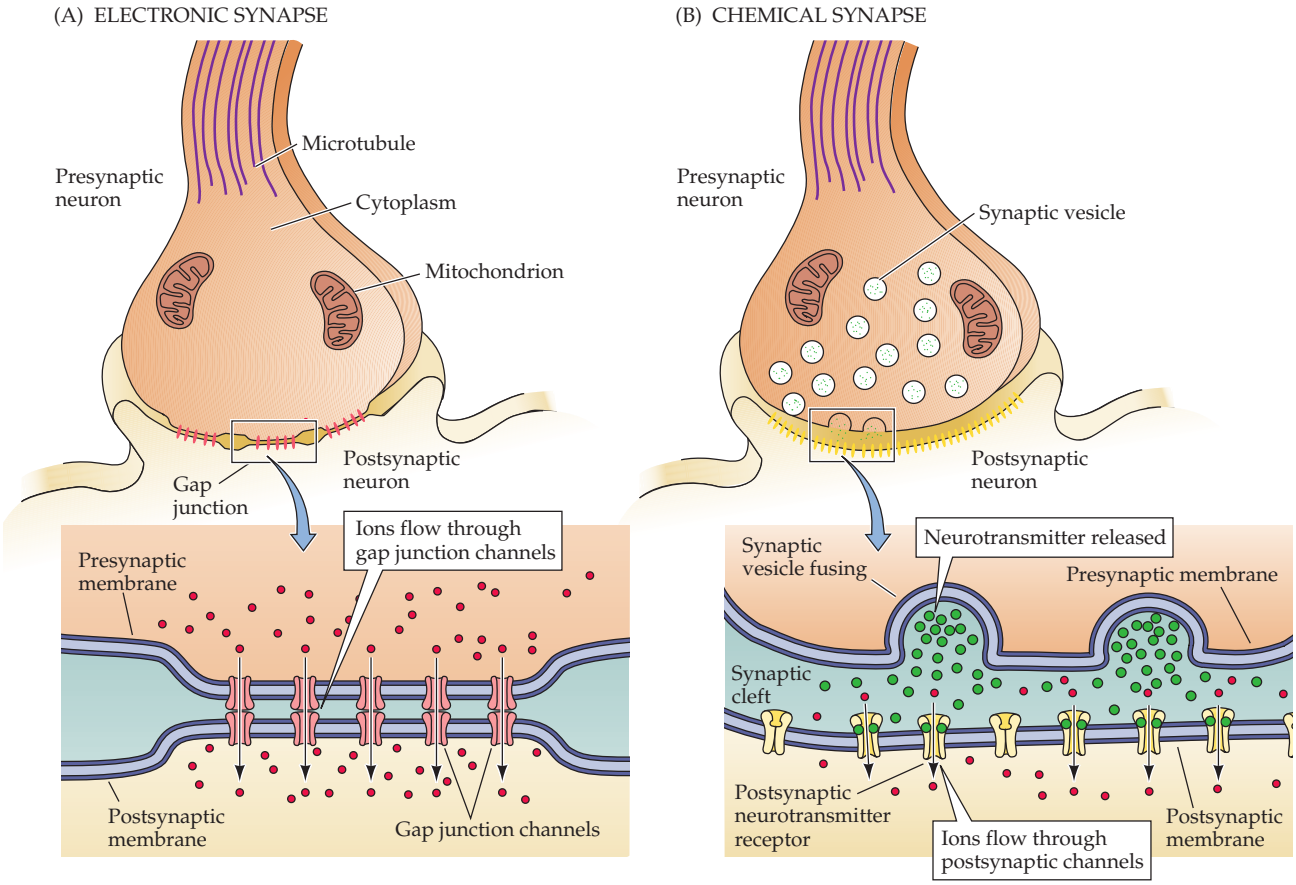


Figure 5.1 Electrical and chemical synapses differ fundamentally in their transmission mechanisms. (A) At electrical synapses, gap junctions between pre- and postsynaptic membranes permit current to flow passively through intercellular channels (blowup). This current flow changes the postsynaptic membrane potential, initiating (or in some instances inhibiting) the generation of postsynaptic action potentials. (B) At chemical synapses, there is no intercellular continuity, and thus no direct flow of current from pre- to postsynaptic cell. 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 (blowup).

The structure of an electrical synapse is shown schematically in Figure 5.1A. The "upstream" neuron, which is the source of current, is called the **presynaptic** element, and the "downstream" neuron into which this current flows is termed **postsynaptic**. The membranes of the two communicating neurons come extremely close at the synapse and are actually linked together by an intercellular specialization called a **gap junction**. Gap junctions contain precisely aligned, paired channels in the membrane of the pre- and postsynaptic neurons, such that each channel pair forms a pore (see Figure 5.2A). The pore of a gap junction channel is much larger than the pores of the voltage-gated ion channels described in the previous chapter. 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 gap junction pores include molecules with molecular weights as great as several hundred daltons. This permits ATP and other important intracellular metabolites, such as second messengers (see Chapter 7), to be transferred between neurons.

Electrical synapses thus work by allowing ionic current to flow passively through the gap junction pores from one neuron to another. The usual source of this current is the potential difference generated locally by the action potential (see Chapter 3). This arrangement has a number of interesting consequences. One is that transmission can be bidirectional; that is, current can flow in either direction across the gap junction, depending on which member of the coupled pair is invaded by an action potential (although

some types of gap junctions have special features that render their transmission unidirectional). Another important feature of the electrical synapse is that transmission is extraordinarily fast: because passive current flow across the gap junction is virtually instantaneous, communication can occur without the delay that is characteristic of chemical synapses.

These features are 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 5.2). In fact, at least part of this brief synaptic delay is caused by propagation of the action potential into the presynaptic terminal, so that 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.

A more general purpose of electrical synapses is 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. Electrical transmission between certain hormone-secreting neurons within the mammalian hypothalamus ensures that all cells fire action potentials at about the same time, thus facilitating a burst of hormone secretion into the circulation. The fact that gap junction pores are large enough to allow molecules such as ATP and second messengers to diffuse intercellularly also permits electrical synapses to coordinate the intracellular signaling and metabolism of coupled cells. This property may be particularly important for glial cells, which form large intracellular signaling networks via their gap junctions.

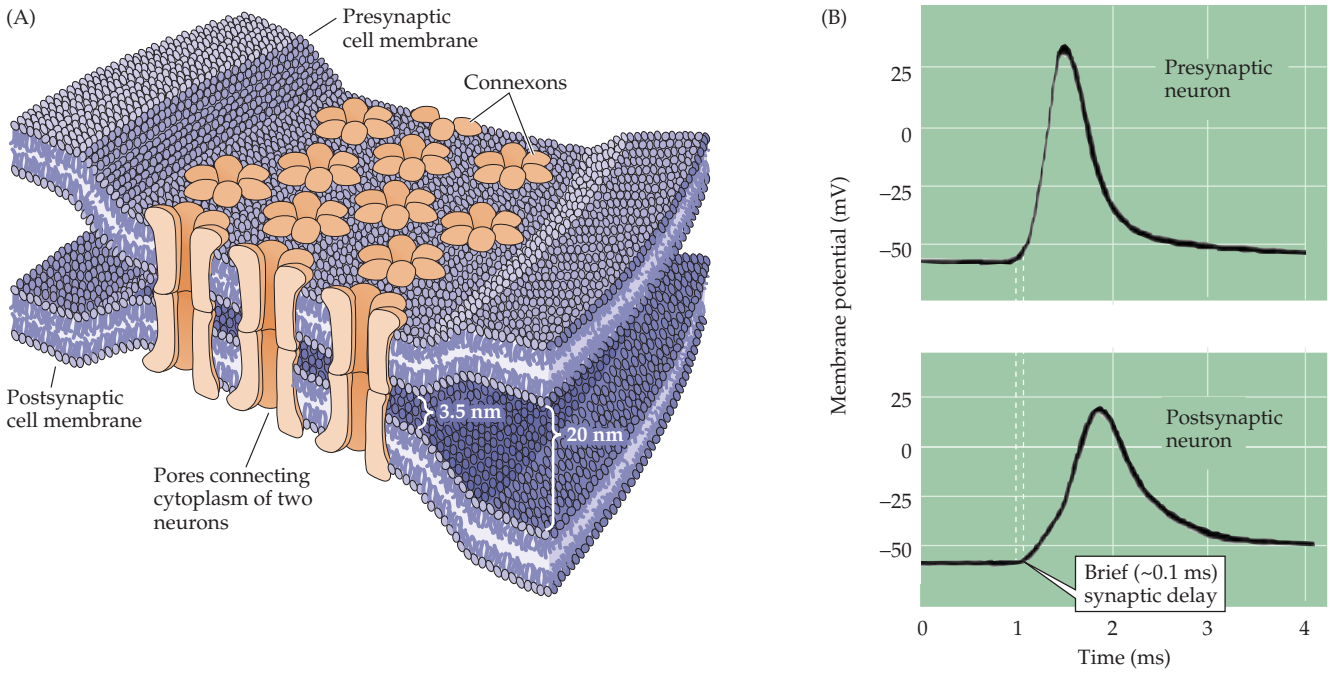


Figure 5.2 Structure and function of gap junctions at electrical synapses. (A) Gap junctions consist of hexameric complexes formed by the coming together of subunits called connexons, which are present in both the pre- and postsynaptic membranes. The pores of the channels connect to one another, creating electrical continuity between the two cells. (B) 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 after Furshpan and Potter, 1959.)

Signal Transmission at Chemical Synapses

The general structure of a chemical synapse is shown schematically in Figure 5.1B. 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 key 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**, the chemical signals secreted from the presynaptic neuron, and it is these chemical agents acting as messengers between the communicating neurons that gives this type of synapse its name.

Transmission at chemical synapses is based on the elaborate sequence of events depicted in Figure 5.3. The process 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.

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 into (or out of) the postsynaptic cells. 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. In this way, information is transmitted 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. A key experiment that supported this idea was performed in 1926 by German physiologist Otto Loewi. 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. He isolated and perfused the hearts of two frogs, monitoring the rates at which they were beating (Figure 5.4). His experiment collected the perfusate flowing through the stimulated heart and transferred this solution to the second heart. When the vagus nerve to the first heart was stimulated, the beat of this heart slowed. Remarkably, even though the vagus nerve of the second heart had not been stimulated, its beat also slowed when exposed to the perfusate from the first heart. This result showed that the vagus nerve regulates the heart rate by releasing a chemical that accumulates in the perfusate. Originally referred to as "vagus substance," the agent was later shown to be **acetylcholine** (**ACh**). ACh is now known to be a neurotransmitter that acts not only in the heart but at a vari-

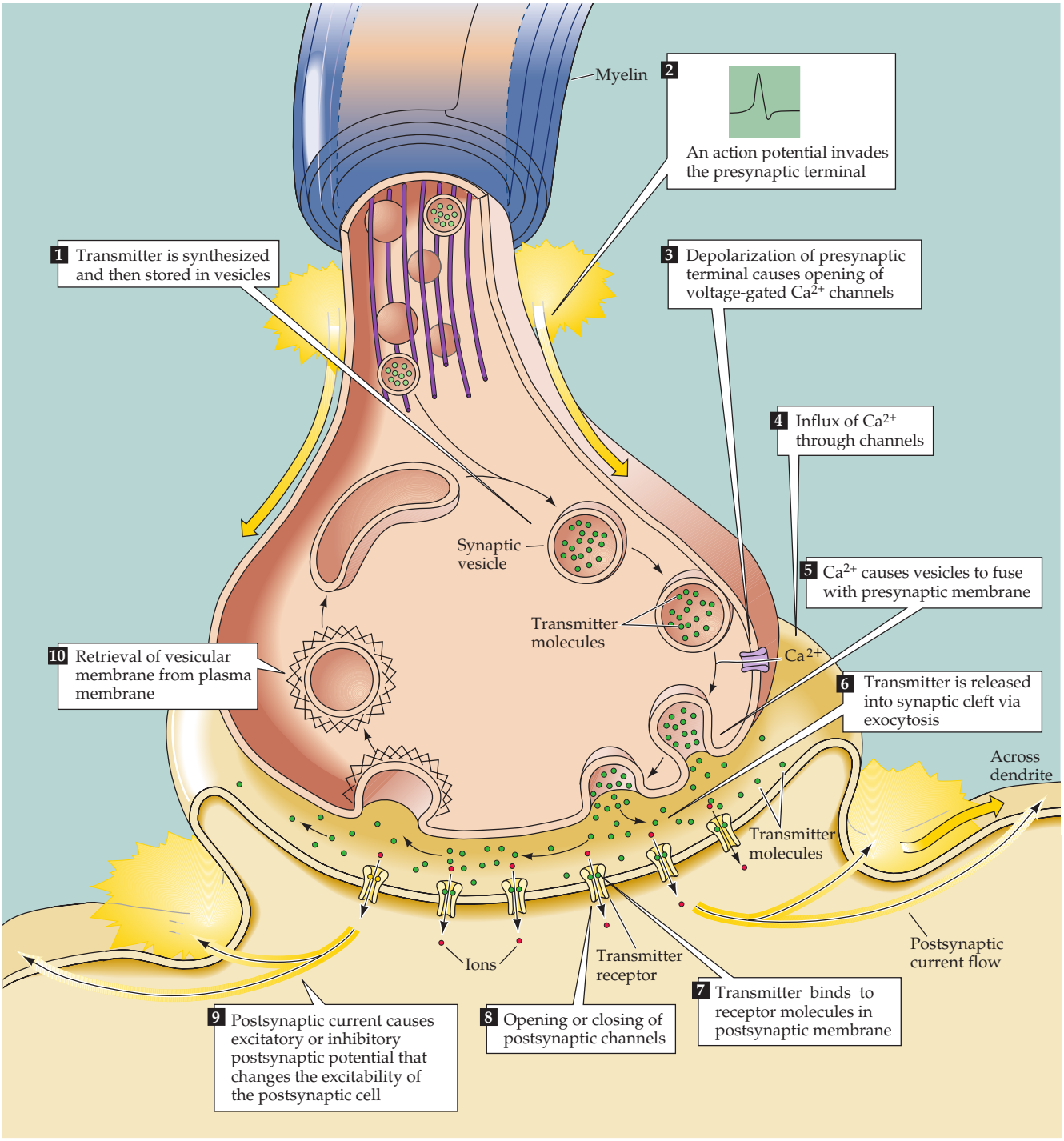


Figure 5.3 Sequence of events involved in transmission at a typical chemical synapse.

ety 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 20).

Over the years, a number of formal criteria have emerged that definitively identify a substance as a neurotransmitter (Box A). These have led to the identification of more than 100 different neurotransmitters, which can be

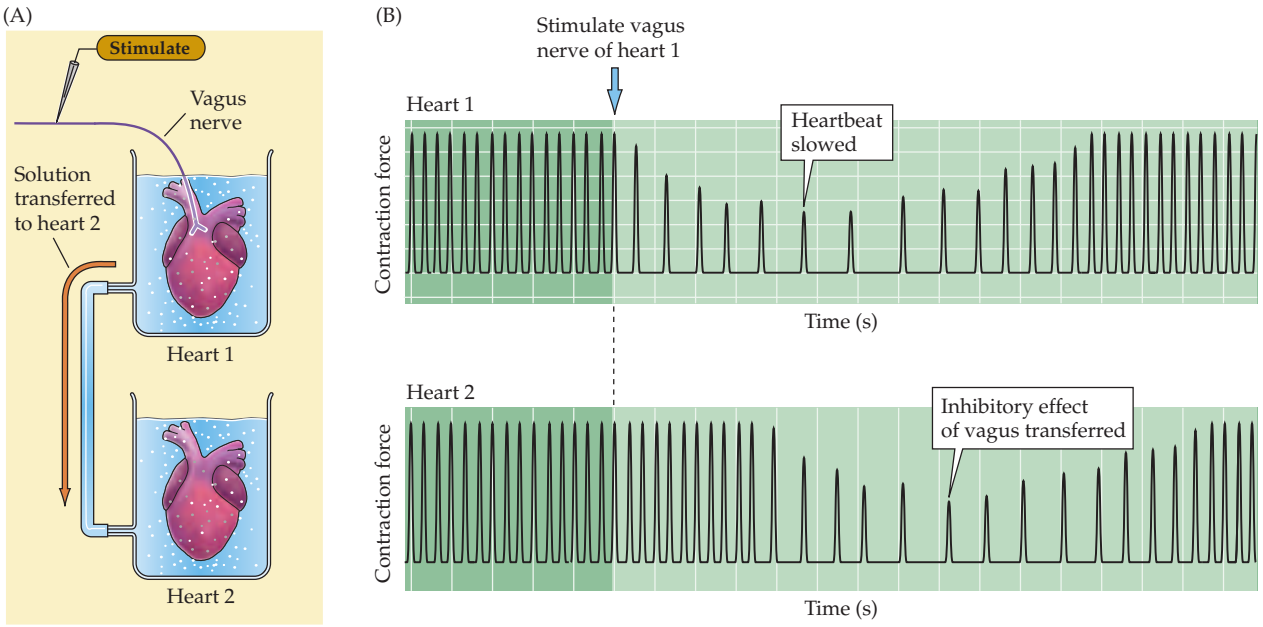


Figure 5.4 Loewi's experiment demonstrating chemical neurotransmission. (A) Diagram of experimental setup. (B) Where the vagus nerve of an isolated frog's heart was stimulated, the heart rate decreased (upper panel). If the perfusion fluid from the stimulated heart was transferred to a second heart, its rate decreased as well (lower panel).

classified into two broad categories: small-molecule neurotransmitters and neuropeptides (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 time scales. In general, small-molecule neurotransmitters mediate rapid synaptic actions, whereas neuropeptides tend to modulate slower, ongoing synaptic functions.

Until relatively recently, it was believed that a given neuron produced only a single type of neurotransmitter. It is now clear, however, that many types of neurons synthesize and release two or more different neurotransmitters. When more than one transmitter is present within a nerve terminal, the molecules are called **co-transmitters**. Because different types of transmitters can be packaged in different populations of synaptic vesicles, co-transmitters need not be released simultaneously. When peptide and small-molecule neurotransmitters act as co-transmitters at the same synapse, they are differentially released according to the pattern of synaptic activity: low-frequency activity often releases only small neurotransmitters, whereas high-frequency activity is required to release neuropeptides from the same presynaptic terminals. As a result, the chemical signaling properties of such synapses change 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

Box A

Criteria That Define a Neurotransmitter

Three primary criteria have been used to confirm that a molecule acts as a neurotransmitter at a given chemical synapse.

1. *The substance must be present within the presynaptic neuron*. Clearly, a chemical cannot be secreted from a presynaptic neuron unless it is present there. Because elaborate biochemical pathways are required to produce neurotransmitters, showing that the enzymes and precursors required to synthesize the substance are present in presynaptic neurons provides additional evidence that the substance is used as a transmitter. Note, however, that since the transmitters glutamate, glycine, and aspartate are also needed for protein synthesis and other metabolic reactions in all neurons, their presence is *not* sufficient evidence to establish them as neurotransmitters.

2. *The substance must be released in response to presynaptic depolarization, and the release must be Ca^{2+}-dependent*. Another essential criterion for identifying a neurotransmitter is to demonstrate that it is released from the presynaptic neuron in response to presynaptic electrical activity, and that this release requires Ca^{2+} influx into the presynaptic terminal. Meeting this criterion is technically challenging, not only because it may be difficult to selectively stimulate the presynaptic neurons, but also because enzymes and transporters efficiently remove the secreted neurotransmitters.

3. *Specific receptors for the substance must be present on the postsynaptic cell*. A neurotransmitter cannot act on its target unless specific receptors for the transmitter are present in the postsynaptic membrane. One way to demonstrate receptors is to show that application of exogenous transmitter mimics the postsynaptic effect of presynaptic stimulation. A more rigorous demonstration is to show that agonists and antagonists that alter the normal postsynaptic response have the same effect when the substance in question is applied exogenously. High-resolution histological methods can also be used to show that specific receptors are present in the postsynaptic membrane (by detection of radioactively labeled receptor antibodies, for example).

Fulfilling these criteria establishes unambiguously that a substance is used as a transmitter at a given synapse. Practical difficulties, however, have prevented these standards from being applied at many types of synapses. It is for this reason that so many substances must be referred to as "putative" neurotransmitters.

Demonstrating the identity of a neurotransmitter at a synapse requires showing (1) its presence, (2) its release, and (3) the postsynaptic presence of specific receptors.

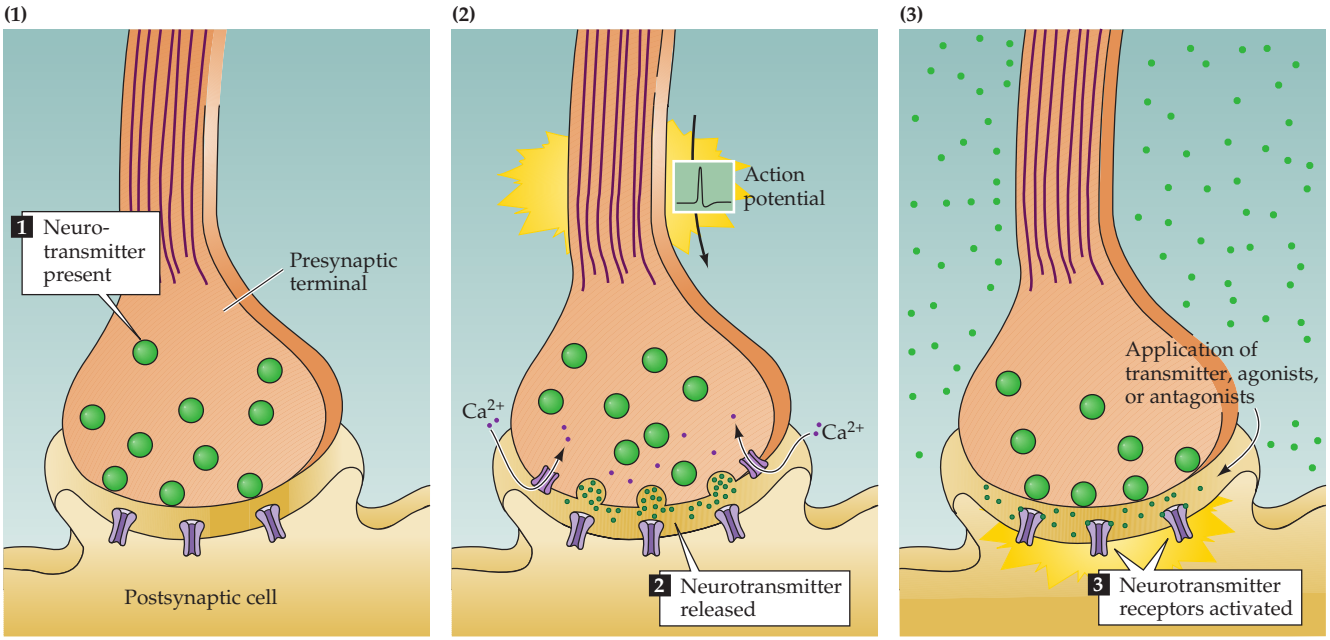
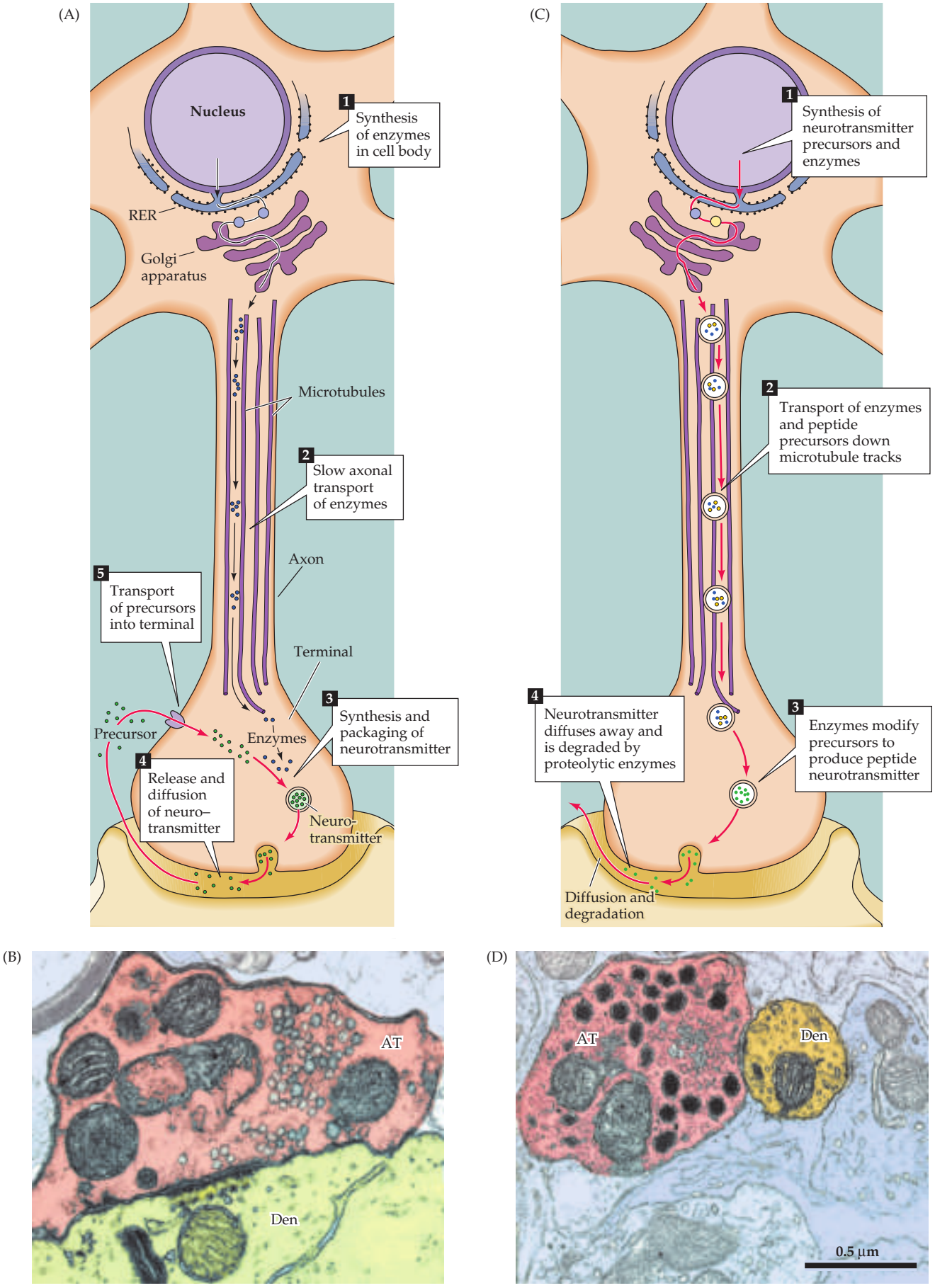


Figure 5.5 Metabolism of small-molecule and peptide transmitters. (A) Small-molecule neurotransmitters are synthesized at nerve terminals. The enzymes necessary for neurotransmitter synthesis are made in the cell body of the presynaptic cell (1) and are transported down the axon by slow axonal transport (2). Precursors are taken up into the terminals by specific transporters, and neurotransmitter synthesis and packaging take place within the nerve endings (3). After vesicle fusion and release (4), the neurotransmitter may be enzymatically degraded. The reuptake of the neurotransmitter (or its metabolites) starts another cycle of synthesis, packaging, release, and removal (5). (B) Small clear-core vesicles at a synapse between an axon terminal (AT) and a dendritic spine (Den) in the central nervous system. Such vesicles typically contain small-molecule neurotransmitters. (C) Peptide neurotransmitters, as well as the enzymes that modify their precursors, are synthesized in the cell body (1). Enzymes and propeptides are packaged into vesicles in the Golgi apparatus. During fast axonal transport of these vesicles to the nerve terminals (2), the enzymes modify the propeptides to produce one or more neurotransmitter peptides (3). After vesicle fusion and exocytosis, the peptides diffuse away and are degraded by proteolytic enzymes (4). (D) Large dense-core vesicles in a central axon terminal (AT) synapsing onto a dendrite (Den). Such vesicles typically contain neuropeptides or, in some cases, biogenic amines. (B and D from Peters, Palay, and Webster, 1991.)

degradation (or removal) of neurotransmitters to achieve the desired levels of transmitter molecules. The synthesis of small-molecule neurotransmitters occurs locally within presynaptic terminals (Figure 5.5A). The enzymes needed to synthesize these transmitters are produced in the neuronal cell body and transported to the nerve terminal cytoplasm at 0.5–5 millimeters a day by a mechanism called **slow axonal transport**. 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. The enzymes synthesize neurotransmitters in the cytoplasm of the presynaptic terminal and the transmitters are then loaded into synaptic vesicles via transporters in the vesicular membrane (see Chapter 4). For some small-molecule neurotransmitters, the final steps of synthesis occur inside the synaptic vesicles. Most small-molecule neurotransmitters are packaged in vesicles 40 to 60 nm in diameter, the centers of which appear clear in electron micrographs; accordingly, these vesicles are referred to as **small clear-core vesicles** (Figure 5.5B). Neuropeptides are synthesized in the cell body of a neuron, meaning that the peptide is produced a long distance away from its site of secretion (Figure 5.5C). To solve this problem, peptide-filled vesicles are transported along an axon and down to the synaptic terminal via **fast axonal transport.** This process carries vesicles at rates up to 400 mm/day along cytoskeletal elements called microtubules (in contrast to the slow axonal transport of the enzymes that synthesize small-molecule transmitters). Microtubules are long, cylindrical filaments, 25 nm in diameter, present throughout neurons and other cells. Peptide-containing vesicles are moved along these microtubule "tracks" by ATP-requiring "motor" proteins such as kinesin. Neuropeptides are packaged into synaptic vesicles that range from 90 to 250 nm in diameter. These vesicles are electron-dense in electron micrographs—hence they are referred to as **large dense-core vesicles** (Figure 5.5D).

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 syn-


(A)
Nucleus
1
Synthesis of enzymes in cell body
RER
Golgi apparatus
Microtubules
2
Slow axonal transport of enzymes
Axon
5
Transport of precursors into terminal
Terminal
3
Synthesis and packaging of neurotransmitter
Precursor
Enzymes
4
Release and diffusion of neuro–transmitter
Neuro-transmitter
(C)
1
Synthesis of neurotransmitter precursors and enzymes
2
Transport of enzymes and peptide precursors down microtubule tracks
4
Neurotransmitter diffuses away and is degraded by proteolytic enzymes
3
Enzymes modify precursors to produce peptide neurotransmitter
Diffusion and degradation
(B)
AT
Den
(D)
AT
Den
0.5 µm

aptic 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.

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 saucer-like appearance of the site on the muscle fiber where the presynaptic axon elaborates its terminals (Figure 5.6A). Most of the pioneering work on neuromuscular transmission was performed by Bernard Katz and his collaborators at University College London during the 1950s and 1960s, and Katz has been widely recognized for his remarkable contributions to understanding synaptic transmission. Though he worked primarily on the frog neuromuscular junction, numerous subsequent experiments have confirmed the applicability of his observations to transmission at chemical synapses throughout the nervous system.

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 for producing a postsynaptic action potential (Figure 5.6B). The postsynaptic action potential triggered by the EPP causes the muscle fiber to contract. Unlike the case for 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. Such a 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 5.6C). These changes have the same shape as EPPs but are much

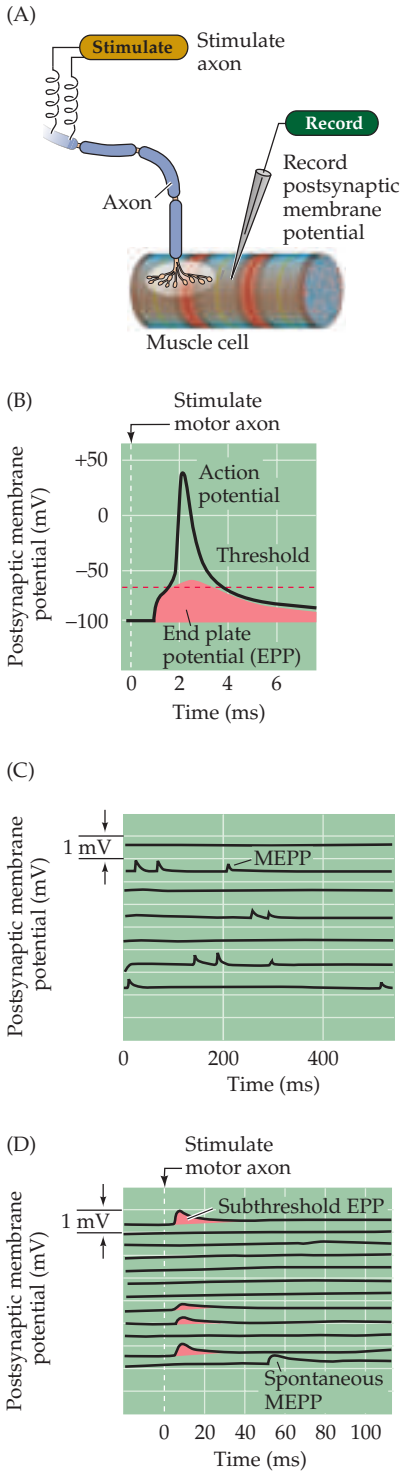


Figure 5.6 Synaptic transmission at the neuromuscular junction. (A) Experimental arrangement, typically using the muscle of a frog or rat. The axon of the motor neuron innervating the muscle fiber is stimulated with an extracellular electrode, while an intracellular microelectrode is inserted into the postsynaptic muscle cell to record its electrical responses. (B) End plate potentials (EPPs) 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.)

smaller (typically less than 1 mV in amplitude, compared to an EPP of perhaps 40 or 50 mV). Both EPPs and these small, spontaneous events are sensitive to pharmacological agents that block postsynaptic acetylcholine receptors, such as curare (see Box B in Chapter 6). 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 5.3, 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 5.6D). 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 response is strikingly similar to the size of single MEPPs (compare Figure 5.6C and D). Further supporting this similarity, increments in the EPP response (Figure 5.7A) occur in units about the size of single MEPPs (Figure 5.7B). These "quantal" fluctuations in the amplitude of EPPs indicated to Katz and colleagues 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 like MEPPs (see Figure 5.7B). 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 5.7A). Such analyses confirmed the idea that release of 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 vesi-

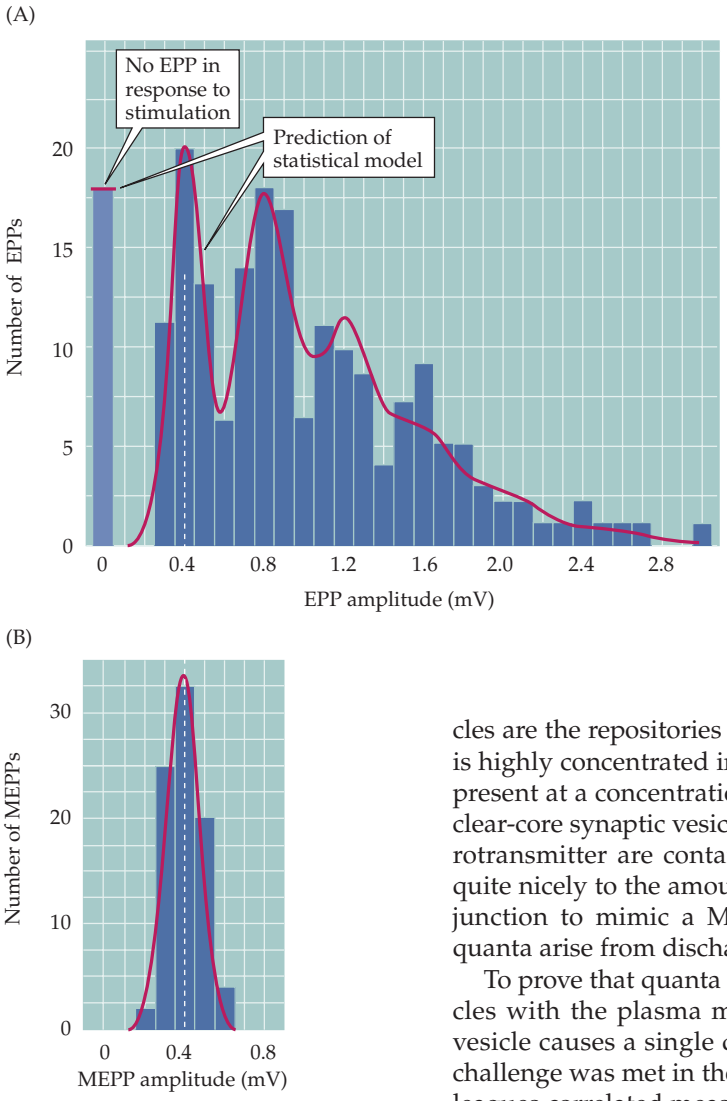
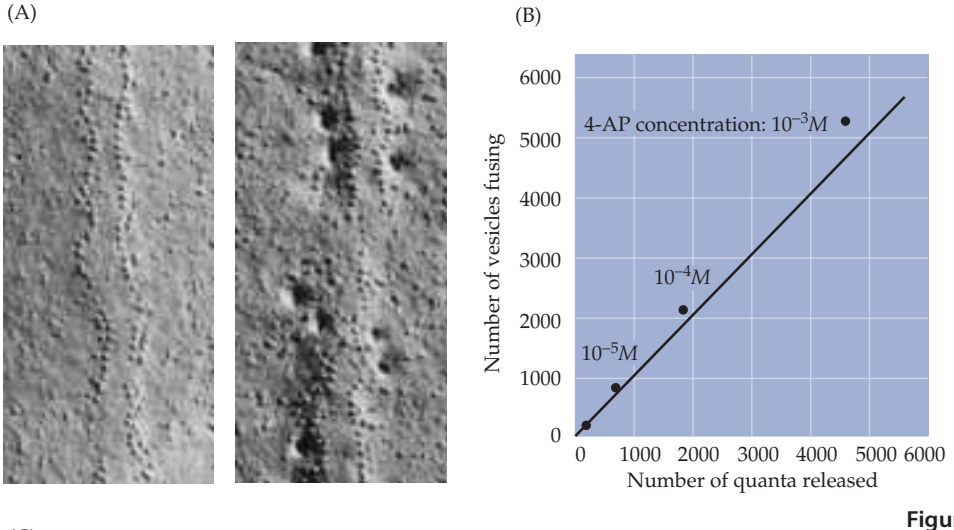


Figure 5.7 Quantized distribution of EPP amplitudes evoked in a low Ca^{2+} solution. Peaks of EPP amplitudes (A) tend to occur in integer multiples of the mean amplitude of MEPPs, whose amplitude distribution is shown in (B). The leftmost bar in the EPP amplitude distribution shows trials in which presynaptic 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.)

cles 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 causes a single quantal event to be recorded postsynaptically. 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. In their experiments, the number of vesicles that fused with the presynaptic plasma membrane was measured by electron microscopy in terminals that had been treated with a drug (4-aminopyridine, or 4-AP) that enhances the number of vesicle fusion events produced by single action potentials (Figure 5.8A). Parallel electrical measurements were made of the quantal content of the EPPs elicited in this way. A comparison of the number of synaptic vesicle fusions observed with the electron microscope and the number of quanta released at the synapse showed a good correlation between these two measures (Figure 5.8B). These results remain one of the strongest lines of support for the idea that a quantum of transmitter release is due to a synaptic vesicle fusing with the presynaptic membrane. Subsequent evidence, based on other means of measuring vesicle fusion, has left no doubt about the validity of this general interpretation of chemical synaptic transmission. Very recent work has identified structures within the presynaptic terminal that connect vesicles to the plasma membrane and may be involved in membrane fusion (Figure 5.8C).



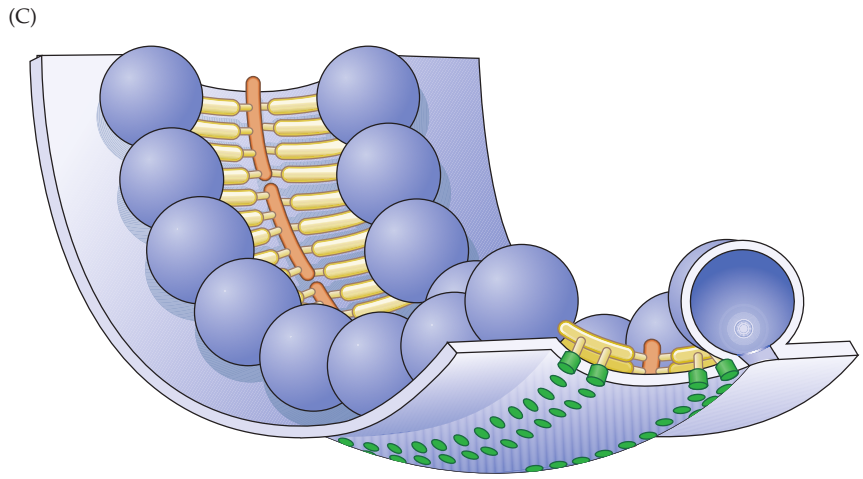


Figure 5.8 Relationship of synaptic vesicle exocytosis and quantal transmitter release. (A) A special electron microscopical technique called freeze-fracture microscopy was used to visualize the fusion of synaptic vesicles in presynaptic terminals of frog motor neurons. *Left:* Image of the plasma membrane of an unstimulated presynaptic terminal. *Right:* Image of the plasma membrane of a terminal stimulated by an action potential. Stimulation causes the appearance of dimple-like structures that represent the fusion of synaptic vesicles with the presynaptic membrane. The view is as if looking down on the release sites from outside the presynaptic terminal. (B) Comparison of the number of observed vesicle fusions to the number of quanta released by a presynaptic action potential. Transmitter release was varied by using 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) Fine structure of vesicle fusion sites of frog presynaptic terminals. Synaptic vesicles are arranged in rows and are connected to each other and to the plasma membrane by a variety of proteinaceous structures (yellow). Green structures in the presynaptic membrane, corresponding to the rows of particles seen in (A), are thought to be Ca^{2+} channels. (A and B from Heuser et al., 1979; C after Harlow et al., 2001)

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 can be made to produce 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 5.9). 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 follow-

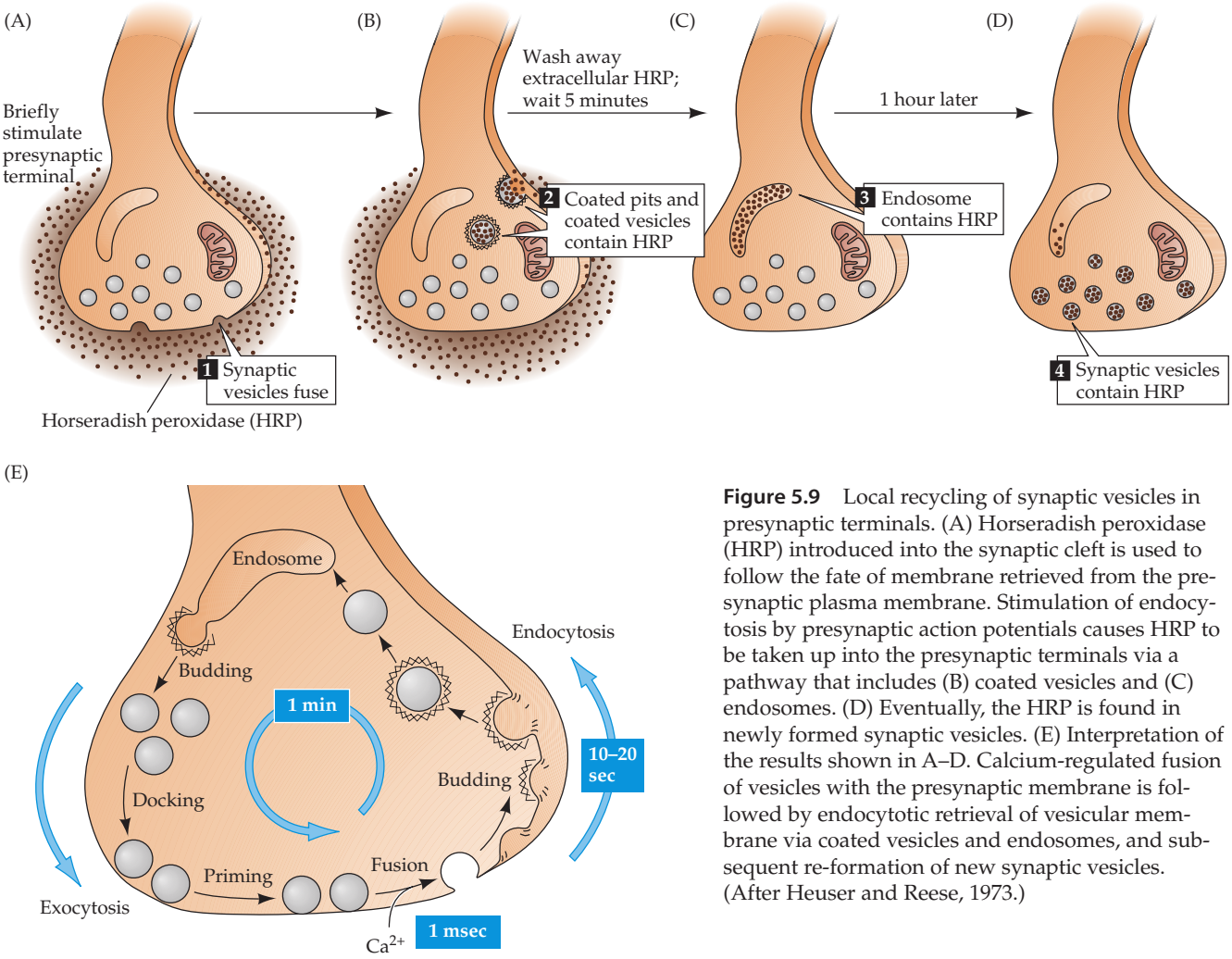


Figure 5.9 Local recycling of synaptic vesicles in presynaptic terminals. (A) Horseradish peroxidase (HRP) introduced into the synaptic cleft is used to follow the fate of membrane retrieved from the presynaptic plasma membrane. Stimulation of endocytosis by presynaptic action potentials causes HRP to be taken up into the presynaptic terminals via a pathway that includes (B) coated 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.)

ing stimulation, the HRP was found within special endocytotic organelles called coated vesicles (Figure 5.9A,B). A few minutes later, however, the coated vesicles had disappeared and the HRP was found in a different organelle, the endosome (Figure 5.9C). Finally, within an hour after stimulating the terminal, the HRP reaction product appeared inside synaptic vesicles (Figure 5.9D).

These observations indicate that synaptic vesicle membrane is recycled within the presynaptic terminal via the sequence summarized in Figure 5.9E. In this process, called the **synaptic vesicle cycle**, the retrieved vesicular membrane passes through a number of 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 10–20 sec-

onds of this time. As can be seen from the 1-millisecond delay in transmission following excitation of the presynaptic terminal (see Figure 5.6B), membrane fusion during exocytosis is much more rapid than budding during endocytosis. Thus, all of the recycling steps interspersed between membrane budding and subsequent refusion of a 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. As might be expected, defects in synaptic vesicle recycling can cause severe neurological disorders, some of which are described in Box B.

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 5.6B 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-sensitive 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 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 5.10A), confirmed

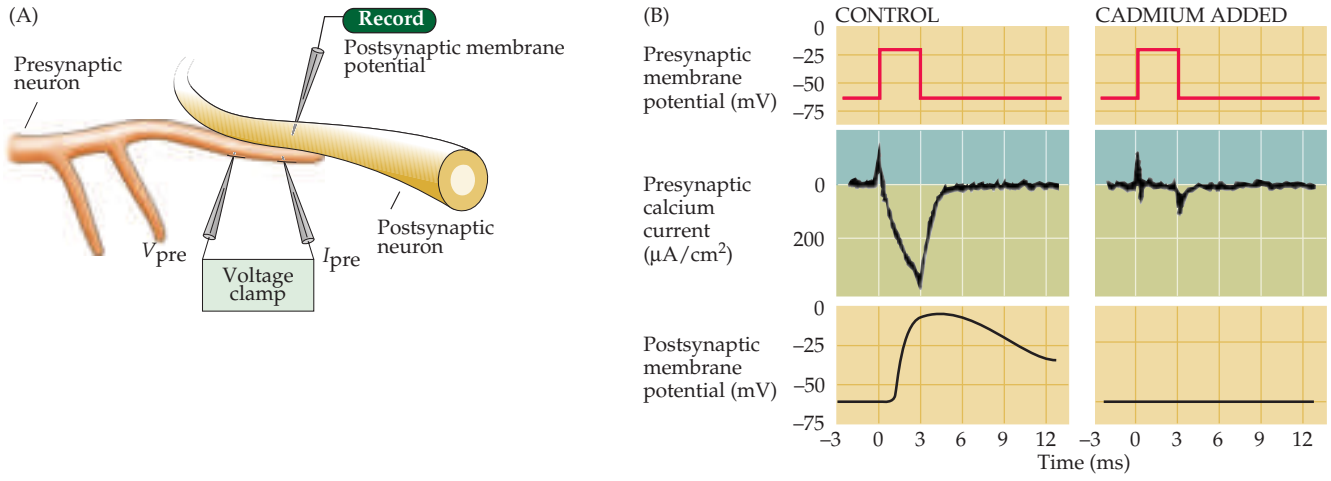


Figure 5.10 The entry of Ca^{2+} through the specific voltage-dependent calcium channels in the presynaptic terminals causes transmitter release. (A) Experimental setup using an extraordinarily large synapse in the squid. The voltage clamp method detects currents flowing across the presynaptic membrane 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.)

Box B

Diseases That Affect the Presynaptic Terminal

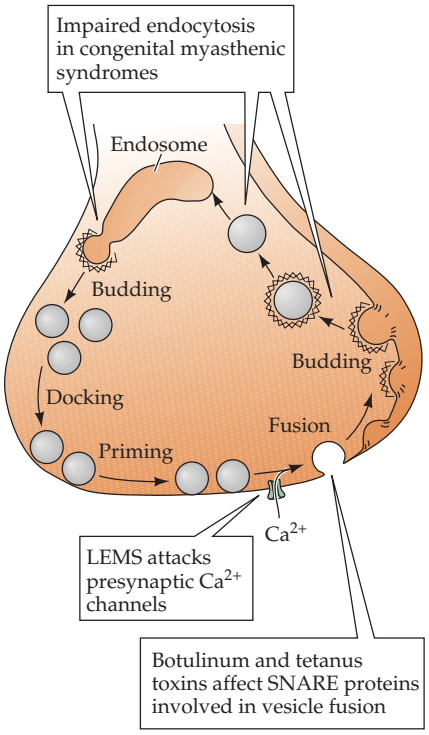
Various steps in the exocytosis and endocytosis of synaptic vesicles are targets of a number of rare but debilitating neurological diseases. Many of these are myasthenic syndromes, in which abnormal transmission at neuromuscular synapses leads to weakness and fatigability of skeletal muscles (see Box B in Chapter 7). One of the best-understood examples of such disorders is the Lambert-Eaton myasthenic syndrome (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 shown in Figure 5.6. Such recordings have shown that when a motor neuron is stimulated, the number of quanta contained in individual EPPs is greatly reduced, although the amplitude of spontaneous MEPPs is normal. Thus, LEMS impairs evoked neurotransmitter release, but does not affect the size of individual quanta.

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). Thus, the defect in neuromuscular transmission can be overcome by increasing the extracellular concentration of Ca^{2+}, and anatomical studies indicate a lower density of Ca^{2+} channel proteins in the presynaptic plasma membrane. The loss of presynaptic Ca^{2+} channels in LEMS apparently arises from a defect in the immune system. The blood of LEMS patients 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 causes a reduction in Ca^{2+} channel currents. 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 also cause muscle weakness by affecting neuromuscular transmission. Some of these syndromes affect the acetylcholinesterase that degrades acetylcholine in the synaptic cleft, whereas others arise from autoimmune attack of acetylcholine receptors (see Box C in Chapter 6). However, a number of congenital myasthenic syndromes arise from defects in acetylcholine release due to altered synaptic vesicle traffic within 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) or from a reduced supply of vesicles 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 normal in number, but smaller in diameter. This finding suggests a different type of genetic lesion that somehow alters formation of new synaptic vesicles following endocytosis, thereby leading to less acetylcholine in each vesicle.

Another disorder of synaptic transmitter release results from poisoning by anaerobic *Clostridium* bacteria. This genus of microorganisms produces some



Presynaptic targets of several neurological disorders.

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 abolition of neurotransmitter release. This interference with neuromuscular transmission causes skeletal muscle weakness, in extreme cases producing respiratory failure due to 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.

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, producing hyperexcitation of skeletal muscle and tetanic contractions in affected muscles (hence the name of the disease).

Although their clinical consequences are dramatically different, clostridial toxins have a common mechanism of action (see figure). Tetanus toxin and botulinum toxins work by cleaving the SNARE proteins involved in fusion of synaptic vesicles with the presynaptic plasma membrane (see Box C). This proteolytic action presumably accounts for the block of transmitter release at the afflicted synapses. The different actions of these toxins on synaptic transmission at excitatory motor versus inhibitory synapses apparently results 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 basis for this differential uptake of toxins is not known, but presumably arises from the presence of different types of toxin receptors on the two types of neurons.

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the presence of voltage-gated Ca^{2+} channels in the presynaptic terminal (Figure 5.10B). Such experiments showed that the amount of neurotransmitter released is very sensitive to the exact amount of Ca^{2+} that enters. Further, blockade of these Ca^{2+} channels with drugs also inhibits transmitter release (Figure 5.10B, right). These observations all confirm that the voltage-gated Ca^{2+} channels are directly involved in neurotransmission. Thus, presynaptic action potentials open voltage-gated Ca^{2+} channels, with a resulting influx of Ca^{2+}.

That Ca^{2+} entry into presynaptic terminals causes a rise in the concentration of Ca^{2+} within the terminal has been documented by microscopic imaging of terminals filled with Ca^{2+}-sensitive fluorescent dyes (Figure 5.11A). The consequences of the rise in presynaptic Ca^{2+} concentration for neurotransmitter release has been directly shown in two ways. First, microinjection of Ca^{2+} into presynaptic terminals triggers transmitter release in the absence of presynaptic action potentials (Figure 5.11B). 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 5.11C). These results prove beyond any doubt that a rise in presynaptic Ca^{2+} concentration is both necessary and sufficient for neurotransmitter release. Thus, as is the case for many other forms of neuronal signaling (see Chapter 7), Ca^{2+} serves as a second messenger during transmitter 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

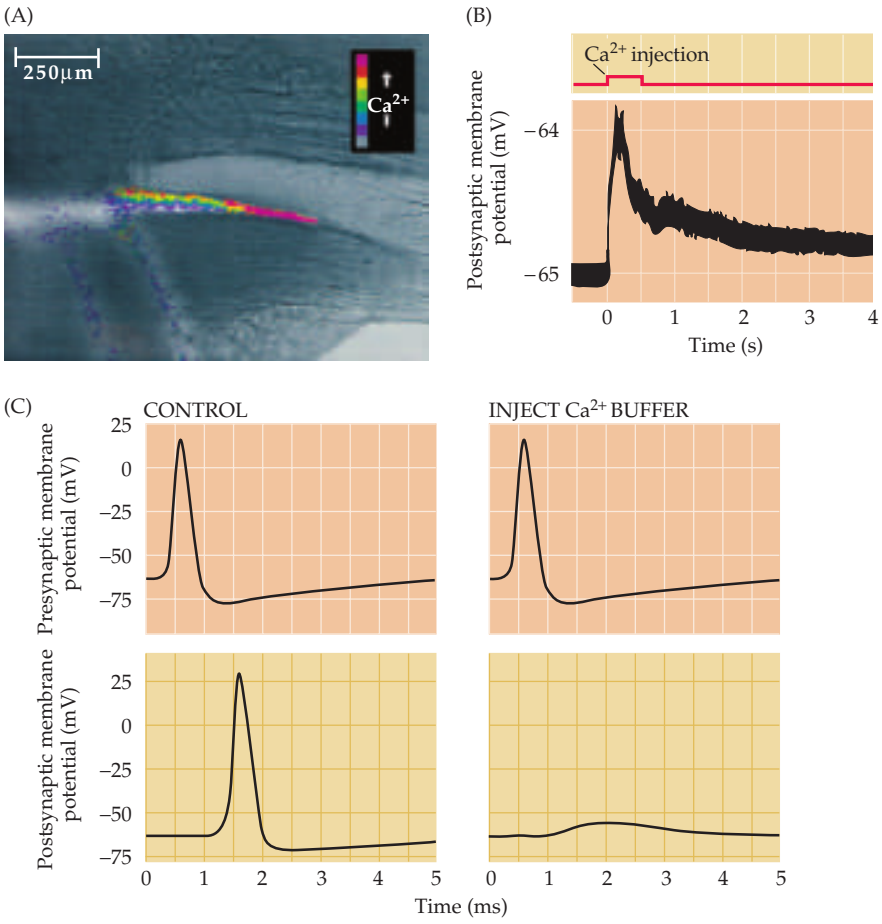


Figure 5.11 Evidence that a rise in presynaptic Ca^{2+} concentration triggers transmitter release from presynaptic terminals. (A) Fluorescence microscopy measurements of presynaptic Ca^{2+} concentration at the squid giant synapse (see Figure 5.8A). A train of presynaptic action potentials causes a rise in Ca^{2+} concentration, as revealed by a dye (called fura-2) that fluoresces more strongly when the Ca^{2+} concentration increases. (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, 1971; C after Adler et al., 1991.)

from motor neurons requires only a fraction of a millisecond (see Figure 5.6), release of neuropeptides require 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. This perhaps is most evident in cases where small molecules and peptides serve as co-transmitters (Figure 5.12). Whereas the small, clear-core vesicles containing small-molecule transmitters are typically docked at the plasma membrane in advance of Ca^{2+} entry, large dense core vesicles containing peptide transmitters are farther away from the plasma membrane (see Figure 5.5D). At low firing frequencies, the concentration of Ca^{2+} may increase only locally at the presynaptic plasma membrane, in the vicinity of open Ca^{2+} channels, limiting release to small-molecule transmitters from the docked small, clear-core vesicles. Prolonged high-frequency stimulation increases the Ca^{2+} concentration throughout the presynaptic terminal, thereby inducing the slower release of neuropeptides.

Molecular Mechanisms of Transmitter Secretion

Precisely how an increase in presynaptic Ca^{2+} concentration goes on to trigger vesicle fusion and neurotransmitter release is not understood. However, many important clues have come from molecular studies that have identified and characterized the proteins found on synaptic vesicles and their binding

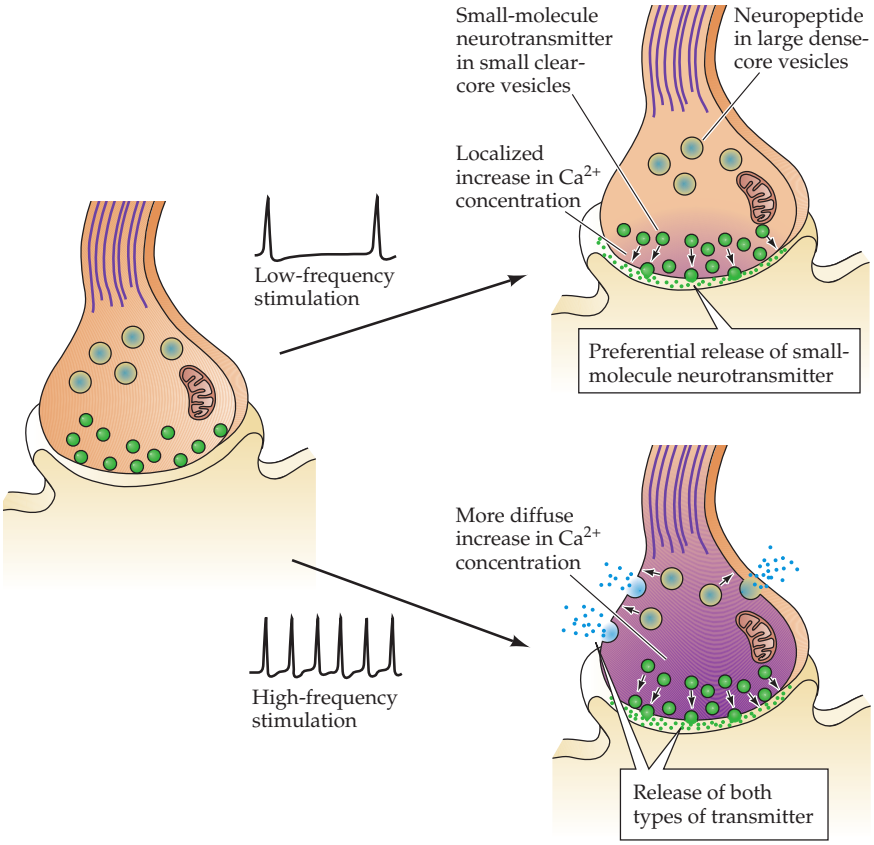


Figure 5.12 Differential release of neuropeptide and small-molecule co-transmitters. Low-frequency stimulation preferentially raises the Ca^{2+} concentration close to the membrane, favoring the release of transmitter from small clear-core vesicles docked at presynaptic specializations. High-frequency stimulation leads to a more general increase in Ca^{2+}, causing the release of peptide neurotransmitters from large dense-core vesicles, as well as small-molecule neurotransmitters from small clear-core vesicles.

partners on the presynaptic plasma membrane and cytoplasm (Figure 5.13). Most, if not all, of these proteins act at one or more steps in the synaptic vesicle cycle. Although a complete molecular picture of neurotransmitter release is still lacking, the roles of several proteins involved in vesicle fusion have been deduced.

Several of the proteins important for neurotransmitter release are also involved in other types of membrane fusion events common to all cells. 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 *p*rotein) 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). One of these 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 membrane. These SNARE proteins can form a macromolecular complex that spans the two membranes, thus bringing them into close apposition (Figure 5.14A). 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 (Box C). In addition, putting SNARE proteins into artificial lipid membranes and allowing these proteins to form complexes with each other causes the membranes to fuse. Many other proteins, such as

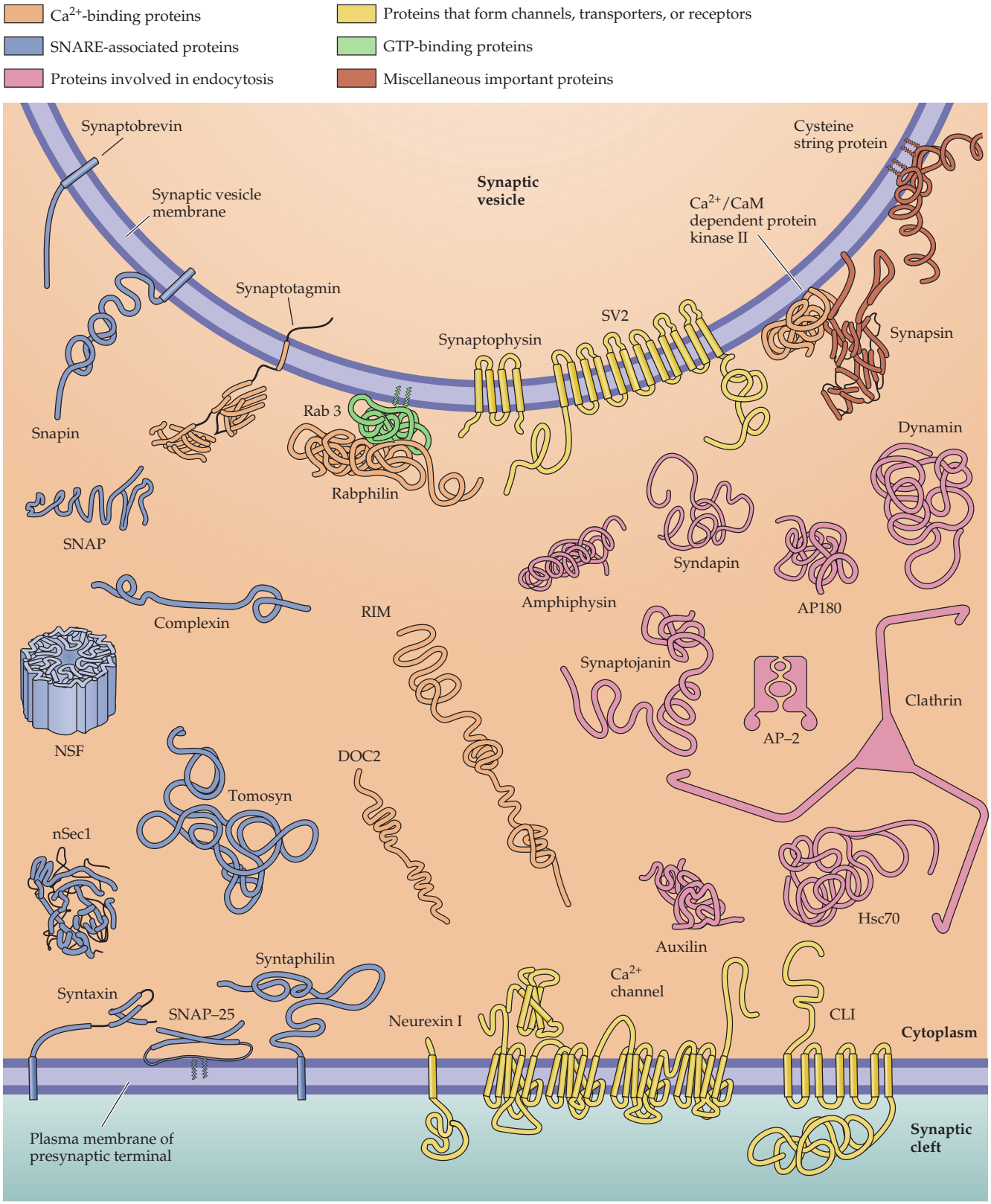


Figure 5.13 Presynaptic proteins implicated in neurotransmitter release. Structures adapted from Brunger (2001) and Brodsky et al. (2001).

complexin, nSec-1, snapin, syntaphilin, and tomosyn, bind to the SNAREs and presumably regulate the formation or disassembly of this complex.

Because the SNARE proteins do not bind Ca^{2+}, still other molecules must be responsible for Ca^{2+} regulation of neurotransmitter release. Several presynaptic proteins, including calmodulin, CAPS, and munc-13, are capable of binding Ca^{2+}. However, the leading candidate for Ca^{2+} regulation of neurotransmitter release is **synaptotagmin**, a protein found in the membrane of synaptic vesicles. Synaptotagmin binds Ca^{2+} at concentrations similar to those required to trigger vesicle fusion within the presynaptic terminal. It may act as a Ca^{2+} sensor, signaling the elevation of Ca^{2+} within the terminal and thus triggering vesicle fusion. In support of this idea, alterations of the properties of synaptotagmin in the presynaptic terminals of mice, fruit flies, squid, and other experimental animals impair Ca^{2+}-dependent neurotransmitter release. In fact, deletion of only one of the 19 synaptotagmin genes of mice is a lethal mutation, causing the mice to die soon after birth. How Ca^{2+} binding to synaptotagmin could lead to exocytosis is not yet clear. It is known that Ca^{2+} changes the chemical properties of synaptotagmin, allowing it to insert into membranes and to bind to other proteins, including the SNAREs. A plausible model is that the SNARE proteins bring the two membranes close together, and that Ca^{2+}-induced changes in synaptotagmin then produce the final fusion of these membranes (Figure 5.14B).

Still other proteins appear to be involved at subsequent steps of the synaptic vesicle cycle (Figure 5.14C). For example, the protein **clathrin** is involved in endocytotic budding of vesicles from the plasma membrane. Clathrin forms structures that resemble geodesic domes (Figure 5.14D); these structures form coated pits that initiate membrane budding. Assembly of individual clathrin triskelia (so named because of their 3-legged appearance) into coats is aided by several other accessory proteins, such as AP2, AP180 and amphiphysin. The coats increase the curvature of the budding membrane until it forms a coated vesicle-like structure. Another protein, called **dynamin**, is at least partly responsible for the final pinching-off of membrane to convert the coated pits into coated vesicles. The coats are then removed by an ATPase, **Hsc70**, with another protein called **auxilin** serving as a co-factor. Other proteins, such as **synaptojanin**, are also important for vesicle uncoating. Several lines of evidence indicate that the protein **synapsin**, which reversibly binds to synaptic vesicles, may cross-link newly formed vesicles to the cytoskeleton to keep the vesicles tethered within the reserve pool. Mobilization of these reserve pool vesicles is caused by phosphorylation of synapsin by proteins kinases (Chapter 7), which allows synapsin to dissociate from the vesicles, thus freeing the vesicles to make their way to the plasma membrane.

In summary, a complex cascade of proteins, acting in a defined temporal and spatial order, allows neurons to secrete transmitters. Although the detailed mechanisms responsible for transmitter secretion are not completely clear, rapid progress is being made toward this goal.

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. Much subsequent work has shown that receptor molecules do indeed account for the ability of neurotransmitters, hormones, and drugs to alter the

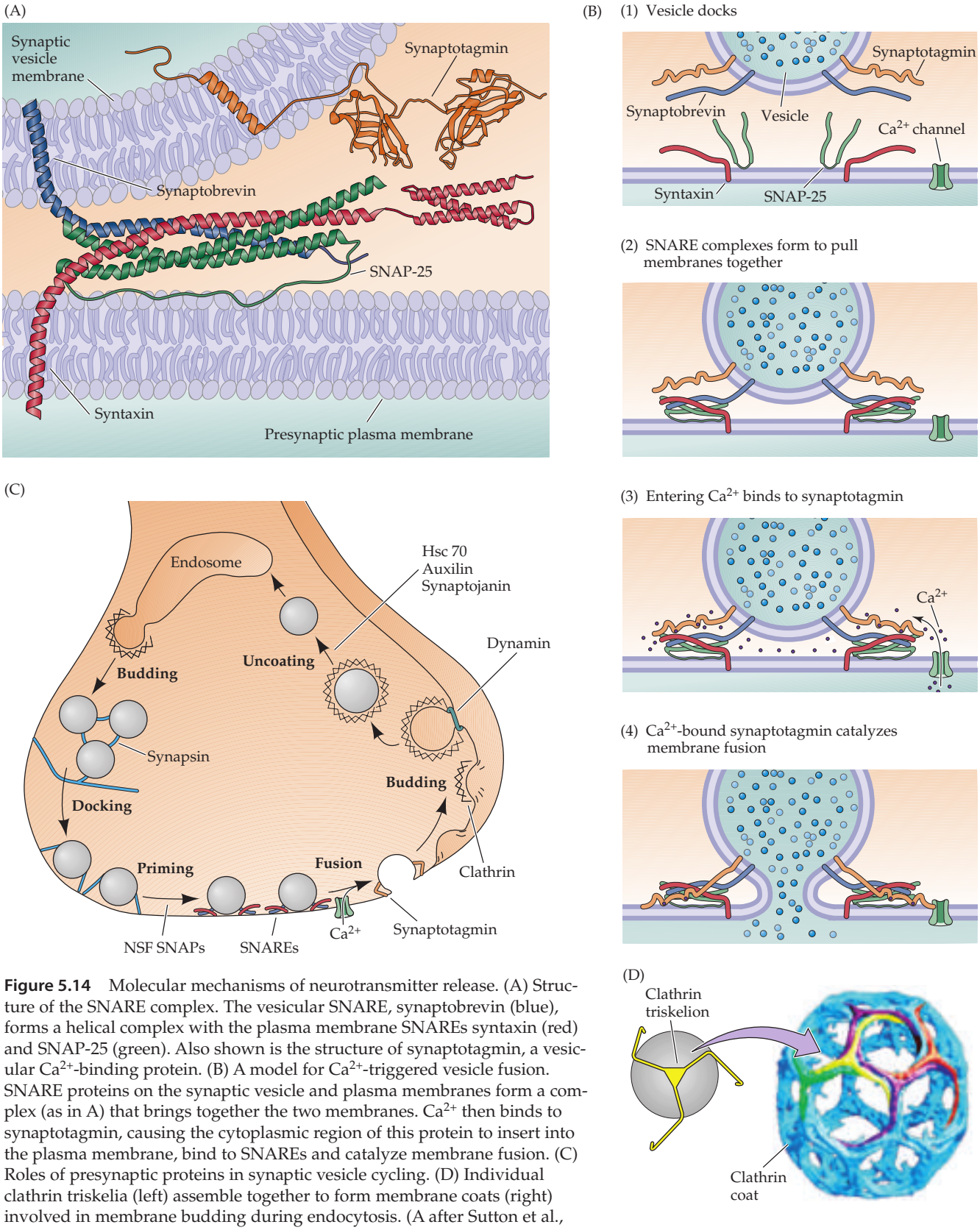


Figure 5.14 Molecular mechanisms of neurotransmitter release. (A) Structure of the SNARE complex. The vesicular SNARE, synaptobrevin (blue), forms a helical complex with the plasma membrane SNAREs syntaxin (red) and SNAP-25 (green). Also shown is the structure of synaptotagmin, a vesicular Ca^{2+}-binding protein. (B) A model for Ca^{2+}-triggered vesicle fusion. SNARE proteins on the synaptic vesicle and plasma membranes form a complex (as in A) that brings together the two membranes. Ca^{2+} then binds to synaptotagmin, causing the cytoplasmic region of this protein to insert into the plasma membrane, bind to SNAREs and catalyze membrane fusion. (C) Roles of presynaptic proteins in synaptic vesicle cycling. (D) Individual clathrin triskelia (left) assemble together to form membrane coats (right) involved in membrane budding during endocytosis. (A after Sutton et al., 1998; C after Sudhof, 1995; D after Marsh and McMahon, 2001.)

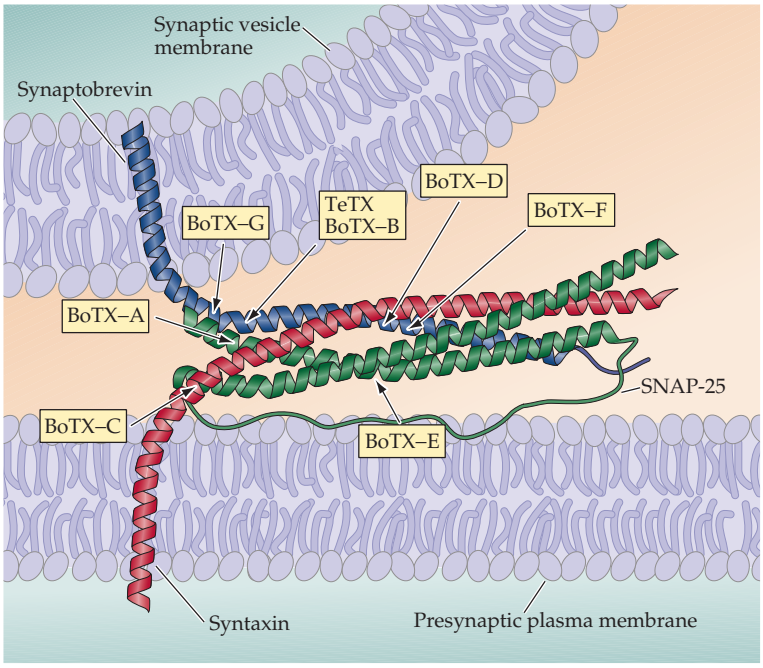
Box C

Toxins That Affect Transmitter Release

Several important insights about the molecular basis of neurotransmitter secretion have come from analyzing the actions of a series of biological toxins produced by a fascinating variety of organisms. One family of such agents is the clostridial toxins responsible for botulism and tetanus (see Box B). Clever and patient biochemical work has shown that these toxins are highly specific proteases that cleave presynaptic SNARE proteins (see figure). Tetanus toxin and botulinum toxin (types B, D, F, and G) specifically cleave the vesicle SNARE protein, synaptobrevin. Other botulinum toxins are proteases that 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 actions of the toxins on neurotransmitter release. The evidence described in the text also implies that these three synaptic SNARE proteins are somehow important in the process of vesicle–plasma membrane fusion.

Another toxin that targets neurotransmitter release is α-latrotoxin, a protein found in the venom of the female black widow spider. Application of this molecule to neuromuscular synapses causes a massive discharge of synaptic vesicles, even when Ca^{2+} is absent from the extracellular medium. While it is not yet clear how this toxin triggers Ca^{2+}-independent exocytosis, 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 (see Figure 5.13). Several lines of evidence implicate binding to neurexins in at least some of the actions of α-latrotoxin. Because the neurexins bind to synaptotagmin, a vesicular Ca^{2+}-binding protein that is known to be important in exocytosis, 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, Ca^{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 Chapter 7). Thus, the binding of α-latrotoxin to CL1 is thought to activate an intracellular signal transduction cascade that may be involved in the Ca^{2+}-independent actions of α-latrotoxin. While more work is needed to establish the roles of neurexins and CL1 in the actions of α-latrotoxin definitively, effects on these two proteins probably account for the potent presynaptic actions of this toxin.

Still other toxins produced by snakes, snails, spiders, and other predatory animals are known to affect transmitter release, but their sites of action have yet to be identified. Based on the precedents described here, it is likely that these biological poisons will continue to provide valuable tools for elucidating the molecular basis of neurotransmitter release, just as they will continue to enable the predators to feast on their prey.



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

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functional properties of neurons. While it has been clear since Langley's day that receptors are important for synaptic transmission, their identity and detailed mechanism of action remained a mystery until quite recently. It is now known that neurotransmitter receptors are proteins embedded in the plasma membrane of postsynaptic cells. Domains of receptor molecules that extend into the synaptic cleft bind neurotransmitters that are released into this space by the presynaptic neuron. The binding of neurotransmitters, either directly or indirectly, causes ion channels in the postsynaptic membrane to open or close. Typically, the resulting ion fluxes change the membrane potential of the postsynaptic cell, thus mediating the transfer of information across the 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 A in Chapter 4) 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, much in the way that changes in membrane potential open voltage-gated ion channels (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 only open briefly, (Figure 5.15B1), the opening of a large number of channels is synchronized by the brief duration during which 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 current flowing during the EPC is normally inward, it causes the postsynaptic membrane potential to depolarize. This depolarizing change in potential is the EPP (Figure 5.15C), which typically triggers a postsynaptic action potential by opening voltage-gated Na^+ and K^+ channels (see Figure 5.6B).

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 (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 poten-

tials, 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 3), the magnitude of the EPC at any membrane potential is given by the product of the ionic 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. Further, 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.

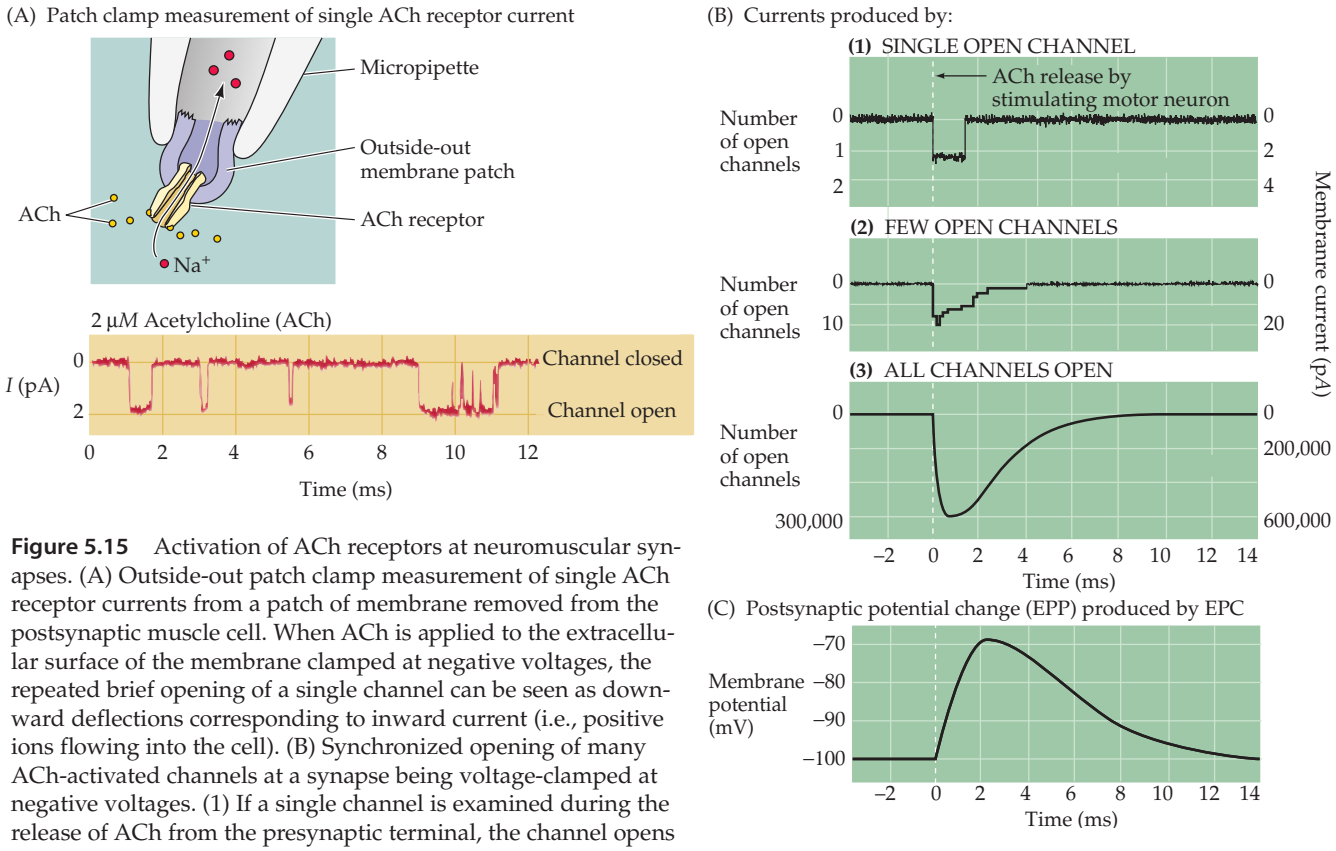


Figure 5.15 Activation of ACh receptors at neuromuscular synapses. (A) Outside-out patch clamp measurement of single ACh receptor currents from a patch of membrane removed from the postsynaptic muscle cell. When ACh is applied to the extracellular surface of the membrane clamped at negative voltages, the repeated brief opening of a single channel can be seen 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 at negative voltages. (1) If a single channel is examined during the release of ACh from the presynaptic terminal, the channel opens transiently. (2) If a number of 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).

Thus, the magnitude and polarity of the postsynaptic membrane potential determines 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. As a result, the identity of the ions that flow during the EPC can be deduced by observing how the reversal potential of the EPC compares to the equilibrium potential for various ion species (Figure 5.17). For example, if ACh were to open an ion channel permeable only to K^+, then the reversal

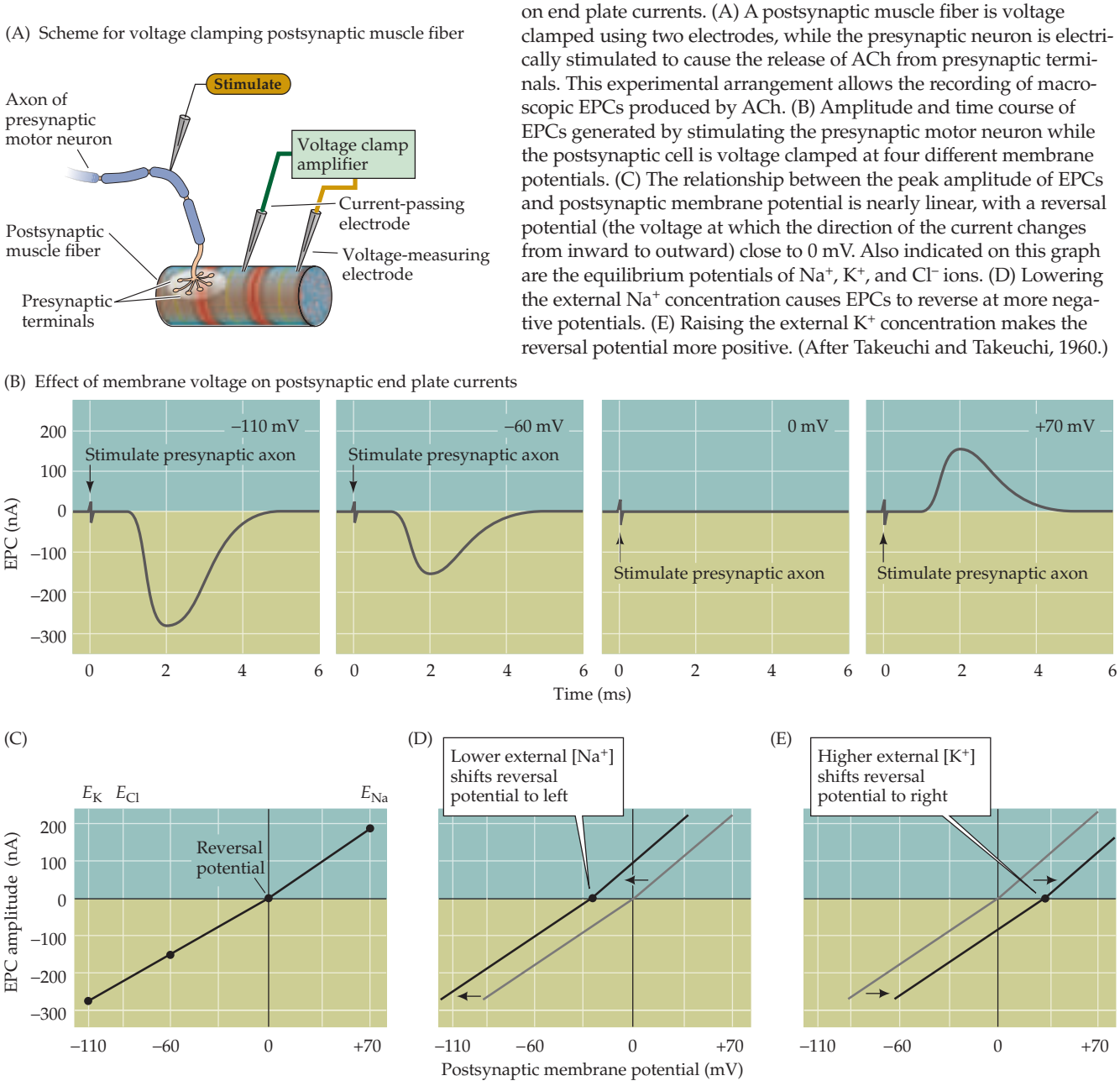


Figure 5.16 The influence of the postsynaptic membrane potential on end plate currents. (A) A postsynaptic muscle fiber is voltage clamped using two electrodes, while the presynaptic neuron is electrically stimulated to cause the release of ACh from presynaptic terminals. This experimental arrangement allows the recording of macroscopic 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) Lowering the external Na^+ concentration causes EPCs to reverse at more negative potentials. (E) Raising the external K^+ concentration makes the reversal potential more positive. (After Takeuchi and Takeuchi, 1960.)

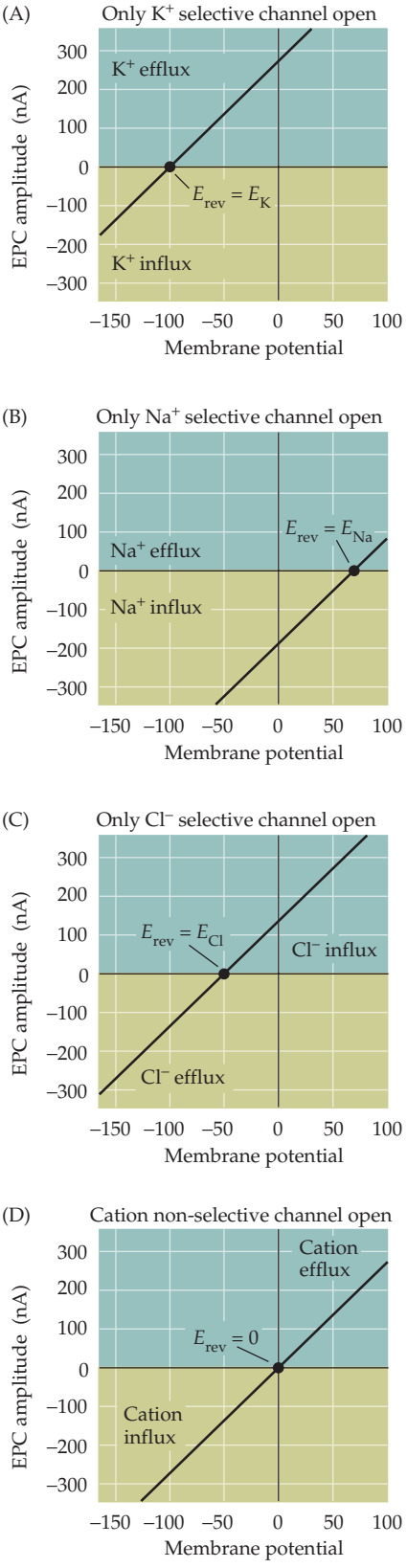


Figure 5.17 The effect of ion channel selectivity on the reversal potential. Voltage clamping a postsynaptic cell while activating presynaptic neurotransmitter release reveals the identity of the ions permeating the postsynaptic receptors being activated. (A) The activation of postsynaptic channels permeable only to K^+ results in currents reversing at E_K, near –100 mV. (B) The activation of postsynaptic Na^+ channels results in currents reversing at E_{Na}, near +70 mV. (C) Cl^--selective currents reverse at E_{Cl}, near –50 mV. (D) Ligand-gated channels that are about equally permeable to both K^+ and Na^+ show a reversal potential near 0 mV.

potential of the EPC would be at the equilibrium potential for K^+, which for a muscle cell is close to –100 mV (Figure 5.17A). 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 (Figure 5.17B); if these channels were permeable only to Cl^-, then the reversal potential would be approximately –50 mV (Figure 5.17C). 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 (Figure 5.17D).

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 was tested in 1960, by the 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 was 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 5.16D), whereas elevating external K^+ concentration, which makes E_K more positive, causes E_{rev} to shift to a more positive potential (Figure 5.16E). Such experiments confirm that the ACh-activated ion channels are in fact permeable to both Na^+ and K^+.

Even though the channels opened by the binding of ACh to its receptors are permeable to both Na^+ and K^+, at the resting membrane potential the EPC is generated primarily by Na^+ influx (Figure 5.18). If the membrane potential is kept at E_K, the EPC arises entirely from an influx of Na^+ because at this potential there is no driving force on K^+ (Figure 5.18A). At the usual muscle fiber resting membrane potential of –90 mV, there is a small driving force on K^+, but a much greater one on Na^+. Thus, during the EPC, much more Na^+ flows into the muscle cell than K^+ flows out (Figure 5.18B); it is the net influx of positively charged Na^+ that constitutes the inward current measured as the EPC. At the reversal potential of about 0 mV, Na^+ influx and K^+ efflux are exactly balanced, so no current flows during the opening of channels by ACh binding (Figure 5.18C). At potentials more positive than E_{rev} the balance reverses; for example, at E_{Na} there is no influx of Na^+ and a large efflux of K^+ because of the large driving force on Na^+ (Figure 5.18D). Even more positive potentials cause efflux of both Na^+ and K^+ and produce an even larger outward EPC.

Were it possible to measure the EPP at the same time as the EPC (of course, the voltage clamp technique prevents this by keeping membrane potential constant), the EPP would be seen to vary in parallel with the amplitude and polarity of the EPC (Figures 5.18E,F). At the usual postsynaptic resting membrane potential of –90 mV, the large inward EPC causes the postsynaptic membrane potential to become more depolarized (see Figure

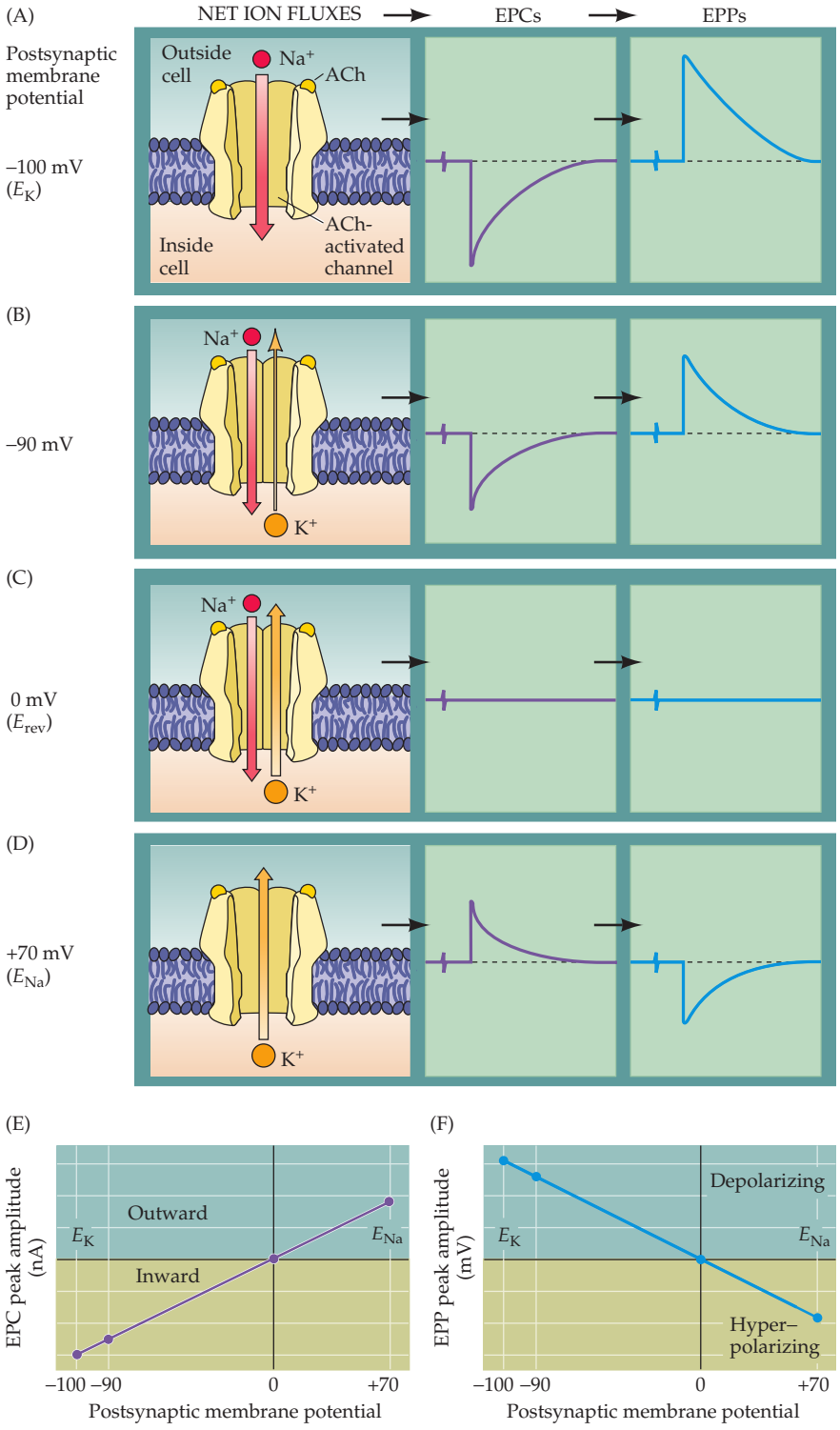


Figure 5.18 Na^+ and K^+ movements during EPCs and EPPs. (A–D) Each of the postsynaptic potentials (V_{post}) indicated at the left results in different relative fluxes of net Na^+ and K^+ (ion fluxes). These ion fluxes determine the amplitude and polarity of the EPCs, which in turn determine the EPPs. 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 currents at potentials more negative than E_{rev} and outward currents 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.

5.18F). However, at 0 mV, the EPP reverses its polarity, and at more positive potentials, the EPP is hyperpolarizing. Thus, the polarity and magnitude of the EPC depend on the electrochemical driving force, which in turn determines the polarity and magnitude of the EPP. 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. The general principle is that 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 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 postsynaptic 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 ionic current flow that changes the membrane potential (see Figure 5.18).

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 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 rest-

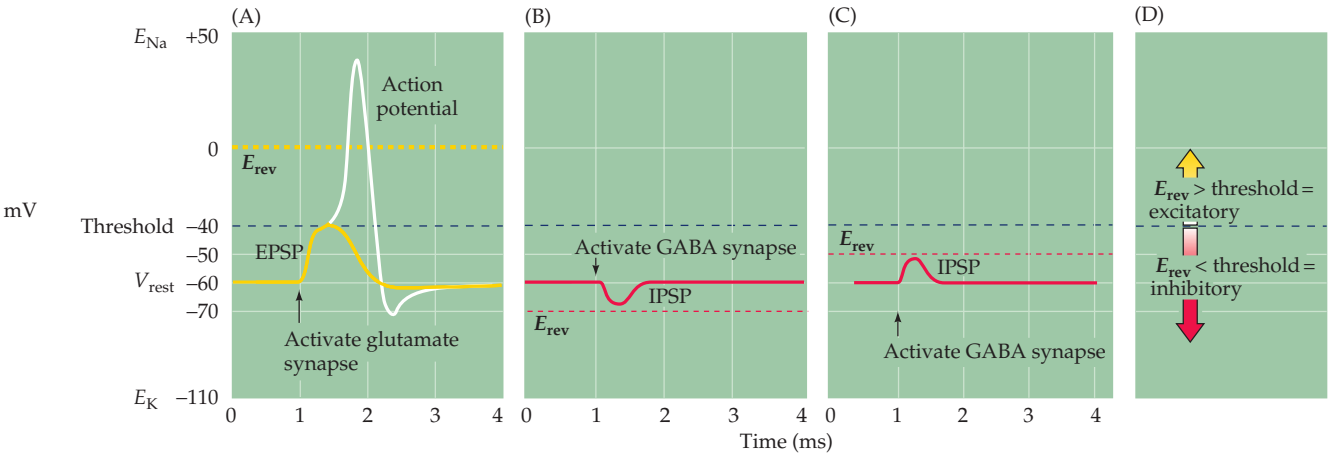


Figure 5.19 Reversal potentials and threshold potentials determine postsynaptic excitation and inhibition. (A) If the reversal potential for a PSP (0 mV) is more positive than the action potential threshold (–40 mV), the effect of a transmitter is excitatory, and it generates EPSPs. (B) If the reversal potential for a PSP is more negative than the action potential threshold, the transmitter is inhibitory and generate 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.

ing 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 typical for many 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 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 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 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 sub-

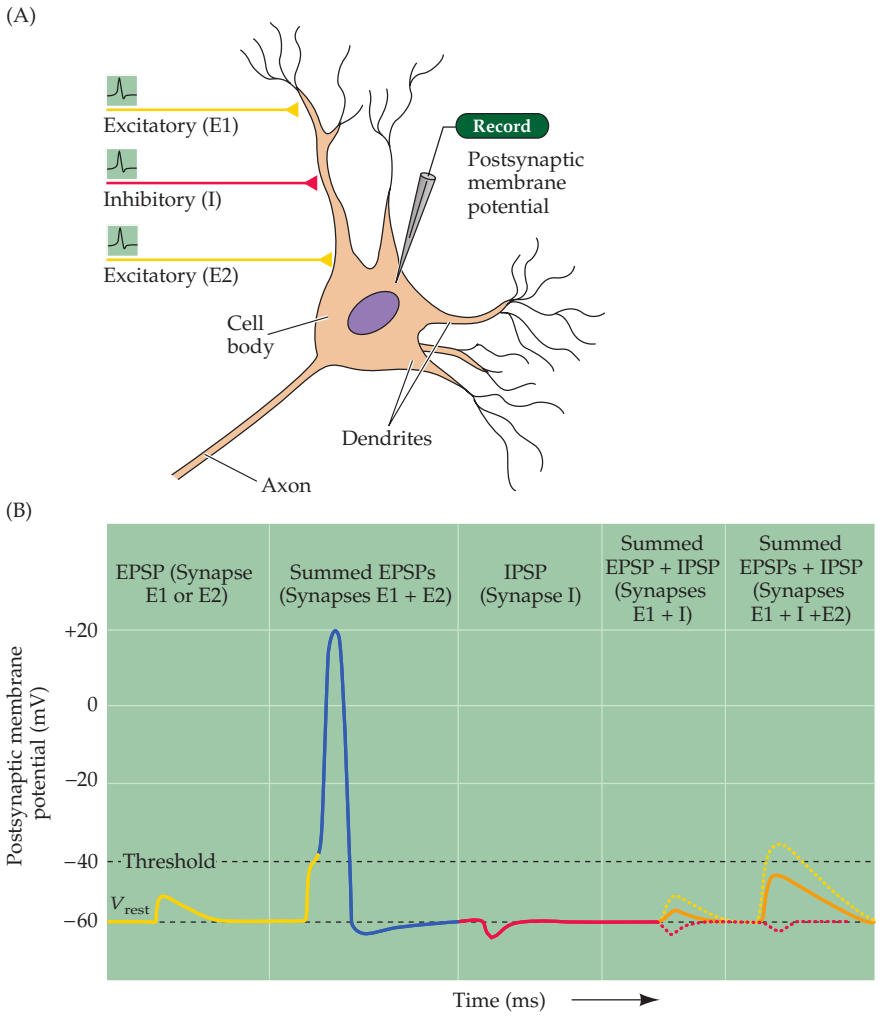


Figure 5.20 Summation of postsynaptic potentials. (A) A microelectrode records the postsynaptic potentials produced by the activity of two excitatory synapses (E1 and E2) and an inhibitory synapse (I). (B) Electrical responses to synaptic activation. Stimulating either excitatory synapse (E1 or E2) produces 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 (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.

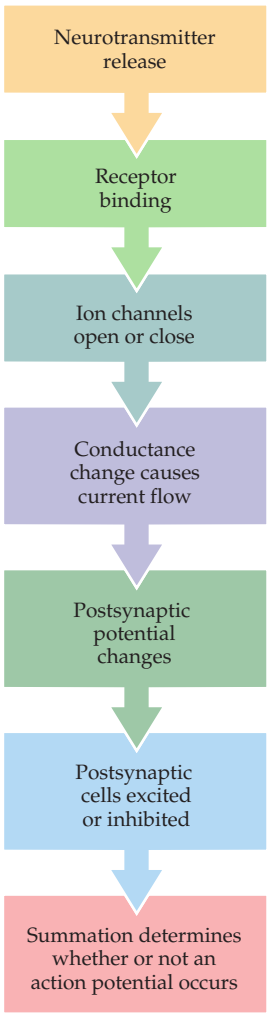


Figure 5.21 Events from neurotransmitter release to postsynaptic excitation or inhibition. Neurotransmitter release at all presynaptic terminals on a cell results in receptor binding, which causes the opening or closing of specific ion channels. The resulting conductance change causes current to flow, 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.

threshold 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 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. Normally, 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 neurotransmitter-induced 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 (Figure 5.21).

Two Families of Postsynaptic Receptors

The opening or closing of postsynaptic ion channels is accomplished in different ways by two broad families of receptor proteins. The receptors in one family—called **ionotropic receptors**—are linked directly to ion channels (the Greek *tropos* means to move in response to a stimulus). These receptors contain two functional domains: an extracellular site that binds neurotransmitters, and a membrane-spanning domain that forms an ion channel (Figure 5.22A). Thus ionotropic receptors combine transmitter-binding and channel functions into a single molecular entity (they are also called **ligand-gated ion channels** to reflect this concatenation). Such receptors are multimers made up of at least four or five individual protein subunits, each of which contributes to the pore of the ion channel.

The second family of neurotransmitter receptors are the **metabotropic receptors,** so called because the eventual movement of ions through a channel depends on one or more metabolic steps. These receptors do not have ion channels as part of their structure; instead, they affect channels by the activation of intermediate molecules called **G-proteins** (Figure 5.22B). For this reason, metabotropic receptors are also called **G-protein-coupled receptors**. Metabotropic receptors are monomeric proteins with an extracellular domain that contains a neurotransmitter binding site and an intracellular domain that binds to G-proteins. Neurotransmitter binding to metabotropic 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 the regulation of postsynaptic ion channels. The postsynaptic signaling events initiated by metabotropic receptors are taken up in detail in Chapter 7.

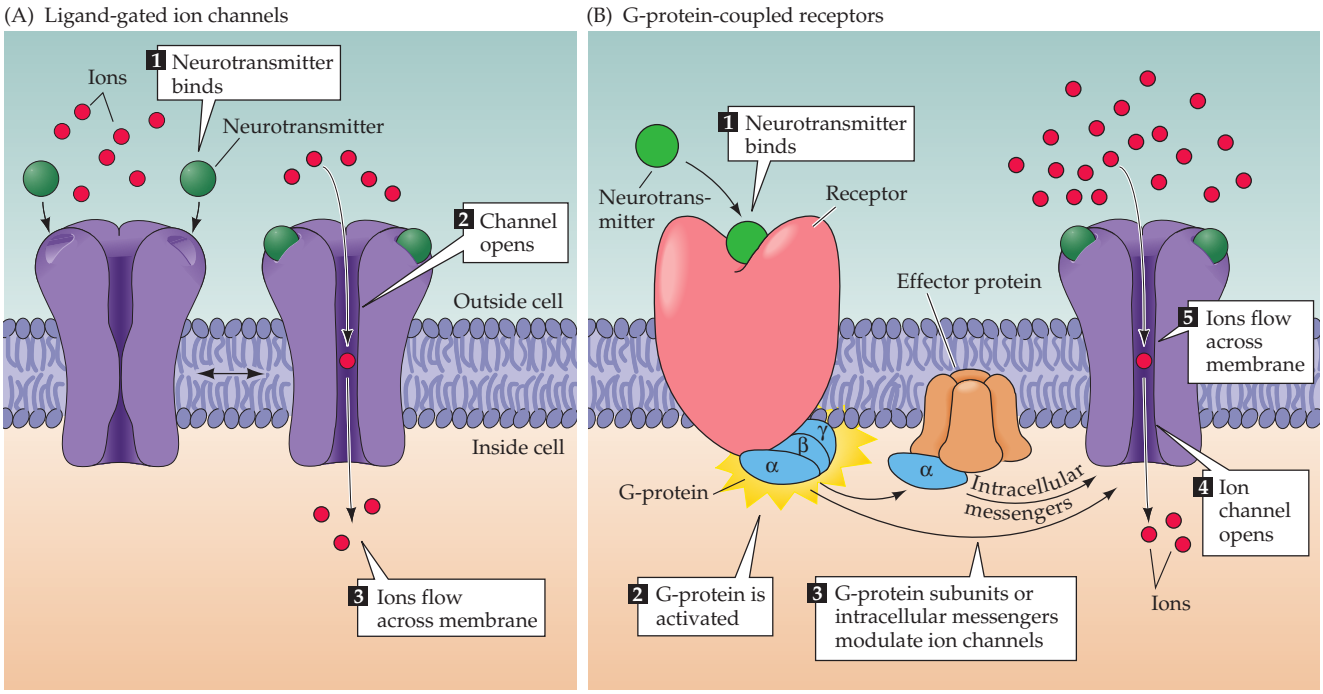


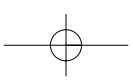
Figure 5.22 A neurotransmitter can affect the activity of a postsynaptic cell via two different types of receptor proteins: ionotropic or ligand-gated ion channels, and metabotropic or G-protein-coupled receptors. (A) Ligand-gated ion channels combine receptor and channel functions in a single protein complex. (B) Metabotropic receptors usually activate G-proteins, which modulate ion channels directly or indirectly through intracellular effector enzymes and second messengers.

These two families of postsynaptic receptors give rise to PSPs with very different time courses, producing 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 5.15), EPSPs produced at certain glutamatergic synapses (Figure 5.19A), and IPSPs produced at certain GABAergic synapses (Figure 5.19B). In all three cases, the PSPs 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, a given transmitter may activate both ionotropic and metabotropic receptors to produce both fast and slow PSPs at the same synapse.

Perhaps the most important principle to keep in mind is that the response elicited at a given synapse depends upon the neurotransmitter released and the postsynaptic complement of receptors and associated channels. The molecular mechanisms that allow neurotransmitters and their receptors to generate synaptic responses are considered in the next chapter.

Summary

Synapses communicate the information carried by action potentials from one neuron to the next in neural circuits. The cellular mechanisms that underlie synaptic transmission are closely related to the mechanisms that generate other types of neuronal electrical signals, namely ion flow through membrane channels. In the case of electrical synapses, these channels are gap junctions; direct but passive flow of current through the gap junctions 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 after release 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 vesicle, and released into the synaptic cleft in a Ca^{2+}-dependent manner. Many synapses release more than one type of neurotransmitter, and multiple transmitters can even be packaged within the same synaptic vesicle. 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. How calcium triggers neurotransmitter release is not yet established, but synaptotagmin, SNAREs, and a number of other proteins found within the presynaptic terminal are clearly involved. Postsynaptic receptors are a diverse group of proteins that transduce binding of neurotransmitters into electrical signals by opening or closing postsynaptic ion channels. The postsynaptic currents produced by the synchronous opening or closing of ion channels changes 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. Two broadly different families of neurotransmitter receptors have evolved to carry out the postsynaptic signaling actions of neurotransmitters. 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.



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