PRINCIPLES OF NEUROBIOLOGY

LIQUN LUO
About the Author

Liqun Luo earned his bachelor degree from the University of Science & Technology of China and PhD from Brandeis University. Since 1997, Dr. Luo has taught neurobiology to undergraduate and graduate students at Stanford University, where he also directs a lab studying the assembly and function of neural circuits. Dr. Luo is a member of the National Academy of Sciences and the American Academy of Arts and Sciences, and an Investigator of the Howard Hughes Medical Institute.

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ISBNs: 978-0-8153-4492-6 (hardcover) 978-0-8153-4494-0 (softcover)
In this chapter, we continue the theme of neuronal communication begun in Chapter 2. We discuss first how the arrival of an action potential at the presynaptic terminal triggers neurotransmitter release from synaptic vesicles, and then how neurotransmitters affect the properties of postsynaptic cells. Collectively, these processes are referred to as synaptic transmission, through which information is transmitted from the presynaptic cell to the postsynaptic cell across the chemical synapse. In the context of studying postsynaptic reception, we also introduce the fundamentals of signal transduction and describe how synaptic inputs are integrated in postsynaptic neurons. Finally we discuss the electrical synapse, an interneuronal communication form in parallel to the chemical synapse.

**HOW IS NEUROTRANSMITTER RELEASE CONTROLLED AT THE PRESYNAPTIC TERMINAL?**

In Chapter 2, we addressed the basic cell biological and electrical properties of neurons that are required to understand how molecules, organelles, and action potentials get to the axon terminals. We will now study the major purpose of these movements: to transmit information across synapses to postsynaptic targets, which can be other neurons or muscle cells.

### 3.1 Action potential arrival at the presynaptic terminal triggers neurotransmitter release

The vertebrate neuromuscular junction, the synapse between the motor neuron axon terminals and the skeletal muscle, has been used as a model synapse to explore many basic properties of synaptic transmission that were later found to be widely applicable to other synapses. Neurotransmitters are molecules released by presynaptic neurons that act across the synaptic cleft on postsynaptic target cells. The neurotransmitter at the vertebrate neuromuscular junction was identified in the 1930s as acetylcholine (ACh) ([Figure 3-1A](#)). An important advantage of studying the neuromuscular synapse is that the postsynaptic muscle cell (also called muscle fiber) is a giant cell that can easily be impaled by a microelectrode for intracellular recording (see Section 13.2.1); this enables synaptic transmission to be assessed in a sensitive and quantitative manner by recording the resulting current or membrane potential changes in the muscle fiber. The neuromuscular junction is also an unusual synapse in that a single motor axon forms many terminal branches, which harbor hundreds of sites releasing neurotransmitter onto its target muscle, making it a strong and reliable synapse for transmitting action potentials in motor neurons to muscle contraction via depolarization-induced action potentials in the muscle fiber (to be discussed in more detail in Chapter 8). Indeed, in experiments described below, researchers typically adjusted the conditions to prevent muscle action potentials and muscle contraction.
In a typical setup for studying synaptic transmission across the neuromuscular junction, an *in vitro* preparation that contains the muscle and its attached motor nerve was bathed in a solution that mimics physiological conditions. The motor nerve was then stimulated with a stimulating electrode to produce action potentials, and the membrane potential of the muscle fiber was recorded with an intracellular electrode (Figure 3-1B). Motor nerve stimulation was found to induce a transient depolarization in the muscle fiber within a few milliseconds (Figure 3-1C, top panel). This transient depolarization is the synaptic potential produced in the postsynaptic muscle cell, and is called an *end-plate potential*, or EPP, as the postsynaptic specialization area of the muscle fiber is also called a motor end plate. We will study the postsynaptic mechanism that produces the EPP in greater detail in the second part of this chapter. For now, we use the EPP as a measure for presynaptic mechanisms that cause neurotransmitter release.

How does motor nerve stimulation produce an EPP? Researchers found that motor nerve stimulation can be mimicked by application of ACh through a micropipette at the contact site between the motor axon terminals and the muscle (Figure 3-1C, bottom panel). (This method is termed *iontophoresis*; here, positively charged ACh is driven out of a micropipette by applying a positive current.) Adding the puffer fish tetrodotoxin (TTX; see Figure 2-29) to the bath, which blocks voltage-gated Na⁺ channels and thus prevents action potential propagation in motor axons, blocked the muscle EPP in response to motor nerve stimulation. However, ACh application could elicit an EPP even when action potentials were blocked by TTX, or when the motor axon was removed altogether. These results indicated that the final effect of action potentials in the motor axon is to trigger ACh release at the axon terminals, and binding of ACh to the muscle membrane triggers depolarization of the muscle fiber in the form of an EPP.

As introduced in Chapter 1, we now know that ACh release is caused by fusion of synaptic vesicles with presynaptic plasma membrane, releasing packets of ACh molecules into the synaptic cleft. The concept that neurotransmitters are released in discrete packets, however, was first deduced prior to the discovery of synaptic vesicles.

### 3.2 Neurotransmitters are released in discrete packets

Bernard Katz and colleagues applied intracellular recording techniques, then newly invented, to the muscle cells to study the mechanisms of neuromuscular synaptic transmission in the early 1950s. While studying the muscle EPPs evoked by nerve stimulation in the frog neuromuscular junction, they observed that
muscle fibers also exhibited small EPPs in the absence of any nerve stimulation; these were termed **miniature end-plate potentials**, or mEPPs. mEPPs had an intriguing property: for a given neuromuscular preparation, they seemed to have either a defined, unitary size or occasionally a multiple of that defined size. The amplitude of mEPPs, hypothesized to be due to spontaneous release of ACh from motor axon terminals, was usually two orders of magnitude lower than EPPs evoked by nerve stimulation. However, when the extracellular solution contained very low concentrations of Ca$^{2+}$ and high concentrations of Mg$^{2+}$ (both of which inhibit neurotransmitter release, as will be discussed in Section 3.4), a condition could be reached in which most nerve stimulations did not evoke any EPPs. When stimuli did trigger EPPs under these conditions, the amplitude of these evoked EPPs were the same size as mEPPs (Figure 3-2A). Further reduction of Ca$^{2+}$ concentrations reduced the frequency of these evoked EPPs, but did not further diminish their amplitude. These observations suggested that mEPPs were the basic unit of synaptic transmission for EPPs induced by nerve stimulation, which under normal conditions were equivalent to the simultaneous occurrence of hundreds of mEPPs. Furthermore, whether spontaneous or triggered by nerve stimulation, most ACh release occurred in the same basic unit with a finite quantal (packet) size, and occasionally two or three times the unit size. These observations led to a **quantal hypothesis of neurotransmitter release**, that is, neurotransmitters are released in discrete quanta of relatively uniform size.

If the quantal hypothesis were true, one could use statistical methods (see Box 3-1 for details) to predict the frequencies of releasing no quantum, a single quantum, or multiple quanta in response to nerve stimulation. When the release probability is small, which is the case when the neuromuscular junction is in low-Ca$^{2+}$, high-Mg$^{2+}$ medium, the frequency ($f$) that $k$ quanta are released per nerve stimulation could be calculated following the **Poisson distribution**:

$$ f = \frac{m^k e^{-m}}{k!} $$

where $m$ is the mean number of units (quanta) that respond to an individual stimulus. Since mEPPs from each spontaneous release correspond to one unit, $m$ can be experimentally determined as the mean EPP amplitude divided by the mean mEPP amplitude. Indeed, the frequency distributions of EPPs calculated above and determined experimentally were an excellent fit; the frequency of the cases when nerve stimulation did not cause any EPP (called synaptic failure) matched precisely with the statistical prediction; there was a prominent peak at around the size of the unitary mEPP, and a small peak at twice the mEPP amplitude (Figure 3-2B). Thus, this statistical analysis provided strong support that neurotransmitters are released in discrete packets.
Box 3-1: Binomial distribution, Poisson distribution, and calculating neurotransmitter release probability

The Poisson distribution and the related **binomial distribution** are both probability distributions that describe the frequency of discrete events that occur independently. Let’s start our discussion with the binomial distribution. **Suppose** the probability that an individual event occurs, such as the head faces up after you toss a coin, is \( p \). The binomial distribution describes the frequency (\( f \)) in which \( k \) events occur (that is, \( k \) times heads facing up after coin toss) after \( n \) trials:

\[
f(k; n, p) = \frac{n!}{k!(n-k)!} p^k (1-p)^{n-k}
\]

where \( k = 0, 1, 2, ... n \), \( ! \) is factorial (for example, \( 4! = 4 \times 3 \times 2 \times 1 = 24 \)), and \( n!/(n-k)! \) is the binomial coefficient. **Suppose** you want to know the likelihood of tossing a coin four times and having only instance of heads. The probability for heads, \( p \), is 0.5 for any given toss of a fair coin. According to the formula above, the binomial coefficient for \( k = 0, 1, 2, 3, 4 \) is respectively 1, 4, 6, 4, 1 (note that \( 0! = 1 \)), and the frequency of occurrence (\( f \)) for the five \( k \) values can be calculated as 0.0625, 0.25, 0.375, 0.25, 0.0625, respectively. In other words, from the four coin tosses, the probability that the head faces up only once (or three times) is 25%; the probability that the head faces up twice is 37.5%, and the probability that heads faces up four times (or zero time) is 6.25%

If neurotransmitter release occurs in discrete packets, and if the release of each packet occurs at a probability of \( p \), we can calculate the frequency that \( k \) packets out of the total \( n \) packets are released using the binomial formula above just as in the example of coin toss. However, researchers did not know the actual values for \( n \) (how many quanta are available to be released) or \( p \) (how likely is an individual quantum to be released), so it was not possible to apply binomial distribution. Fortunately, according to probability theory, when \( n \) is large (>20) and \( p \) is small (<0.05), the binomial distribution can be approximated by the Poisson distribution, in which the frequency (\( f \)) that \( k \) events occur can be determined by a single parameter \( \lambda \) (which equals the product of \( n \) and \( p \) in the binomial distribution) according to the following formula:

\[
f(k; \lambda) = \frac{\lambda^k}{k!} e^{-\lambda}
\]

One can experimentally estimate \( \lambda \) (same as \( n \) in Section 3.2) because as the product of \( n \) and \( p \), it equals the mean number of packets that are released in response to a stimulus, and thus is equivalent to the ratio of evoked EPP and mEPP (assumed to be the quantal unit). Thus, researchers can calculate the probability of release in response to nerve stimulation—estimating the likelihood that no release occurs (\( k = 0 \)), that a single packet is released (\( k = 1 \)), that two packets are released (\( k = 2 \)), and so on—and can then compare these calculations with the actual experimental data, as shown in Figure 3-2B.

Note that in order to apply the Poisson distribution, the release probability (\( p \)) must be small and the number of available packets (\( n \)) must be large so that \( p \) does not change during the measurement of \( \lambda \). While researchers cannot control \( n \) as this is determined by nature (as it turns out, \( n \) is very large in the vertebrate neuromuscular junction because there are typically hundreds of neurotransmitter release sites between a motor axon and its muscle target), they can experimentally reduce \( p \) by studying neurotransmitter release in low-Ca\(^{2+}\) and high-Mg\(^{2+}\) media. Synaptic transmission at the neuromuscular junction also follows closely other assumptions required for the Poisson distribution: independent release of each quantum (because of the large number of release sites), the uniformity of the population (\( p \) is the same for all quanta), and the relative uniformity of their size (each vesicle contains similar amount of neurotransmitter molecules). In many CNS synapses the assumptions either fail (for example, \( n \) is often too small) or cannot be tested adequately. The probability of neurotransmitter release may not follow the Poisson distribution.

3.3 Neurotransmitters are released when synaptic vesicles fuse with the presynaptic plasma membrane

Physiological and anatomical studies often complement each other in driving neuroscience discoveries. The physical basis of the quantal neurotransmitter release became evident when electron microscopy was first applied to the nervous system in the mid 1950s. Thin sections across the nerve terminals revealed that they contain abundant vesicles that are ~40 nm in diameter. At the neuromuscular junction, many such vesicles appear stacked near the presynaptic membrane juxtaposed to the muscle membrane (Figure 3-3A). These synaptic vesicles were immediately hypothesized to be vesicles that are filled with neurotransmitters. The relatively uniform size of synaptic vesicles explained why neurotransmitters are released in packets with a uniform quantal size. (The quantal size at the frog neuromuscular junction has been estimated to be about 7000 ACh molecules.) The unitary release of neurotransmitters occurs when a single synaptic vesicle fuses with the plasma membrane, dumping its neurotransmitter content into the synaptic cleft and producing an mEPP in the muscle cell. Nerve stimulation under normal conditions (not in low Ca\(^{2+}\)) causes hundreds of these vesicle fusion
How is neurotransmitter release controlled at the presynaptic terminal?

How is neurotransmitter release controlled at the presynaptic terminal?

(A) (B) (C)

Figure 3-3 Structures of synapses revealed by electron microscopy. All images share the scale bar. Red asterisks indicate postsynaptic density. Pairs of arrows define the extent of the active zones in the presynaptic terminals. Note the abundance of ~40 nm diameter synaptic vesicles in each presynaptic terminal; some of these vesicles are 'docked' at the active zone ready for release. (A) A frog neuromuscular junction. The synaptic cleft is considerably wider than at the CNS synapses shown in the other two panels. SC indicates a Schwann cell process that wraps around the motor axon terminal. A typical motor axon forms hundreds of such presynaptic terminals onto a muscle fiber. (B) Two synapses formed between a single axon and two Purkinje cell dendritic spines in rat cerebellar cortex. (C) A synapse from human cerebral cortex. AS indicates an astrocyte process that wraps around many CNS synapses. (A, courtesy of Jack McMahan; B & C, courtesy of Josef Spacek and Kristen M. Harris, Synapse Web.)

Even events at a given neuromuscular junction, therefore producing EPPs two orders of magnitude higher than when neurotransmitter is released from a single vesicle. Thus, the neuromuscular junction has a high quantal yield (that is, a large number of synaptic vesicle exocytosis events per action potential) of several hundreds. By contrast, many synapses in the CNS have much lower quantal yield (a few or just one).

The basic structural elements of chemical synapses are highly similar across the entire nervous system and in different animal species (Figure 3-3B, C). In all cases presynaptic terminals have an electron-dense region called the active zone, with clusters of synaptic vesicles 'docked' at the presynaptic membrane ready for release. Across the synaptic cleft from the active zone and underneath the postsynaptic membrane is a structure called postsynaptic density, also concentrated with electron-dense structures. We will study the molecular composition of the active zone and postsynaptic density later in the chapter.

While EM studies found plenty of vesicles in the presynaptic terminals, observing a fusion event was necessary to establish the synaptic vesicle hypothesis. Because fusion of synaptic vesicles with the presynaptic plasma membrane, a necessary intermediate step for neurotransmitter release, occurs very transiently, it is difficult to detect such events in an electron microscopic preparation from a steady-state nervous system. To maximize the chance of visualizing such fusion events, experiments were designed to fix the neuromuscular junction samples immediately after the nerve stimulation. This was achieved by stimulating a neuromuscular preparation while the entire sample was falling toward a block that would freeze the tissue immediately upon contact, so that nerve stimulation could be achieved within a few milliseconds prior to fixation. Fusion between synaptic vesicles and the presynaptic plasma membrane were indeed caught in action (Figure 3-4). Such studies provide definitive evidence that neurotransmitter release is caused by fusion of synaptic vesicles with the presynaptic plasma membrane.

Figure 3-4 Synaptic vesicle fusion caught in action. This electron micrograph was taken from a frog neuromuscular junction preserved 3-5 ms after nerve stimulation, revealing the fusion of two synaptic vesicles (red asterisks) with the presynaptic plasma membrane. (Courtesy of John Heuser. See also Heuser JE & Reese TS [1981] J Cell Biol 88:564-580.)
3.4 Neurotransmitter release is controlled by Ca\textsuperscript{2+} entry into the presynaptic terminal

How does action potential arrival cause synaptic vesicle fusion in the presynaptic terminal? As noted in Section 3.2, external Ca\textsuperscript{2+} is essential for action potential-triggered neurotransmitter release: bathing neuromuscular junction preparations in solutions with progressively lower concentrations of Ca\textsuperscript{2+} rendered the stimulation of motor axons increasingly ineffective in generating EPP in muscles. Supplying Ca\textsuperscript{2+} locally at the neuromuscular synapse through iontophoresis provided a means to test when Ca\textsuperscript{2+} was required during action potential-induced synaptic transmission. It was found that a brief application of extracellular Ca\textsuperscript{2+} enabled neurotransmitter release if it occurred immediately before the depolarization pulse, but exogenous Ca\textsuperscript{2+} became ineffective if applied after the depolarization pulse. Thus, extracellular Ca\textsuperscript{2+} is required during a brief period when depolarization occurs, preceding the transmitter release itself.

How does external Ca\textsuperscript{2+} participate in neurotransmitter release? This question was answered with the help of the squid giant synapse, whose presynaptic as well as postsynaptic terminals are large such that researchers can insert electrodes into both compartments for intracellular recordings. (One of the postsynaptic target cells is the neuron that extends the giant axon featured in Chapter 2.) It was found that action potentials could be replaced simply by depolarization, which opens voltage-gated Ca\textsuperscript{2+} channels (see Box 2-4) in the presynaptic plasma membrane, causing an inward flow of Ca\textsuperscript{2+} that triggers neurotransmitter release.

Let's study in more detail one specific experiment (Figure 3-5), which showcased the Ca\textsuperscript{2+} dependence of neurotransmitter release and provided information about the timing of different steps of neurotransmitter release. In this experiment, the voltage clamp technique was applied to both the presynaptic terminal and postsynaptic target of the squid giant synapse in the presence of the Na\textsuperscript{+} and K\textsuperscript{+} channel blockers, such that the only cation that could cross the presynaptic membrane was Ca\textsuperscript{2+}. From a resting potential at ~70 mV, a depolarizing voltage step to ~25 mV (Figure 3-5A, top) triggered Ca\textsuperscript{2+} influx as measured by presynaptic current (Figure 3-5A, middle), resulting in effective synaptic transmission as measured by an inward postsynaptic current (Figure 3-5A, bottom; we will study the nature of such postsynaptic current in later sections). However, a voltage step to +50 mV did not trigger presynaptic Ca\textsuperscript{2+} influx or postsynaptic current (Figure 3-5B, left portion). This is likely because the +50 mV was close to the equilibrium potential of Ca\textsuperscript{2+} in the presynaptic terminal under the experimental condition, such that even though voltage-gated Ca\textsuperscript{2+} channels were open, there was no driving force for Ca\textsuperscript{2+} influx (see Section 2.5). However, returning the presynaptic membrane potential from +50 mV to ~70 mV produced a presynaptic 'tail current' (Figure 3-5B, middle). This is because the membrane potential change was faster than the closure...
of voltage-gated Ca\(^{2+}\) channels; thus there was a transient period when there was a driving force for Ca\(^{2+}\) influx while Ca\(^{2+}\) channels remained open. The Ca\(^{2+}\) influx due to the presynaptic tail current produced a corresponding postsynaptic current response (Figure 3-5B, bottom). Interestingly, the Ca\(^{2+}\) tail current triggered a postsynaptic response more rapidly than the presynaptic depolarization did (compare the time interval between the two dotted lines in the two panels). This suggested that the normal synaptic delay between presynaptic depolarization and postsynaptic response consists of two components: a delay between depolarization and opening of voltage-gated Ca\(^{2+}\) channels (which was bypassed in the tail current condition as the channels were already open), and a delay between Ca\(^{2+}\) entry and the neurotransmitter-triggered postsynaptic response.

The Ca\(^{2+}\) hypothesis of neurotransmitter release was further validated by other techniques. In one type of experiment, a chemical dye used as an indicator for changes in Ca\(^{2+}\) concentration (see Section 13.22 for more details) was injected into the presynaptic terminal of the squid giant synapse. Nerve stimulation was found to cause a rise of intracellular Ca\(^{2+}\) concentration at the presynaptic terminal (Figure 3-6). The Ca\(^{2+}\) concentration was highest in specific regions of the presynaptic terminal. As will be discussed in Section 3.7, this is because the voltage-gated Ca\(^{2+}\) channels are highly concentrated at the active zone, where synaptic vesicles dock and fuse with presynaptic membrane. In another type of experiment, chemical compounds were synthesized that 'cage' Ca\(^{2+}\) to prevent the ion's effects; such cages can be triggered by light to release Ca\(^{2+}\). When caged Ca\(^{2+}\) was introduced into the presynaptic terminal of the squid giant axon, light could trigger neurotransmitter release in the absence of action potentials or Ca\(^{2+}\) entry from the extracellular media.

Together, these experiments firmly established a sequence of events from action potential to neurotransmitter release:

\[
\text{Action potential from the axon} \rightarrow \text{Depolarization of the presynaptic terminal} \rightarrow \text{Opening of voltage-gated Ca}^{2+} \text{channels} \rightarrow \text{Ca}^{2+} \text{entry into the presynaptic terminal} \rightarrow \text{Fusion of synaptic vesicle with presynaptic plasma membrane} \rightarrow \text{Neurotransmitter release}
\]

This sequence of events, which was originally worked out in the frog neuromuscular junction and the squid giant synapse, applies universally to all chemical synapses across the animal kingdom, regardless of the type of synapse and neurotransmitter used.

The short latency between Ca\(^{2+}\) entry into the presynaptic terminal and postsynaptic events (~2 ms in Figure 3-5B and often shorter) indicates that there must be a pool of synaptic vesicles that are ready to fuse with the presynaptic plasma membrane immediately upon a rise of intracellular Ca\(^{2+}\) concentration. This is consistent with observations in electron microscopy (see Figure 3-3). Furthermore, membrane fusion is energetically unfavorable because breaking two membranes and resealing them necessitates exposing hydrophobic surfaces to water, thus requiring external energy such as ATP hydrolysis. However, the final step of synaptic vesicle fusion is so fast that it is unlikely to involve an ATP hydrolysis-dependent catalytic process. Instead, as we will soon learn, synaptic vesicles are primed for fusion by a specialized protein complex already existing in a high-energy configuration, simply waiting for Ca\(^{2+}\) to trigger the sudden change of configuration that permits fusion.

### 3.5 SNARE and SM proteins mediate synaptic vesicle fusion

We now turn to the molecular mechanisms that mediate the fusion of synaptic vesicles with the plasma membrane (a process also called neurotransmitter **exocytosis**: see Figure 2-2). Our current understanding of these mechanisms came from a convergence of multiple experimental approaches. The first is a biochemical approach to identify presynaptic protein components. Because of the uniform size and buoyancy of synaptic vesicles and their abundance, researchers can purify them to a high degree, which permitted the identification of their
The synaptic vesicle is one of the best-characterized organelles in the cell, with quantitative information about the protein and lipid compositions (Figure 3-7; Movie 3-1). We will encounter some of the synaptic vesicle proteins in this and subsequent sections.

The second is a stunning convergence of yeast genetics to identify genes required for secretion and biochemical reconstitution of mammalian vesicle fusion reactions in vitro, which led to the conclusion that the fundamentals of membrane fusion were highly conserved and that neurotransmitter exocytosis was a specialized form of membrane fusion that occurs in all cells and in many parts of the cells (see Figure 2-2); we will return to this topic in Chapter 12 in the context of the evolutionary origin of neuronal communication. This convergence led to the identification of many evolutionarily conserved vesicle fusion components and their regulators that will be discussed below. The third is the ability to disrupt genes in C. elegans, Drosophila, and mice to test the in vivo function of these evolutionarily conserved proteins in synaptic transmission. The fourth is studies of toxins that block specific steps of neurotransmitter release and identification of their protein targets. Together, these approaches have given rise to our current understanding of the neurotransmitter release mechanisms summarized below.

At the core of vesicle fusion are three **SNARE** proteins (SNARE stands for soluble NSF-attachment protein receptor; NSF is N-ethylmaleimide-sensitive fusion protein, named after a chemical inhibitor that blocks vesicle fusion reactions in vitro) and **SM proteins** (for Sec1/Munc18-like proteins; Sec1 was originally identified in a genetic screen in yeast for its requirement in secretion; Munc18 is the mammalian homology of Unc18, originally identified in a genetic screen in C. elegans for mutants that exhibit an uncoordinated phenotype). The first SNARE is a transmembrane protein on the synaptic vesicle called **synaptobrevin** (also named VAMP for vesicle-associated membrane protein), which is the most abundant synaptic vesicle protein. As a vesicular protein, synaptobrevin is designated as a v-SNARE. The second SNARE is a transmembrane protein on the plasma membrane called **syntaxin**. Owing to its location on the target membrane for vesicle fusion, syntaxin is called a t-SNARE. The third SNARE, also a t-SNARE named **SNAP-25** (synaptosomal-associated protein with a molecular weight of 25 kDa), is anchored onto the cytoplasmic face of the plasma membrane via lipid modification. Once the synaptic vesicle is in the vicinity of the presynaptic plasma membrane, the cytoplasmic domains of synaptobrevin, syntaxin, and SNAP-25 assemble into a very tight complex. How the SNARE complex mediates the fusion is still an active area of research. Current data indicate that the assembly of the SNARE complex proceeds from the membrane-distal to membrane-proximal ends of the SNARE proteins like zipping a zipper. The force generated by the assembly of the SNARE complex drives the synaptic vesicle membrane even closer to the plasma membrane and leads the lipid bilayers to fuse, such that the contents of the synaptic vesicle are exposed to the extracellular space (Figure 3-8A; Movie 3-2).

The structure of the SNARE complex has been determined at atomic resolution by X-ray crystallography. Three SNARE proteins form a four-helix bundle, with synaptobrevin and syntaxin each contributing one helix and SNAP-25 contributing two helices (Figure 3-8B). Many naturally occurring protease toxins that inhibit neuronal communication target these three SNARE proteins at specific amino acid residues (see Box 3-2). Proteolytic cleavage by these proteases is predicted to inhibit the attachment of the four-helix bundle to the membrane, thereby blocking neurotransmitter release.

The SNARE-based mechanism of membrane fusion applies to many fusion reactions in intracellular vesicle trafficking. The v- and t-SNAREs for other specific fusion events (for example, fusion of ER-derived vesicles with the Golgi membrane; see Figure 2-2) resemble the v- and t-SNAREs for synaptic vesicle exocytosis. These findings suggest that the mechanism of synaptic vesicle exocytosis was co-opted from general vesicle trafficking. In all of these reactions, including synaptic vesicle fusion, however, SNARE proteins were found to be insufficient
to mediate fusion. A partner for SNARE proteins in all fusion reactions is an SM protein, which at the mammalian synapse is a protein called Munc18. This protein binds to SNAREs throughout the fusion reaction and is essential for fusion. The precise function of SM proteins is incompletely understood; a leading hypothesis is that SM proteins act as catalysts for SNARE-mediated fusion.

Box 3–2: From toxins to medicines

Research in neurobiology has benefited tremendously from naturally occurring toxins that have evolved to block specific steps of neuronal communication. These toxins are produced by organisms from a wide range of phylogenetic groups, including bacteria, protists, plants, fungi, and animals. Despite the energy costs of producing them, toxins offer adaptive advantages such as deterring herbivores, fending off predators, or immobilizing prey. Scientists have used these toxins to study the biological functions and mechanisms of action of their target proteins. Some of these toxins have been further developed into medicines.

Virtually all steps of neuronal communication are targets for toxins. Action potentials are potently blocked by tetrodotoxin (TTX, see Figure 2–29), an inhibitor of voltage-gated Na⁺ channels produced by symbiotic bacteria in puffer fish, rough-skinned newt, and certain octopi. Synaptic transmission is blocked by a number of proteases produced by the bacteria 
*Clostridium tetani* and 
*Clostridium botulinum*. Tetanus and botulinum toxins specifically cleave SNARE proteins, with each toxin cleaving a specific SNARE at a specific residue, thereby preventing synaptic vesicle fusion with presynaptic membrane (see Figure 3–8). Indeed, identification of the protein targets of tetanus and botulinum toxins was instrumental in establishing that SNARE proteins play a central role in synaptic vesicle fusion. A small peptide from marine snails, o-conotoxin, specifically blocks presynaptic voltage-gated Ca²⁺ channels essential for neurotransmitter release. Other toxins target neurotransmitter receptors that will be discussed later in this chapter. For instance, curare, a plant toxin used by native Americans on poisonous arrows, and α-bungarotoxin and cobratoxin from snakes, are all potent competitive inhibitors of the acetylcholine receptor at the vertebrate neuromuscular junction, and thereby block motor neuron-triggered muscle contraction. Picrotoxin, another plant toxin, is a potent blocker of the GABA_A receptors that mediate fast inhibition in vertebrates and invertebrates alike. Muscimol, produced by toxic mushrooms, is a potent activator of the GABA_A receptors. The venoms of predators such as snakes, scorpions, cone snails, and spiders have been a rich source of tools for investigating neuronal communication. The fact that most toxins affect many different animal species also indicates that the molecular machinery of neuronal communication is highly conserved across animals.

Natural toxins and their derivatives have also been used extensively in medicine. Channel blockers have been used to treat epilepsy and intractable pain. Synaptic transmission blockers have been used as muscle relaxants. For example, botulinum toxin A, commonly known as Botox, can be injected into specific eye muscles to treat strabismus (misaligned eyes). Botox injections have also become a popular cosmetic procedure to temporarily remove wrinkles.
3.6 Synaptotagmin serves as a \( \text{Ca}^{2+} \) sensor to trigger synaptic vesicle fusion

How does \( \text{Ca}^{2+} \) entry regulate neurotransmitter exocytosis? A prime candidate that links these two events is a class of transmembrane proteins on the synaptic vesicle called synaptotagmins (see Figure 3–7), which possess up to five \( \text{Ca}^{2+} \)-binding sites on their cytoplasmic domain. To test the function of synaptotagmin in synaptic transmission, \textit{knockout} mice were created in which synaptotagmin-1, the predominant form of synaptotagmin expressed in forebrain neurons, was disrupted using the gene targeting method in embryonic stem cells (see Section 13.7 for details of the knockout method). To assay for synaptic transmission, embryonic hippocampal neurons from control or knockout mice were dissociated and cultured \textit{in vitro} to allow synapse formation, and pairs of synaptically connected neurons were subjected to a variation of the patch clamp technique called \textit{whole-cell patch recording}. (In whole-cell patch recording, the membrane underneath the patch pipette is ruptured such that the patch pipette is connected to the entire neuron; see Section 13.21 and Box 13–2 for details.) Depolarization of a wild-type presynaptic neuron, which caused it to fire action potentials, resulted in an inward current of the postsynaptic neuron, an indication of successful synaptic transmission. Depolarization of synaptotagmin-1 knockout neurons elicited much smaller postsynaptic responses, indicating that synaptotagmin-1 is required for normal synaptic transmission (Figure 3–9A). Earlier studies in \textit{Drosophila} and \textit{C. elegans} indicated that disruption of synaptotagmin homologs in these invertebrates also impaired synaptic transmission.

The knockout experiment did not prove that synaptotagmin acts as a \( \text{Ca}^{2+} \) sensor, as disrupting other genes encoding proteins essential for synaptic transmission, such as the v-SNARE synaptobrevin, similarly blocked synaptic transmission. Subsequent experiments have provided strong evidence that synaptotagmin is a major \( \text{Ca}^{2+} \) sensor that regulates neurotransmitter release. For example, a mutant synaptotagmin-1 with a single amino acid change was identified that reduces \( \text{Ca}^{2+} \) binding by 50% in an \textit{in vitro} biochemical assay. When this mutant synaptotagmin-1 was used to replace the endogenous synaptotagmin-1 in a variation of the knockout procedure called \textit{knock-in} (see Section 13.7), neurons derived from the knock-in mice exhibited a corresponding 50% reduction in the \( \text{Ca}^{2+} \) sensitivity of neurotransmitter release (Figure 3–9B). Another protein involved in neurotransmitter release is complexin, which has a complex role of both activating the SNARE complex and clamping it at an intermediate step. One

![Figure 3–9 Synaptotagmin serves as a \( \text{Ca}^{2+} \) sensor in synaptic transmission.](image-url)

(A) Left, schematic of experimental preparation to examine the role of synaptotagmin-1 in synaptic transmission. Both hippocampal neurons in culture were subjected to whole-cell patch recording. A depolarizing current was injected into the presynaptic neuron to cause it to fire an action potential, and the postsynaptic response was recorded as an inward current when the membrane potential was clamped at -70 mV. Right, compared with the inward current triggered by a presynaptic action potential between a pair of wild-type neurons (top trace), the synaptic response between a pair of neurons from Syt1 knockout mice (lacking synaptotagmin-1; meaning both copies of the Syt1 gene were disrupted) was greatly diminished (bottom trace). (B) A point mutation in Syt1 that reduced \( \text{Ca}^{2+} \) binding by 50% also reduced the sensitivity of neurotransmitter release of cultured hippocampal neurons to \( \text{Ca}^{2+} \) by about 50%, as indicated by the downward shift of the mutant curve compared with the wild-type curve, each plotting normalized synaptic transmission amplitude against \( \text{Ca}^{2+} \) concentration. This finding supports the notion that synaptotagmin-1 acts as a \( \text{Ca}^{2+} \) sensor for synaptic vesicle fusion in hippocampal neurons. (A, adapted from Geppert M, Goda Y, Hammer RE et al. [1994] Cell 79:717–727. With permission from Elsevier Inc.); B, adapted from Fernandez-Chacon R, Koenigsfoder A, Gerber SH et al. [2001] Nature 410:41–49. With permission from Macmillan Publishers Ltd.)
current model is that synaptotagmin releases the inhibitory clamp of complexin in a Ca\(^{2+}\)-dependent manner, thus allowing SNAREs to complete the vesicle fusion reaction in response to a rise of intracellular Ca\(^{2+}\) concentration.

In fast mammalian CNS synapses at physiological temperatures, action potential arrival can cause neurotransmitter release within as little as 150 µs, as measured by postsynaptic depolarization. This interval includes about 90 µs to open voltage-gated Ca\(^{2+}\) channels during the action potential upstroke to allow Ca\(^{2+}\) influx, and 60 µs in total for Ca\(^{2+}\) to trigger vesicle fusion and for neurotransmitter molecules to diffuse across the synaptic cleft and act on postsynaptic cells. To enable this rapid action, synaptic vesicles are docked at the active zone ready for release (see Figure 3-3), with their SNARE proteins already partially preassembled in a high-energy configuration but clamped, waiting for the action of a Ca\(^{2+}\) sensor such as synaptotagmin to release the clamp and complete SNARE assembly that drives membrane fusion.

In addition to rapid release of synaptic vesicles after Ca\(^{2+}\) entry, it is also important that transmitter release is transient so that the presynaptic terminal can respond to future action potentials with more neurotransmitter release. This requires free Ca\(^{2+}\) to be rapidly removed after their entry, and that the Ca\(^{2+}\) sensor has a low binding affinity for Ca\(^{2+}\). Indeed, Ca\(^{2+}\)-binding proteins and pumps rapidly sequester free Ca\(^{2+}\) upon entry. Moreover, synaptotagmin employs multiple low-affinity Ca\(^{2+}\)-binding sites that bind Ca\(^{2+}\) cooperatively (that is, the binding of one Ca\(^{2+}\) facilitates the binding of a second Ca\(^{2+}\)); only when multiple sites bind to Ca\(^{2+}\) would it be able to trigger neurotransmitter release. Together, these mechanisms ensure that neurotransmitter release is only triggered transiently and locally at the site of Ca\(^{2+}\) entry.

### 3.7 The presynaptic active zone is a highly organized structure

The fast action and transient nature of Ca\(^{2+}\)-induced neurotransmitter release relies on the proximity of voltage-gated Ca\(^{2+}\) channels and docked synaptic vesicles in the active zone. Indeed, Ca\(^{2+}\) imaging of the presynaptic terminals (for example, see Figure 3-6) suggested that the rise of intracellular Ca\(^{2+}\) concentration in response to depolarization is highly restricted to microdomains near the active zone. Although the intracellular Ca\(^{2+}\) concentration is normally very low (∼0.1 µM), it can shoot up transiently to tens or even hundreds of micromolar in the microdomain; this facilitates cooperative binding of Ca\(^{2+}\) to multiple Ca\(^{2+}\)-binding sites of synaptotagmin to achieve the conformational change necessary for triggering vesicle fusion.

The molecular machinery that organizes the active zone has been extensively characterized in vertebrate neurons (Figure 3-10), and many components are conserved in invertebrates. The cytoplasmic domain of the voltage-gated Ca\(^{2+}\) channel binds to two active zone core components, RIM (Rab3-interacting molecule) and RIM-BP (RIM-binding protein). RIM also binds to a synaptic vesicle associated protein Rab3, a small GTPase of the Rab subfamily, thus bringing the synaptic vesicle into proximity with Ca\(^{2+}\) channels. In addition, RIM and RIM-BP interact with other active zone proteins, which in turn associate with the actin cytoskeleton that supports the structural integrity of the presynaptic terminal and transports molecules into the presynaptic terminal (see Figure 2-6). The active zone protein complex is also associated with synaptic adhesion molecules. These include the cadherins (Ca\(^{2+}\)-dependent cell adhesion proteins) present on both pre- and postsynaptic membranes that bind each other (termed homophilic binding), and neurexin on the presynaptic membrane binding to neuroligin on the postsynaptic membrane (termed heterophilic binding). These cell-adhesion molecules bring the presynaptic and postsynaptic plasma membranes together, and align the active zone with the postsynaptic membrane rich in neurotransmitter receptors (to be discussed later in the chapter), thus minimizing the distance neurotransmitters need to travel to act on their receptors (Figure 3-10).

Recent studies using super-resolution fluorescent microscopy (see Section 13.17 for more details) have begun to determine where specific molecules are located with respect to each other at the active zone. For example, according to a
Figure 3-10 Molecular organization of the presynaptic terminal. Left, a magnified model of the presynaptic cell's active zone. The RIM/RIM-BP protein complex binds to the voltage-gated Ca\(^{2+}\) channel directly, and to the synaptic vesicle via the Rab3 protein; this allows Ca\(^{2+}\) entry to activate synaptotagmin with minimal diffusion, which in turn releases the complex inhibitory clamp on the SNARE/SV complex and causes neurotransmitter release (the SNARE complex is represented as in Figure 3-8A; Munc18 is the SM protein in mammalian synapses). RIM and RIM-BP are also associated with other presynaptic scaffolding proteins, which are in turn associated with the actin cytoskeleton and with synaptic adhesion molecules. Right, a lower-magnification model of a chemical synapse showing presynaptic and postsynaptic cells. Trans-synaptic adhesion molecules (blue, homophilic binding between cadherins; yellow and red, heterophilic binding between presynaptic neurexin and postsynaptic neulin) align the active zone with a postsynaptic density enriched for neurotransmitter receptors, facilitating the rapid action of neurotransmitters. (Adapted from Sudhof TC [2012] Neuron 75:11-22. With permission from Elsevier Inc.)

Figure 3-11 A model of the organization of selected proteins in the Drosophila neuromuscular synapse. This model is based on super-resolution localization of molecules in the Drosophila neuromuscular junction (Figure 3-11), the RIM-BP proteins form a ring around a cluster of voltage-gated Ca\(^{2+}\) channels at the active zone presynaptic membrane. An active zone scaffolding protein called Bruchpilot (corresponding to a mammalian protein called ELKS) extends from the center of the active zone to the periphery. Glutamate receptors are enriched in the postsynaptic density aligned with the presynaptic active zone (as discussed in Section 3.11, glutamate is used as a neurotransmitter in the Drosophila neuromuscular synapse). Future studies on synapses in the central nervous system and in other species will help determine whether all synapses share a similar structural organization, and what variations might exist among different synapses.

3.8 Neurotransmitters are efficiently cleared from the synaptic cleft by enzymatic cleavage or transport into presynaptic and glial cells

In order for the postsynaptic neurons to continually respond to the firing of presynaptic neurons, neurotransmitters released in response to each presynaptic action potential must be cleared from the synaptic cleft efficiently. While diffusion of neurotransmitters away from the synaptic cleft is a major mechanism of clearance, additional mechanisms are employed for neurotransmitter clearance.
How is neurotransmitter release controlled at the presynaptic terminal?  

Depending on the neurotransmitter system (we will introduce different neurotransmitter systems in more detail in Section 3.11).

ACh at the neuromuscular junction is rapidly degraded by acetylcholinesterase, an enzyme enriched in the synaptic cleft. Indeed, this enzyme is so active that most ACh molecules released by motor axon terminals are degraded while diffusing across the short distance of the synaptic cleft. Some of the physiology experiments involving mEPP measurement described in earlier sections actually included acetylcholinesterase inhibitors in the saline to boost the mEPP amplitude.

For most other neurotransmitter systems, excess transmitter molecules in the synaptic cleft are recycled. In a process called neurotransmitter reuptake, excess neurotransmitters are first taken back into the presynaptic cytosol using the plasma membrane neurotransmitter transporters, which derive energy from co-transporting Na⁺ into the presynaptic cell down the Na⁺ electrochemical gradient (Figure 3-12; see Movie 3-1). Once in the cytosol, neurotransmitters refill new and recycled synaptic vesicles (see Section 3.9) utilizing a second transporter: the vesicular neurotransmitter transporter on the synaptic vesicle (see also Figure 3-7). The energy for the vesicular transporters derives from transporting protons in the opposite direction down the proton gradient. The proton gradient (high in the vesicle and low in the cytosol) is created by V-ATPase, the largest molecule on the synaptic vesicle membrane (see Figure 3-7), which pumps protons (H⁺) into the synaptic vesicle against an electrochemical gradient using energy derived from ATP hydrolysis. In some neurotransmitter systems, excess transmitters are mostly taken up by neurotransmitter transporters on the plasma membrane of glial cells, which wrap around many synapses (see Figure 3-3). In Chapter 11, we will learn more about the neurotransmitter reuptake mechanisms, because drugs altering these mechanisms are widely used to treat psychiatric disorders.

### 3.9 Synaptic vesicle recycling by endocytosis is essential for continual synaptic transmission

In order to maintain the ability to respond to sustained neuronal firing, presynaptic terminals must be able to replenish the stockpile of synaptic vesicles filled with neurotransmitters. While the synaptic vesicle membrane and proteins are mostly synthesized in the soma (see Sections 2.2–2.3), vesicles are rapidly recycled locally at the synaptic terminals. Considering the distance between the synaptic terminal and the soma, the recycling of synaptic vesicles is critical in order to rapidly recover synaptic vesicles for future rounds of synaptic transmission.

![Figure 3-12](image-url)  
**Figure 3-12** Clearance and recycling of neurotransmitters. After being released into the synaptic cleft as a result of synaptic vesicle fusion with the presynaptic plasma membrane, excess transmitters are taken up by plasma membrane transporters (PMTs) on the presynaptic membrane or on the nearby glial plasma membrane; both are symporters that utilize energy from Na⁺ entry down its electrochemical gradient. Within the presynaptic cytosol, neurotransmitters are transported into synaptic vesicles by the vesicular neurotransmitter transporters (VTs), which are antiporters that use the energy by transporting protons (H⁺) out of the synaptic vesicle down an electrochemical gradient. The V-ATPase on the synaptic vesicle membrane establishes the H⁺ gradient in the vesicle using energy from ATP hydrolysis. (See Blakey RD & Edwards RH [2012] Cold Spring Harb Perspect Biol 4:a005595.)
After the fusion of synaptic vesicle membrane with presynaptic plasma membrane resulting in the release of neurotransmitter molecules, at least two mechanisms have been proposed to retrieve synaptic vesicles back to the presynaptic cytosol. The first mechanism, called ‘kiss and run,’ involves a very transient fusion of the synaptic vesicle with the presynaptic plasma membrane to release the neurotransmitters, followed by rapid reformation of the vesicle so that mixing of the vesicle’s protein and lipid content with the presynaptic plasma membrane is limited. In the second mechanism, synaptic vesicle membrane becomes part of the presynaptic plasma membrane after full fusion, and is retrieved back to the presynaptic terminal by clathrin-mediated endocytosis. (Clathrin is a protein that assembles into a cage on the cytoplasmic side of a membrane to form a coated pit, which buds off to form a clathrin-coated vesicle.) Whereas full fusion likely applies to most cases of synaptic vesicle recycling, the degree to which the kiss-and-run mechanism is used is still a subject of debate. In both cases, the SNARE complexes are disassembled by NSF in an ATP-dependent manner. (Recall from Section 3.5 that the name SNARE derives from NSF.) Synaptobrevin returns to the synaptic vesicle, while syntaxin and SNAP-25 remain in the presynaptic plasma membrane. The vesicles are then acidified by the proton pump V-ATPase and refilled with neurotransmitters (see Figure 3-12). Filled vesicles join the reserve pool of synaptic vesicles. A synaptic vesicle protein called synapsin, a commonly used marker for identifying synapses, is involved in regulating the size of the reserve pool. A small subset of synaptic vesicles constitutes the readily releasable pool, which are docked at the active zone, primed by an ATP-dependent process to achieve the high-energy configuration of pre-assembled SNARE complex, and readied for another round of neurotransmitter release in response to depolarization-induced Ca\textsuperscript{2+} entry (Figure 3-13).

We use a specific example to illustrate the importance of synaptic vesicle retrieval for continual synaptic transmission and neuronal communication. To identify genes necessary for neuronal communication, forward genetic screens (see Section 13.6) were carried out in the fruit fly Drosophila to isolate mutations that caused paralysis when flies were shifted to high temperatures. This led to the discovery of a temperature-sensitive mutation called Shibire\textsuperscript{3}. Shibire\textsuperscript{3} flies behave normally at room temperature (\(<20^\circ\text{C}\)), but are paralyzed shortly after shifting to elevated temperatures (\(>29^\circ\text{C}\)); their motility returns to normal within a few minutes after the temperature is returned to 20°C. Molecular-genetic analysis
How is neurotransmitter release controlled at the presynaptic terminal?

**Figure 3-14** Electron micrographs of synapses in temperature-sensitive Shibire<sup>ts</sup> mutant fruit flies. (A) A Shibire<sup>ts</sup> mutant fly neuromuscular junction fixed at 19°C. The presynaptic terminal is abundant in synaptic vesicles (sv). Arrows indicate active zones. (B) Neuromuscular junction fixed 8 minutes after raising the temperature to 29°C. Note the reduced number of synaptic vesicles in the presynaptic terminal compared with panel A, and the presence of 'collared' vesicles (arrows, see inset for higher magnification) indicating a block of the last step of endocytosis. (From Koeng JH & Ikeda K [1989] J Neurosci 9:3844-3860. With permission from the Society for Neuroscience.)

identified that the Shibire gene encodes a protein called dynamin, which is essential for clathrin-mediated endocytosis of synaptic vesicles. The Shibire<sup>ts</sup> mutation causes reversible destabilization of dynamin at elevated temperatures. Without vesicle recycling, presynaptic terminals were rapidly deprived of synaptic vesicles after the reserved pool is exhausted ([Figure 3-14](#)), and became unable to release neurotransmitters in response to further action potentials, thus causing paralysis. The Shibire<sup>ts</sup> mutation has provided a useful tool for rapidly and reversibly silencing specific neurons in vivo to analyze their function in information processing within neural circuits (see Section 13.23).

As a summary of what we have learned so far, [Table 3-1](#) provides a list of molecules that play key roles in mediating and regulating the sequence of events required for neurotransmitter release.

### 3.10 Synapses can be facilitating or depressing

Because synaptic transmission is a key mechanism of interneuronal communication, the **efficacy of synaptic transmission**, measured by the magnitude of the postsynaptic response to a presynaptic stimulus, is subject to many forms of regulation. The ability to change the efficacy of synaptic transmission, or **synaptic plasticity**, is an extremely important property of the nervous system. Depending on the temporal scale, synaptic plasticity is usually divided into **short-term synaptic plasticity**, which occurs within milliseconds to minutes, and **long-term synaptic plasticity**, which can extend from hours to the lifetime of an animal. We discuss below the two simplest forms of short-term plasticity involving changes of neurotransmitter release probability. Long-term synaptic plasticity will be a subject of focus in Chapter 10 in the context of memory and learning.

Although Ca<sup>2+</sup>-dependent synaptic vesicle fusion provides an essential link between action potential arrival and neurotransmitter release, not every action potential results in the same amount of neurotransmitter release. As discussed earlier, the quantal yield of CNS synapses is much lower than that of the neuromuscular junction because a presynaptic axon may form only a few or a single active zone onto a postsynaptic partner neuron. In some mammalian CNS *in vivo*, the average release probability, defined as the probability that an active zone of
Table 3-1: A molecular cast for neurotransmitter release

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Location</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synaptic vesicle fusion with presynaptic membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synaptobrevin/VAMP</td>
<td>synaptic vesicle</td>
<td>mediates vesicle fusion (v-SNARE)</td>
</tr>
<tr>
<td>Syntaxin</td>
<td>presynaptic plasma membrane</td>
<td>mediates vesicle fusion (t-SNARE)</td>
</tr>
<tr>
<td>SNAP-25</td>
<td>presynaptic plasma membrane</td>
<td>mediates vesicle fusion (t-SNARE)</td>
</tr>
<tr>
<td>Sec1/Munc18 (SM)</td>
<td>presynaptic cytosol</td>
<td>likely acts as a catalyst for SNARE-mediated vesicle fusion</td>
</tr>
<tr>
<td>Ca²⁺ regulation of synaptic transmission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voltage-gated Ca²⁺ channel</td>
<td>active zone of presynaptic membrane</td>
<td>allows Ca²⁺ entry in response to action potential-triggered depolarization</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>entering from extracellular space to presynaptic cytosol</td>
<td>triggers synaptic vesicle fusion</td>
</tr>
<tr>
<td>Synaptotagmin</td>
<td>synaptic vesicle</td>
<td>senses Ca²⁺ to trigger vesicle fusion</td>
</tr>
<tr>
<td>Complexin</td>
<td>presynaptic cytosol</td>
<td>binds and regulates SNARE-mediated vesicle fusion</td>
</tr>
<tr>
<td>Organization of presynaptic terminal (and alignment with postsynaptic density)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIM</td>
<td>active zone</td>
<td>organizes presynaptic scaffold</td>
</tr>
<tr>
<td>RIM-BP</td>
<td>active zone</td>
<td>organizes presynaptic scaffold</td>
</tr>
<tr>
<td>ELKS/Bruchpilot</td>
<td>active zone</td>
<td>organizes presynaptic scaffold</td>
</tr>
<tr>
<td>Rab3</td>
<td>synaptic vesicle</td>
<td>interacts with active zone components</td>
</tr>
<tr>
<td>Cadherin</td>
<td>presynaptic and postsynaptic plasma membranes</td>
<td>trans-synaptic adhesion</td>
</tr>
<tr>
<td>Neuroxin</td>
<td>presynaptic plasma membrane</td>
<td>trans-synaptic adhesion</td>
</tr>
<tr>
<td>Neuriligin</td>
<td>postsynaptic plasma membrane</td>
<td>trans-synaptic adhesion</td>
</tr>
<tr>
<td>Neurotransmitter and vesicle recycling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>synaptic cleft</td>
<td>degrades neurotransmitter acetylcholine</td>
</tr>
<tr>
<td>Plasma membrane neurotransmitter transporter (PMT)</td>
<td>synpatic plasma membrane, glial membrane</td>
<td>transports excess neurotransmitter molecules back to presynaptic cytosol or to nearby glia</td>
</tr>
<tr>
<td>Vesicular neurotransmitter transporter (VT)</td>
<td>synaptic vesicle</td>
<td>transports neurotransmitters from presynaptic cytosol to the synaptic vesicle</td>
</tr>
<tr>
<td>V-ATPase</td>
<td>synaptic vesicle</td>
<td>establishes proton gradient within the synaptic vesicle</td>
</tr>
<tr>
<td>Synapsin</td>
<td>synaptic vesicle</td>
<td>regulates the size of the reserve pool</td>
</tr>
<tr>
<td>Clathrin</td>
<td>presynaptic cytosol</td>
<td>retrieves vesicles from presynaptic plasma membrane via endocytosis</td>
</tr>
<tr>
<td>Shibire/dynamin</td>
<td>presynaptic cytosol</td>
<td>retrieves vesicles from presynaptic plasma membrane via endocytosis</td>
</tr>
<tr>
<td>NSF</td>
<td>presynaptic cytosol</td>
<td>disassembles SNARE complex after fusion</td>
</tr>
</tbody>
</table>

A presynaptic terminal releases the transmitter contents of one or more synaptic vesicles following an action potential, estimated to be far smaller than 1. If many active zones exist between a presynaptic and a postsynaptic cell, as is the case for the vertebrate neuromuscular junction, the probability that at least one active zone releases a vesicle is close to 1; however, the magnitude of postsynaptic response still depends on the release probability of each active zone.

The release probability can be affected by prior usage of the synapse. In facilitating synapses, successive action potentials trigger larger and larger postsynaptic responses. By contrast, in depressing synapses, successive action potentials result in smaller and smaller postsynaptic responses (Figure 3-15). These changes can be caused by a postsynaptic mechanism, such as altered sensitivity to the release of the same amount of neurotransmitters, as we will discuss later in
the chapter and in Chapter 10, but fast facilitation and depression are most often caused by a presynaptic mechanism such as the altered amount of neurotransmitter release. The same synapse can be facilitating or depressing depending on its intrinsic property and its prior history of usage.

In the simplest case, facilitating synapses have a low starting release probability. The amount of release increases during repeated action potentials as active zone Ca^{2+} builds up. Depressing synapses, on the other hand, are usually characterized with a high starting release probability that results in a large amount of release at the beginning of a stimulus train; this exhausts the number of vesicles readily available for release and leads to a decline in the amount of release as the stimulus train proceeds. Because there are typically large numbers of vesicles in the reserve pool that can replenish depleted vesicles in the readily releasable pool, this sort of depression can recover in seconds. In the course of this book, we will encounter many additional mechanisms that adjust synaptic strength using distinct mechanisms and at different temporal scales.

### 3.11 The nervous system uses many neurotransmitters

To illustrate the basic principles of synaptic transmission, we have focused primarily on the vertebrate neuromuscular junction, which utilizes acetylcholine as the neurotransmitter. The principles we have learned thus far apply to virtually all chemical synapses, regardless of the neurotransmitter they use (Figure 3-16; Table 3-2). Two major neurotransmitters used in the vertebrate central nervous system are glutamate (glutamic acid), a natural amino acid, and GABA (γ-aminobutyric acid), derived from glutamate by the enzyme glutamic acid decarboxylase (GAD). Glutamate is the predominant excitatory neurotransmitter in the vertebrate nervous system because its release depolarizes postsynaptic neurons and makes them more likely to fire action potentials. GABA is the predominant inhibitory neurotransmitter because its release usually renders postsynaptic neurons less likely to fire action potentials. The amino acid glycine, another inhibitory neurotransmitter, is used in a subset of inhibitory neurons in the brainstem and spinal cord of the vertebrate nervous system.

GABA appears to be the major inhibitory neurotransmitter across different species, including many invertebrates such as the nematode *C. elegans*, the fruit fly *Drosophila melanogaster*, and crustaceans (GABA’s inhibitory action was first established in the crab). Like vertebrates, *C. elegans* also uses glutamate as the major excitatory neurotransmitter and ACh as the transmitter at the neuromuscular junction. Curiously, *Drosophila* utilizes ACh as the major excitatory neurotransmitter in the CNS and glutamate as the transmitter at the neuromuscular junction (see Figure 3-11). It is important to note that although it is convenient to label a particular neurotransmitter excitatory or inhibitory by its action on postsynaptic cells in most cases, we will see in the next part of the chapter that
CHAPTER 3  Signaling across synapses

Figure 3-16 Structures of a subset of small-molecule neurotransmitters. Glutamate and glycine are natural amino acids. GABA (γ-aminobutyric acid) is produced from glutamate. Dopamine is derived from the amino acid tyrosine. Norepinephrine is produced from dopamine and is a precursor for the hormone epinephrine. Serotonin is derived from the amino acid tryptophan. Histamine is derived from the amino acid histidine. See Figure 3-1A for the structure of acetylcholine.

the same transmitter can be excitatory or inhibitory depending on the properties of its receptor and the ionic composition of the postsynaptic cell.

Another important class of neurotransmitters plays a predominantly modulatory role. Modulatory neurotransmitters (also called neuromodulators) can up- or down-regulate the membrane potential, excitability (how readily a neuron fires an action potential), or neurotransmitter release by their postsynaptic target neurons, depending on the type of receptors that their postsynaptic neurons express and the subcellular localization of these receptors. Classic neuromodulators include serotonin (also called 5-HT for 5-hydroxytryptamine), dopamine, norepinephrine (also called noradrenaline), and histamine (see Figure 3-16). They are all derived from aromatic amino acids and are collectively called monoamine neurotransmitters. In addition to being released into the synaptic cleft, these neurotransmitters can also be secreted into the extracellular space outside the confines of morphologically defined synapses to affect nearby cells; this property is referred to as volume transmission. In vertebrates, the cell bodies of neurons that synthesize monoamine neurotransmitters are mostly clustered in discrete nuclei in the brainstem or hypothalamus. They send profuse axons that collectively innervate a large fraction of the nervous system (see Box 8–1). Dopamine and serotonin act as neuromodulators throughout the animal kingdom. In place of norepinephrine, a chemically similar molecule called octopamine is used in some invertebrate nervous systems.

Some neurotransmitters have multiple roles in different parts of the nervous system (see Table 3-2). In vertebrates, ACh is used as an excitatory neurotransmitter by motor neurons to control skeletal muscle contraction at the neuromuscular junction. It is also one of the two neurotransmitters employed in the autonomic nervous system for neural control of visceral functions such as heart rate, respiration, and digestion. In the brain, ACh can act both as an excitatory neurotransmitter and as a neuromodulator much like the monoamine neurotransmitters. Likewise, norepinephrine functions as the autonomic nervous system’s other neurotransmitter, but acts as a neuromodulator in the brain.

The type of neurotransmitter a neuron uses is often used as a major criterion for neuronal classification. Neurons can be broadly classified as excitatory, inhibitory, or modulatory as discussed earlier, and more specifically as glutamatergic, GABAergic, cholinergic, dopaminergic, and so on. Neurons of a given neurotransmitter type express a specific set of genes associated with that type, including enzyme(s) that synthesize the neurotransmitter, a vesicular transporter that pumps the neurotransmitter into synaptic vesicles, and in many cases a plasma-membrane transporter that retrieves the neurotransmitter from the synaptic cleft after release (see Figure 3-12). Some neurons utilize more than one of the neurotransmitters discussed above. For example, some mammalian CNS
neurons can co-release a modulatory neurotransmitter and the excitatory neurotransmitter glutamate, or a modulatory neurotransmitter and the inhibitory neurotransmitter GABA.

In addition to the small-molecule neurotransmitters we have discussed thus far, some neurons also secrete neuropeptides that can act as neurotransmitters to communicate with postsynaptic neurons. The mammalian nervous system utilizes dozens of neuropeptides, with lengths ranging from a few amino acids to several dozen. As we will learn in Chapters 8 and 9, neuropeptides regulate diverse and vital physiological functions such as eating, sleeping, and sexual behaviors. Neuropeptides are usually produced by proteolytic cleavage of precursor proteins in the secretory pathway (see Figure 2-2). They are packaged into dense-core vesicles (which are larger than synaptic vesicles and contain electron-dense materials) after vesicles containing neuropeptides bud off from the Golgi apparatus, and are delivered via fast axonal transport to presynaptic terminals. Because they cannot be locally synthesized or recovered after release, but must be transported across long distances from the soma to axon terminals, neuropeptides are used more sparingly. The probability of neuropeptide release seems to be much lower than that of small-molecule neurotransmitters even when they are present in the same terminals. Compared to synaptic vesicles that release small-molecule neurotransmitters, we know far less about the mechanisms that control neuropeptide release from dense-core vesicles. In most cases, neuropeptides play modulatory roles, and are released from neurons that use a small-molecule neurotransmitter. As we will learn in Chapter 8, some neurons secrete neuropeptides into the bloodstream; in these cases, neuropeptides act as hormones to influence the physiology of recipient cells remotely.

The reason why different neurotransmitters have different effects is because the receptors on the postsynaptic membrane have different properties. We now turn to the next step of neuronal communication: how neurotransmitters act on postsynaptic neurons.

### HOW DO NEUROTRANSMITTERS ACT ON POSTSYNAPTIC NEURONS?

In the first part of the chapter, we used postsynaptic responses, such as the end-plate potential (see Figure 3-1) or postsynaptic inward current (see Figures 3-5 and 3-9) as assays to investigate the mechanisms of presynaptic neurotransmitter release. In the following sections, we will discuss the mechanisms by which postsynaptic neurons produce these responses. We first discuss rapid responses that
occur within milliseconds involving direct change of ion conductance. We then study responses that occur in tens of milliseconds to seconds, involving intracellular signaling pathways. We further highlight responses that occur in hours to days involving new gene expression. Finally, we discuss how postsynaptic neurons integrate different presynaptic input to determine their own firing pattern and neurotransmitter release properties, thus completing a full round of neuronal communication.

3.12 Acetylcholine opens a nonselective cation channel at the neuromuscular junction

We now begin our journey across the synaptic cleft to the postsynaptic side of the synapse, returning first to the vertebrate neuromuscular junction as our model. In Section 3.1, we learned that ACh released from motor axon terminals depolarizes the muscle membrane, and that iontophoretic application of ACh to muscle can substitute for ACh release from presynaptic terminals (see Figure 3-1). How does ACh accomplish this? By locally applying ACh to different regions of muscle fibers, researchers found that exogenous ACh produced the most effective depolarization near the motor axon terminal. These experiments implied that there must be receptors for ACh that are present on the muscle membrane and are concentrated at the neuromuscular junction. Upon ACh binding, ACh receptors trigger a rapid change in the muscle membrane’s ion conductance.

To explore the underlying mechanisms, voltage clamp experiments analogous to those carried out on squid giant axons (see Section 2.10) were performed on the muscle fibers to test how ACh release induced by motor axon stimulation changes ion flow across the muscle membrane (Figure 3-17A). In these experiments, two electrodes were inserted into the muscle cell, one to measure the membrane potential ($V_m$) and compare it to a desired command voltage ($V_{CMD}$), and a second to pass feedback current into the muscle to maintain $V_m$ at the same value as $V_{CMD}$. The current injected into the muscle, which can be experimentally measured, equals the current that passes through the muscle membrane in response to ACh release, or the end-plate current. (Under physiological conditions, that is, when the muscle is not voltage-clamped, the end-plate current would produce a membrane potential change, which is the end-plate potential

![Figure 3-17 Properties of an acetylcholine (ACh)-induced current studied by voltage clamp. (A) Experimental setup. Two intracellular electrodes were inserted into the muscle cell at the frog neuromuscular junction. The first (left) was to record the membrane potential ($V_m$), which was compared with an experimenter-determined commanding potential ($V_{CMD}$). The second electrode injected feedback current into the muscle to maintain $V_m$ at $V_{CMD}$. The end-plate current in response to ACh release caused by motor axon stimulation can be determined from the feedback current that was injected into the muscle cell in order to hold $V_m$ at $V_{CMD}$. (B) The end-plate current elicited by single motor axon stimulation was measured at the six different membrane potentials indicated. At negative potentials the end-plate current was inward (positive ions flowing into the muscle cell), whereas at positive potentials the end-plate current was outward. (C) Peak end-plate current ($I_p$) as a function of the muscle membrane potential ($V_m$, $x$ axis). Experimental data (represented as dots) fell on a curve (the $I$-$V$ curve) that was close to linear (dotted line), indicating that the conductance (represented by the slope of the $I$-$V$ curve) was mostly unaffected by voltage. Note that the current switched sign between negative (inward) and positive (outward) at 0 mV, which is the reversal potential of the channel opened by ACh. (B & C, adapted from Magleby KL & Stevens CF [1972] J Physiol 223:173–197.)}
we have used to measure neurotransmitter release in Section 3.1). It was found that ACh release caused an inward current at negative membrane potentials and an outward current at positive membrane potentials (Figure 3-17B). The current-voltage relationship (called an I-V curve) is nearly linear. The membrane potential at which the current flow reversed direction (called the reversal potential) was approximately 0 mV (Figure 3-17C).

If the ACh-induced current were carried by a single ion, the reversal potential should equal the equilibrium potential of that ion, as both reversal potential and equilibrium potential define a state in which the net current is zero. However, the reversal potential of the ACh-induced current is unlike the Na⁺, K⁺, or Cl⁻ currents discussed in Section 2.5, with equilibrium potentials around -85 mV, and -79 mV, respectively. Indeed, experiments that measured the reversal potentials in response to varying extracellular K⁺, Na⁺, and Cl⁻ concentrations suggested that ACh opens a channel that is permeable to both K⁺ and Na⁺ and other cations but not to anions such as Cl⁻. Further evidence indicated that ACh acts on a single channel that is permeable simultaneously to Na⁺ and K⁺. At positive membrane potentials, the driving force for K⁺ efflux is greater than the driving force for Na⁺ influx (because the V_m is further from E_K than from E_Na), and hence K⁺ efflux exceeds Na⁺ influx, causing a net outward current. At negative membrane potentials, the driving force for Na⁺ influx exceeds that for K⁺ efflux, causing a net inward current. Ca²⁺ influx also makes a small contribution to the inward current. Importantly, since the reversal potential of 0 mV is far above the muscle membrane’s resting potential (around -75 mV) or the threshold for action potential production (which is usually 10-20 mV more depolarized than the resting potential), the end-plate current under physiological conditions is always inward, carried by more Na⁺ influx than K⁺ efflux (Figure 3-18A). This depolarizes the muscle membrane, resulting in the end-plate potential (EPP) we introduced in Section 3.1.

The action of an ACh-induced current can be represented by an electrical circuit model of the muscle membrane, in which the ACh-induced current can be considered as an added branch to the resting muscle membrane (Figure 3-18B). Immediately after the switch is on (representing ACh release), I_Na = g_Na(V_m - E_Na), and I_K = g_K(V_m - E_K). Because at rest V_m is around -75 mV, the absolute value [V_m - E_Na] far exceeds [V_m - E_K]. Assuming that the ACh-activated channel has similar conductance for Na⁺ and K⁺ (see below), the inward current from the Na⁺ branch far exceeds the outward current from the K⁺ branch. Thus, the ACh release activates a net inward current.

The reversal potential, designated as \( E_{rev} \), is an important property of ion channels that are permeable to more than one ion. It is determined by the relative conductance and equilibrium potential of each ion. Using the electrical circuit model in Figure 3-18B, we can determine their relationship as follows. At the reversal potential \( V_m = E_{rev} \), Na⁺ influx equals K⁺ efflux, thus \( I_K = I_{Na} \). Since \( I_K = g_K(V_{in} - E_K) \), and \( I_{Na} = g_{Na}(V_{in} - E_{Na}) \), we have

\[
E_{rev} = \frac{g_{Na}E_{Na} + g_{K}E_{K}}{g_{Na} + g_{K}} = \frac{\frac{g_{Na}E_{Na}}{g_{Na} + g_{K}} + \frac{g_{K}E_{K}}{g_{Na} + g_{K}}}{\frac{1}{g_{Na} + g_{K}} + \frac{1}{g_{Na}} + \frac{1}{g_{K}}}
\]

Figure 3-18 ACh opens a nonselective cation channel on the muscle membrane. (A) Schematic of how ACh release causes depolarization of the muscle membrane. At rest (left), the membrane potential of the muscle cell is around -75 mV, similar to the resting membrane potential of many neurons, with higher K⁺ concentration inside the cell and higher Na⁺ concentration outside (see Figure 2-12A). ACh binding opens a cation channel on the muscle membrane permeable to both Na⁺ and K⁺. This allows more Na⁺ influx than K⁺ efflux because of the larger driving force on Na⁺, thus depolarizing the muscle membrane. (B) An electrical circuit model. The left part represents the resting muscle membrane, which includes a membrane capacitance branch (C_m) and a membrane resistance branch (R_m) with a battery representing the resting potential (E_r) (see Sections 2.7 and 2.8). The right part represents the ACh-induced current, with a K⁺ path and a Na⁺ path (with a resistance of 1/g_K and 1/R_m, respectively) in parallel. After the switch is turned on (green arrow) by ACh release, the current that passes through the Na⁺ path is much larger than the current that passes through the K⁺ path because the driving force for Na⁺ is much greater than the driving force for K⁺, where E_K is the equilibrium potential for K⁺, and E_Na is the membrane potential (see Section 2.5) is far greater than the driving force for K⁺ (= E_K - E_m).
We can see from the above formula that if the conductance for Na⁺ and K⁺ were equal \( (g_{Na}/g_K = 1) \), \( E_{rev} \) would simply be an average of \( E_{Na} \) and \( E_K \). Suppose the ionic concentrations across the muscle membrane are the same as our model neuron in Figure 2-12A, with \( E_K = -85 \text{ mV} \) and \( E_{Na} = +58 \text{ mV} \), then \( E_{rev} \) should be \(-13.5\text{ mV} \). However, since \( E_{rev} = 0 \text{ mV} \) as determined in Figure 3-17C, we can calculate that \( g_{Na}/g_K \) is approximately 1.5; in other words, the channel that is opened upon ACh binding has higher conductance for Na⁺ than for K⁺.

### 3.13 The skeletal muscle acetylcholine receptor is a ligand-gated ion channel

A deeper understanding of the nature of the ACh-induced conductance change requires the identification of the postsynaptic acetylcholine receptor (AChR) and the ion channel whose conductance is coupled to ACh binding. Further studies indicated that muscle AChR is itself the ion channel. Just as the neuromuscular junction served as a model synapse because of its experimental accessibility, the AChR became a model neurotransmitter receptor because of its abundance, particularly in the electric organ of the Torpedo ray, which is highly enriched for an AChR similar to that from the skeletal muscle. Biochemical purification and subsequent cloning of the Torpedo AChR revealed that it consists of five subunits: two \( \alpha \), one \( \beta \), one \( \gamma \), and one \( \delta \) (Figure 3-19A). Each AChR contains two ACh binding sites, which are respectively located at the \( \alpha-\gamma \) and \( \alpha-\delta \) subunit interfaces. Both sites need to bind ACh in order for the channel to open. Evidence in support of this heteropentameric receptor as the ACh-activated channel came from a reconstitution experiment in the Xenopus oocyte. Co-injection of mRNAs encoding all four AChR subunits into the frog oocyte caused the oocyte, which normally does not respond to ACh, to produce an inward current in response to ACh iontophoresis in voltage clamp experiments. This ACh-induced inward current was reversibly blocked by the AChR antagonist (agent that acts to counter the action of an endogenous molecule) curare (see Box 3-2 for more details on curare); washing out the curare restored the inward current (Figure 3-19B). Omitting mRNA for any of the AChR subunits abolished the ACh-induced inward current in the oocyte expression system.

The three-dimensional structure of the Torpedo AChR has been determined by high-resolution electron microscopy (Figure 3-20). All AChR subunits contain four transmembrane helices, with the M2 helices from all subunits lining the ion conduction pore. The transmembrane helices form a hydrophobic barrier or 'gate' when AChR is closed, preventing ion flow. ACh binding induces the rotation of the \( \alpha \) subunits, which causes an alternative conformation of the M2 helices and opens the gate to allow the passage of cations.

To summarize synaptic transmission at the vertebrate neuromuscular junction: action potentials trigger ACh release from motor axon terminals. ACh molecules diffuse across the synaptic cleft and bind to postsynaptic AChRs, which are highly concentrated on the muscle membrane directly apposing the motor axon terminal. Upon ACh binding, muscle AChRs produce a nonselective cation

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**Figure 3-19 Composition of the acetylcholine receptor (AChR).** (A) Schematic illustrating the subunit composition of AChR. The two ACh binding sites are at the \( \alpha-\gamma \) and \( \alpha-\delta \) subunit interfaces. (B) Functional expression of AChR was achieved by injecting mRNAs encoding the four AChR subunits into Xenopus oocytes. Top traces, current used for iontophoresis of ACh. Bottom traces, inward current measured in a voltage clamp setup in response to ACh application: ACh application led to an inward current (left), which was blocked by curare, an AChR inhibitor (middle), but was reversed after curare was washed out (right). The membrane potential was held at \(-60\text{ mV} \). (B, adapted from Mishina M, Kurosaki T, Tobimatsu T et al. [1984] Nature 307:604–605. With permission from Macmillan Publishers Ltd.)
How do neurotransmitters act on postsynaptic neurons?

3.14 Neurotransmitter receptors are ionotrophic or metabotropic

Following the pioneering work on vertebrate skeletal muscle AChRs, receptors for several other neurotransmitters were found to be ion channels. All neurotransmitter-gated ion channels in vertebrates belong to one of three subfamilies. GABA-, glycine-, and serotonin-gated ion channels are in the same subfamily as muscle AChRs (Figure 3-21, left), with five subunits each possessing four transmembrane segments. Glutamate-gated ion channels constitute a second subfamily, with four subunits each possessing three transmembrane segments (Figure 3-21, middle). Finally, ATP can be used as a neurotransmitter in some neurons, and ATP-gated ion channels are trimers each having just two transmembrane segments (Figure 3-21, right).

Neurotransmitter receptors that function as ion channels, which allow rapid communication across the synapse, are also called **ionotropic receptors** (Figure 3-22A). For example, the direct gating of the muscle AChR channel by ACh transmits electrical signals from presynaptic neuron to postsynaptic muscle within a few milliseconds (see Figure 3-1C; Figure 3-17B). Ionotropic receptors are mostly synonymous with the ligand-gated ion channels introduced in the previous section. Both terms encompass receptors that are gated by ligands...
Figure 3-21 Three families of ionotropic receptors in vertebrates. Left, like the ionotropic AChR (see Figure 3-20), each subunit of the ionotropic GABA receptor, glycine receptor, and serotonin receptor spans the membrane four times. Five subunits constitute a functional receptor with two neurotransmitter-binding sites (stars). Middle, an ionotropic glutamate receptor has four subunits and four neurotransmitter-binding sites; each subunit spans the membrane three times. Right, an ionotropic P2X receptor consists of three subunits, each of which features an ATP-binding site and spans the membrane twice. (Hille [2001] Ion Channels of Excitable Membranes. With permission from Sinauer.)

(neurotransmitters) and conduct ion passage across the membrane; the choice of which term to use depends on whether the properties of the receptor or the channel are being emphasized.

In contrast to the fast-acting ionotropic receptors, metabotropic receptors (Figure 3-22B), when activated by neurotransmitter binding, trigger intracellular signaling cascades to regulate ion channel conductance, and thus modulate membrane potential indirectly. (The intracellular signaling molecules are often referred to as ‘second messengers,’ as opposed to the ‘first messengers,’ the extracellular ligands.) Accordingly, they operate over a longer timescale ranging from tens of milliseconds to seconds. In addition, unlike ionotropic receptors, which are mostly concentrated in the postsynaptic density across the synaptic cleft from the presynaptic active zone, metabotropic receptors are typically not concentrated at the postsynaptic membrane apposing the synaptic presynaptic active zone, and therefore are termed “extrasynaptic.”

Many neurotransmitters have both ionotropic and metabotropic receptors (Table 3-3). For example, ACh can act on metabotropic receptors in addition to the ionotropic AChR we just studied. To distinguish between the two receptor

Figure 3-22 Ionotropic and metabotropic neurotransmitter receptors. (A) Ionotropic receptors are ion channels that are gated by neurotransmitters. Neurotransmitter binding causes membrane potential change within a few milliseconds. (B) Metabotropic receptors act through intracellular second messenger systems to regulate ion channel conductance. Neurotransmitter binding causes membrane potential change in tens of milliseconds to seconds.
types, we refer to them according to their specific agonists (agents that mimic the action of an endogenous molecule such as a neurotransmitter). Hence, ionotropic AChRs are called nicotinic AChRs because they are potentially activated by nicotine. Nicotinic AChRs are expressed not only in muscles, but also in many neurons in the brain, where nicotine acts as an addictive stimulant. Metabotropic AChRs are called muscarinic AChRs because they are activated by muscarine, a compound enriched in certain mushrooms.

In the following sections, we highlight the actions of key ionotropic and metabotropic receptors for major neurotransmitters in the CNS (Table 3-3).

### 3.15 AMPA and NMDA glutamate receptors are activated by glutamate under different conditions

Ionotropic glutamate receptors are responsible for the fast action of glutamate, the major excitatory neurotransmitter in the vertebrate CNS. Indeed, glutamatergic excitative synapses account for the vast majority of synapses in the vertebrate CNS: virtually all neurons—whether they are excitatory, inhibitory, or modulatory—express ionotropic glutamate receptors and are excited by glutamate.

Similar to muscle AChR, ionotropic glutamate receptors are cation channels that do not select between Na⁺ and K⁺, with a reversal potential near 0 mV. Under physiological conditions, glutamate binding to ionotropic glutamate receptors produces an inward current called the excitatory postsynaptic current (EPSC) (Figure 3-23, top), as more positively charged ions flow into the cell than out of it. This is analogous to the end-plate current we saw at the neuromuscular junction (see Figure 3-17). The inward current produces a transient depolarization in the postsynaptic neuron called the excitatory postsynaptic potential (EPSP) (Figure 3-23, bottom), analogous to the EPP at the neuromuscular junction. The recordings shown in Figure 3-23 were made in acutely prepared brain slices (fresh sections of brain tissue about a few hundred micrometers thick) that preserve local three-dimensional architecture and neuronal connections while allowing experimental access, such as whole-cell patch recording of individual neurons and control of extracellular media.

Historically, ionotropic glutamate receptors have been divided into three subtypes that are named for their selective responses to three agonists: AMPA

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**Table 3-3: Ionotropic and metabotropic neurotransmitter receptors encoded by the human genome**

<table>
<thead>
<tr>
<th>Neurontransmitter</th>
<th>Ionotropic</th>
<th>Metabotropic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td><strong>Number of genes</strong></td>
<td><strong>Name</strong></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>16</td>
<td>muscarinic ACh receptor</td>
</tr>
<tr>
<td>Glutamate</td>
<td>NMDA receptor</td>
<td>7</td>
</tr>
<tr>
<td>AMPA receptor</td>
<td>4</td>
<td>others</td>
</tr>
<tr>
<td>GABA</td>
<td>GABA&lt;sub&gt;A&lt;/sub&gt; receptor</td>
<td>19</td>
</tr>
<tr>
<td>Glycine</td>
<td>glycine receptor</td>
<td>5</td>
</tr>
<tr>
<td>ATP</td>
<td>P2X receptor</td>
<td>7</td>
</tr>
<tr>
<td>Serotonin (5-HT)</td>
<td>5-HT&lt;sub&gt;2&lt;/sub&gt; receptor</td>
<td>5</td>
</tr>
<tr>
<td>Dopamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine (epinephrine)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropeptides</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: GABA, γ-aminobutyric acid; P2X receptor, ATP-gated ionotropic receptor; P2Y, ATP-gated metabotropic receptor; 5-HT<sub>1</sub>, serotonergic (5-hydroxytryptamine) receptor subtype 1; ACh, acetylcholine; NMDA, N-methyl-D-aspartate; AMPA, 2-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid.

Data from the IUPHAR (International Union of Basic and Clinical Pharmacology) database (www.iuphar-db.org).
The NMDA receptor conductance is nearly constant between -60 mV and +60 mV as indicated by the near linear slope of the I-V curve. This indicates that the NMDA receptor permeability to Na⁺ is not affected by the membrane potential, as can be seen by the near linear I-V curve. In contrast, AMPA receptor opening causes a net influx of positively charged ions, resulting in depolarization of postsynaptic neurons. AMPA receptors are the fast glutamate-gated ion channels that conduct Na⁺ and K⁺. Depending on the subunit composition, some AMPA receptors are also permeable to Ca²⁺ in addition to Na⁺ and K⁺. They mediate synaptic transmission at the glutamatergic synapses when the postsynaptic neuron is near the resting potential because the driving force of Na⁺ is much greater than that of K⁺ near the resting potential. AMPA receptor opening causes a net influx of positively charged ions, resulting in depolarization of postsynaptic neurons.

NMDA receptors have the unusual property of not only being gated by glutamate but also being influenced by the membrane potential; they also require glycine as a co-agonist. The mechanism by which membrane potential affects NMDA receptor conductance is different from the voltage-gated Na⁺ and K⁺ channels discussed in Chapter 2. At the extracellular face of the membrane, the mouth of the NMDA receptor is blocked by Mg²⁺ at negative potentials, such that the channel remains closed despite glutamate binding. However, depolarization of the postsynaptic membrane relieves the Mg²⁺ block. In the absence of external Mg²⁺, the NMDA receptor conductance is not affected by the membrane potential, as can be seen by the near linear I-V curve. However, the NMDA receptor acts as a coincidence detector, and opens only in response to concurrent presynaptic glutamate release and postsynaptic depolarization. This property is very important in synaptic plasticity and learning as well as activity-dependent wiring of the nervous system, subjects we will return to in Chapters 5 and 10. Once opened, the NMDA receptors have high Ca²⁺ conductance. While AMPA receptors provide initial depolarization to release the Mg²⁺ block of nearby NMDA receptors—these two glutamate receptors are often co-expressed in the same postsynaptic site—NMDA receptors contribute additional depolarization alongside AMPA receptors. Importantly, Ca²⁺ influx via NMDA

**Figure 3-24** Properties of AMPA and NMDA subtypes of ionotropic glutamate receptors. (A) When the postsynaptic neuron (represented by a dendritic spine) is near the resting potential, glutamate (GLU) released from the presynaptic neuron opens only the AMPA receptor channel (AMPAR), causing Na⁺ entry and producing excitatory postsynaptic potentials (EPSPs). The NMDA receptor channel (NMDAR) is blocked by external Mg²⁺ and therefore cannot be opened by glutamate binding alone. (B) When the postsynaptic neuron is depolarized, the Mg²⁺ block is relieved. Both NMDAR and AMPAR can now be opened by glutamate binding. The NMDAR is highly permeable to Ca²⁺. For simplicity, the smaller K⁺ efflux through open AMPAR and NMDAR channels is omitted. (Adapted from Cowan WM, Sudhof TC & Stevens CF [2001] Synapses. Johns Hopkins University Press.)

**Figure 3-25** Current-voltage relationship of the NMDA receptor in the presence or absence of external Mg²⁺. Blue curve, in Mg²⁺-free media, the conductance of the NMDA receptor is nearly constant between -60 mV and +60 mV as indicated by the near linear slope of the I-V curve. This indicates that the NMDA receptor permeability to Na⁺ is not affected by voltage, similar to the nicotinic acetylcholine receptor (see Figure 3-17C). Red curve, the presence of physiological concentrations of extracellular Mg²⁺ markedly diminishes the inward current at negative membrane potentials, because Mg²⁺ blocks cation influx. Data were obtained using whole-cell patch recording of cultured mouse embryonic neurons. (Adapted from Nowak L, Bregerovski P & Ascher P [1984] Nature 307:462-465. With permission from Macmillan Publishers Ltd.)

(2-amino-3-hydroxy-5-methylisoxazol-4-propanoic acid), kainate (kainic acid), and NMDA (N-methyl-D-aspartate). Molecular cloning of these receptors revealed that they are encoded by distinct gene subfamilies of ionotropic glutamate receptors (see Table 3-3). Because the properties of AMPA and kainate receptors are more similar to each other, they are collectively called non-NMDA receptors. In contrast, NMDA receptors have distinctive properties. Below we use the AMPA and NMDA receptors to illustrate these differences.

AMPA receptors are the fast glutamate-gated ion channels that conduct Na⁺ and K⁺. Depending on the subunit composition, some AMPA receptors are also permeable to Ca²⁺ in addition to Na⁺ and K⁺ (see below). They mediate synaptic transmission at the glutamatergic synapses when the postsynaptic neuron is near the resting potential. Because the driving force of Na⁺ is much greater than that of K⁺ near the resting potential, AMPA receptor opening causes a net influx of positively charged ions, resulting in depolarization of postsynaptic neurons (Figure 3-24A).
receptors contributes to many biochemical changes in postsynaptic cells, as will be discussed later in the chapter.

Structural studies have revealed how glutamate receptor subunits are arranged and how ligand binding might trigger channel opening. All ionotropic glutamate receptors are composed of four subunits (see Figure 3–21). Each subunit consists of several modular domains (Figure 3–26): an amino terminal domain, a ligand-binding domain, a transmembrane domain that comprises three membrane-spanning helices (M1, M3, and M4) plus an additional pore loop (M2), and a carboxy-terminal intracellular domain. AMPA receptors can form functional homo-tetramers (composed of four identical subunits) although they are usually found in vivo as hetero-tetramers of two or more of the four variants, GluA1, GluA2, GluA3, and GluA4. Crystal structures of tetramers composed of GluA2 suggest that glutamate binding results in a large conformational change in the ligand-binding domain, which causes a corresponding conformational change in the adjacent transmembrane domain to open the ion conductance pore. NMDA receptors are obligatory hetero-tetramers composed of two GluN1 (also called NR1) subunits, each with a binding site for the co-agonist glycine, and two GluN2 (also called NR2) subunits, each with a glutamate-binding site. GluN1 is encoded by a single gene, whereas GluN2 has four variants, GluN2A, GluN2B, GluN2C, and GluN2D, encoded by four separate genes.

The subunit composition of both AMPA and NMDA receptors has important functional consequences. For example, most AMPA receptors contain the GluA2 subunit; most GluA2-containing AMPA receptors are impermeable to Ca\(^{2+}\) due to a post-transcriptional modification called RNA editing, which changes the mRNA sequence encoding a key residue in GluA2’s channel pore. AMPA receptors that lack GluA2 or contain unedited GluA2 subunits are permeable to Ca\(^{2+}\) (though not as permeable as are the NMDA receptors). AMPA receptors that lack GluA2 are also susceptible to a voltage-dependent block by intracellular polyamines, preventing Na\(^+\) influx when the neuron becomes more depolarized. These AMPA receptors are thus inward-rectifiers analogous to the inward-rectifier K\(^+\) channels we discussed in Box 2–4. NMDA receptors containing different GluN2 variants also have distinct channel conductances and cytoplasmic signaling properties, and bind differentially to postsynaptic scaffolding proteins (see the next section). Combinations of different subunits thus offer both AMPA and NMDA receptors a rich repertoire of functional and regulatory properties. Indeed, the subunit compositions of AMPA and NMDA receptors differ in different types of neurons, undergo developmental changes in the same types of neurons, and can be regulated by synaptic activity.

### 3.16 The postsynaptic density is organized by scaffolding proteins

Just as the presynaptic terminal is highly organized by active-zone scaffold proteins (see Section 3.7), the postsynaptic density is highly organized by postsynaptic proteins. At the glutamatergic synapses, for example, the postsynaptic density consists of not only glutamate receptors but also a large number of associated proteins (Figure 3–27). These include (1) trans-synaptic adhesion proteins that align active zones with postsynaptic densities (see also Section 3.7), (2) proteins that participate in signal transduction cascades, and (3) a diverse array of scaffolding proteins that connect the glutamate receptors and trans-synaptic adhesion molecules to signaling molecules and cytoskeletal elements. The resulting protein network controls glutamate receptor localization, density, trafficking, and signaling, all of which affect synaptic transmission and synaptic plasticity. Scaffolding proteins are also present in GABAergic postsynaptic terminals, utilizing scaffolding proteins that only partially overlap with those found in glutamatergic synapses. We will learn more about the postsynaptic density protein network in the context of development and synaptic plasticity in Chapters 7 and 10, and how their dysfunction contributes to brain disorders in Chapter 11.
The organization of postsynaptic density at the glutamatergic synapse. At the cell surface, the postsynaptic density of a mature glutamatergic synapse is enriched in AMPA and NMDA glutamate receptors (AMPAR and NMDAR), as well as trans-synaptic cell adhesion molecules such as cadherins and neuroligins (which respectively bind to presynaptic cadherins and neurexins; see Figure 3-10). Named for their localization to the postsynaptic density and their molecular weight, the scaffolding proteins of the PSD-95 family bind to many proteins, including the GluN2 subunit of the NMDAR, the AMPAR-associated TARPs (transmembrane AMPAR regulatory proteins), the synaptic adhesion molecule neuroligins, the signal-transducing enzyme CaMKII, and other scaffolding proteins that bind to metabotropic glutamate receptors (mGluR) and other postsynaptic density proteins (not shown). The diagram only depicts a subset of known components and interactions in the postsynaptic density. (Adapted from Sheng M & Kim E [2011] Cold Spring Harb Perspect Biol 3:a005678.)

We use one of the most abundant scaffolding proteins at the glutamatergic synapse, PSD-95 (postsynaptic density protein-95 kDa), to illustrate the organizational role of scaffolding proteins in the dendritic spine where glutamatergic synapses are usually located (Figure 3-27). PSD-95 contains multiple protein-protein interaction domains, including three PDZ domains, which bind to C-terminal peptides with a specific sequence motif that occurs in many transmembrane receptors. (PDZ is an acronym for three proteins that share this domain: PSD-95 originally identified from biochemical analysis of the postsynaptic density; Discs-large in Drosophila that regulates cell proliferation and is also associated with the postsynaptic density; and ZO-1, an epithelial tight junction protein.) These protein-protein interaction domains enable PSD-95 to bind directly to the GluN2 subunit of the NMDA receptor, a family of AMPA receptor-associated proteins called TARPs (transmembrane AMPA receptor regulatory proteins), the trans-synaptic adhesion molecule neuroligin, and Ca2+/calmodulin-dependent protein kinase II (CaMKII, an enzyme highly enriched in postsynaptic densities, whose role in signal transduction will be introduced in Section 3.20). PSD-95 also binds other PDZ-domain-containing scaffolding proteins that in turn associate with other postsynaptic components such as metabotropic glutamate receptors and the actin cytoskeleton. Thus, the scaffold protein network stabilizes neurotransmitter receptors at the synaptic cleft by placing them close to the trans-synaptic adhesion complex apposing the active zone (see Figure 3-10), brings enzymes (for example, CaMKII) close to their upstream activators (for example, Ca2+ entry through the NMDA receptor) and downstream substrates, and organizes the structure of the dendritic spine by bridging the trans-synaptic adhesion complex and the underlying actin cytoskeleton.

3.17 Ionotropic GABA and glycine receptors are Cl− channels that mediate inhibition

The role of inhibition in nervous system function was first established in the study of spinal cord reflex over a century ago (see Section 1.9). In the 1950s, when intracellular recording techniques were applied to the study of spinal motor neurons, it was found that stimulating their inhibitory input axons resulted in a rapid
membrane potential change due to current flow across the motor neuron membrane. These current and membrane potential changes are called the inhibitory postsynaptic current (IPSC) and inhibitory postsynaptic potential (IPSP), respectively. In a revealing experiment (Figure 3–28), the membrane potential of the motor neuron was set by the experimenter at different initial values by injecting currents through an electrode, while the membrane potential was measured by a second electrode in response to stimulation of its inhibitory input axons. It was found that when the initial membrane potential was equal to or more depolarized than the resting potential of −70 mV or so, stimulation of the inhibitory input caused hyperpolarization, whereas when the initial membrane potential was set more hyperpolarized than −80 mV, stimulation of the inhibitory input produced depolarization. The reversal potential, around −80 mV, is close to the equilibrium potential for Cl⁻ ($E_{Cl}$), suggesting that the IPSC is carried by Cl⁻ flow. Indeed, by increasing intracellular Cl⁻ concentration, the reversal potential became less negative following the change of $E_{Cl}$ as predicted from the Nernst equation. This experiment suggested that inhibition of the spinal motor neuron is mediated by an increase of Cl⁻ conductance across the motor neuron membrane.

Subsequent studies have shown that the fast inhibitory action is mediated by the neurotransmitters glycine (used by a subset of inhibitory neurons in the spinal cord and brainstem) and GABA (used by most inhibitory neurons), which act on ionotropic glycine receptors or GABA$_A$ receptors, respectively. The structure of GABA$_A$ receptors is similar to that of the nicotinic AChRs (see Figure 3–20), consisting of a pentamer with two α subunits, two β subunits, and one γ subunit. Each subunit has multiple isoforms encoded by several genes (see Table 3–3), and other subunits such as δ and ε can be used in lieu of γ. Many pharmaceutical drugs act on GABA$_A$ receptors to modulate inhibition in the brain. As we will learn in Chapter 11, the most widely used anti-epilepsy, anti-anxiety, and sleep-promoting drugs bind to and enhance the functions of GABA$_A$ receptors. Glycine receptors are also composed of a pentamer with two α subunits. Both GABA$_A$ and glycine receptors are ligand-gated ion channels that are selective for anions, primarily Cl⁻.

How does an increase of Cl⁻ conductance that results from the opening of GABA$_A$ (or glycine) receptor channels on postsynaptic neurons cause inhibition? In most neurons, $E_{Cl}$ is slightly more hyperpolarized than the resting potential as in the case of the spinal motor neuron we just studied. Thus, an increase of Cl⁻ conductance causes Cl⁻ influx (which is equivalent to an outward current because Cl⁻ carries a negative charge), resulting in a small hyperpolarization (Figure 3–29A, left panel). Importantly, if the neuron also receives simultaneously an excitatory input (for example, opening of glutamate receptor channels), which produces an EPSP, the relatively depolarized potential increases the driving force for Cl⁻ influx. This increases the outward current triggered by GABA, which counts the EPSP-producing inward current, making it more difficult for the cell’s membrane potential to reach the threshold for firing action potentials (Figure 3–29A, middle and right panels).

The interaction of excitatory and inhibitory input can also be seen in an electrical circuit model, where each is represented by a branch consisting of a switch (representing neurotransmitter release), a conductance ($g_e$ or $g_i$ representing EPSC or IPSC conductance), and a battery (representing the reversal potential for the excitatory glutamate receptors, $E_{rev}^{e}$ or the GABA$_A$ receptor, which equals $E_{Cl}$). When only the inhibitory input is switched on, because $E_{Cl}$ is more hyperpolarized than the resting potential ($E_r$), a small outward current is produced from the $g_e$ branch, resulting in a small hyperpolarizing IPSP (Figure 3–29B, left). When only the excitatory input is switched on, a large inward current is produced from the $g_e$ branch because $E_{rev}^{e}$ is much more depolarized than $E_r$, resulting in a large depolarizing EPSP (Figure 3–29B, middle). When both the excitatory and the inhibitory inputs are switched on, part of the inward current in the $g_e$ branch flows outward through the $g_i$ branch (Figure 3–29B, right), leading to a smaller depolarization effect than when the $g_e$ branch is active alone. Indeed, as can be seen from the circuit model, even when $E_{Cl}$ equals $E_r$, which means that there is no net influx or efflux of Cl⁻ at rest, GABA$_A$ receptor opening creates an

![Figure 3-29](https://example.com/figure329.png)

**Figure 3-29 Inhibitory postsynaptic potentials (IPSPs).** Top, experimental setup. Two electrodes were inserted into a spinal motor neuron, one for passing current to change the holding membrane potential, and the other to measure the membrane potential in response to electrical stimulation of the inhibitory input. Bottom, IPSPs recorded at four different holding membrane potentials. Each record represents the superposition of about 40 traces. At the membrane potentials of −74 mV or above, stimulation of inhibitory input resulted in hyperpolarizing IPSPs, with increasing amplitudes as the membrane potentials became less negative. At the membrane potentials of −82 mV or below, stimulation of inhibitory input resulted in depolarizing IPSPs, with increasing amplitudes as the membrane potentials became more negative. (Graphs adapted from Coombs JS, Eccles JC & Fatt P [1955] J Physiol 130:326–373.)
**Figure 3-29** The inhibitory effect of Cl⁻ conductance mediated by GABA<sub>A</sub> receptor. (A) In this neuron, the Cl⁻ equilibrium potential, \(E_{Cl}\), is slightly more hyperpolarized than the resting potential. Left, IPSP from GABA<sub>A</sub> receptors causes hyperpolarization of this postsynaptic neuron toward \(E_{Cl}\). Middle, EPSP from glutamate receptors causes depolarization of the postsynaptic neuron, as the reversal potential at \(-0 \text{ mV}\) is far above the resting potential. If the amplitude of EPSP exceeds the threshold, it produces an action potential. Right, IPSP can cancel the effect of EPSP when both excitatory and inhibitory inputs are present in the same neuron, thus preventing the postsynaptic neuron from firing. (B) Circuit models for the three situations in panel A. To the resting neuronal model represented by the membrane capacitance \((C_m)\), resistance \((R_m)\) and resting potential \((E_r)\), two additional branches are added, which represent the inhibitory and excitatory neurotransmitter receptors with conductance of \(g_i\) and \(g_e\) when neurotransmitter binding opens the receptor channels. Left, when only the inhibitory branch is switched on (GABA release activating GABA<sub>A</sub> receptors), a small outward current results (vertical upward arrow) because \(E_{Cl}\) is more hyperpolarized than \(E_r\). This causes more charges to build up at \(C_m\), thus hyperpolarizing the membrane potential \((V_m)\). Middle, when only the excitatory branch is switched on (glutamate release activating glutamate receptors); a large inward current results (vertical downward arrow) because the reversal potential for the excitatory ionotropic glutamate receptors \((E_{rev})\) is far more depolarized than \(E_r\). This causes discharge of \(C_m\) thus depolarizing the membrane potential. Right, when both the inhibitory and excitatory branches are switched on (GABA and glutamate are released at the same time), a small fraction of the inward current in the excitatory branch is diverted by the outward current in the inhibitory branch. As a result, the current to discharge \(C_m\) (dashed arrow) is smaller. (Note that the more depolarized \(V_m\) is, the larger the outward current is due to the larger driving force for \(Cl^-\)).

A noteworthy exception to GABA's inhibitory effects can occur in developing neurons. The intracellular Cl⁻ concentration is high in many developing neurons because their Cl⁻ exchangers (see Figure 2-12B) are not yet fully expressed. When the intracellular Cl⁻ concentration is sufficiently elevated, \(E_{Cl}\) is substantially more depolarized than the resting potential so that an increase of Cl⁻ conductance results in Cl⁻ efflux, causing depolarization that can exceed the threshold for action potential generation. Under these circumstances, GABA can act as an excitatory neurotransmitter.

As we will learn soon, another inhibitory action of GABA is mediated by metabotropic GABA<sub>B</sub> receptors, which usually act through intracellular signaling pathways to cause the opening of K⁺ channels. Because \(E_K\) is always more negative than the resting potential, opening of K⁺ channels always causes hyperpolarization, making the neurons less likely to reach the threshold for an action potential in response to excitatory input. GABA<sub>B</sub> receptors are not only distinct from GABA<sub>A</sub> receptors in the channels that they open but as metabotropic receptors they are also distinct in their mode of action.
3.18 All metabotropic neurotransmitter receptors trigger G protein cascades

We now turn to metabotropic receptors, which act through intracellular signaling pathways rather than mediating ion conduction directly (see Figure 3–22B). These receptors, all of which belong to the G-protein-coupled receptor (GPCR) superfamily, participate in signaling cascades that involve a heterotrimeric guanine nucleotide-binding protein (trimeric GTP-binding protein, or simply G protein). ACh, glutamate, and GABA all bind to their own metabotropic receptors: muscarinic AChRs, metabotropic GluRs (mGluRs), and GABA<sub>B</sub> receptors, respectively, each with several variants. Additional GPCRs include the receptors for dopamine, norepinephrine, serotonin (most subtypes), ATP (P2Y subtypes), adenosine, and all neuropeptides (see Table 3–3), as well as the sensory receptors for vision, taste, and olfaction that we will study in Chapters 4 and 6. Indeed, GPCRs constitute the largest gene family in mammals that encompass receptors of diverse functions (Figure 3–30). GPCRs are crucial for neuronal communication, for responding to external stimuli, and for regulating many other physiological processes. Many pharmaceutical drugs currently in use target GPCRs, demonstrating their importance to human physiology and health.

All GPCRs share a common structure with seven transmembrane helices (Figure 3–31A). Almost all GPCRs are activated by binding of specific extracellular ligands. (The notable exception is rhodopsin in photoreceptors, which is activated by light absorption, as will be discussed in greater detail in Chapter 4.) Ligand binding triggers conformational changes in the transmembrane helices and allows the cytoplasmic domain to associate with a trimeric G protein complex consisting of three different subunits: Ga, Gβ, and Gγ (Figure 3–31B).

Prior to GPCR activation, the G protein heterotrimer preassembles and binds GDP via the Ga nucleotide-binding site (Figure 3–31C, Resting state). Because Ga and Gγ are both lipid-modified, this ternary complex associates with the plasma membrane. Ligand activation of the GPCR triggers the binding of its cytoplasmic domain to Ga. This stabilizes a nucleotide-free conformation of Ga and thereby catalyzes the replacement of GDP with GTP (Figure 3–31C, Steps 1 and 2). Next, GTP binding causes Ga to dissociate from Gβγ. Depending on the cellular context, Ga-GTP, Gβγ, or both can trigger downstream signaling cascades (Figure 3–31C, Step 3). Ga not only binds to GDP and GTP but also carries an intrinsic GTPase activity that hydrolyzes GTP to GDP. This GTPase activity provides a built-in termination mechanism for G protein signaling (Figure 3–31C, Step 4), and is often facilitated by additional proteins (see Box 3–3). GDP-bound Ga has a strong affinity for Gβγ that promotes the reassembly of the ternary complex; this return to the resting state (Figure 3–31C, Step 5) readies the trimeric G protein for the next
3.19 A GPCR signaling paradigm: \(\beta\)-adrenergic receptors activate cAMP as a second messenger

\(\beta\)-Adrenergic receptors (see Figure 3-31A, B) are the most extensively studied ligand-activated GPCRs. They are activated by epinephrine and norepinephrine (also known as adrenaline and noradrenaline, from which the name of the receptors originates). Whereas norepinephrine is produced by neurons and acts as a neurotransmitter in both the CNS and the autonomic nervous system
Box 3–3: G proteins are molecular switches

The G protein cycle outlined in Figure 3–31C is a universal signaling mechanism of GPCRs, which are widely used in many biological contexts. Indeed, the switch between a GDP-bound form and a GTP-bound form defines the G protein superfamily, which includes not only trimeric G proteins but also small monomeric GTPases such as the Rab, Ras, and Rho families. These small GTPases resemble part of the Ga subunit of the trimeric G protein. Rab GTPases regulate different steps of intracellular vesicular trafficking (see Section 2.1); we have encountered a family member, Rab3, in the context of bridging the synaptic vesicle with the presynaptic active zone scaffolding proteins (see Figure 3–10). The Ras family of GTPases contains key signaling molecules involved in cell growth and differentiation. As will be discussed in Box 3–4, Ras GTPases play crucial roles in transducing signals from the cell surface to the nucleus. Rho GTPases are pivotal regulators of the cytoskeleton; we will study them in the context of nervous system wiring in Chapter 5.

All members of the G protein superfamily are molecular switches. For the trimeric G proteins as well as the Ras and Rho families of GTPases, the GDP-bound form is inactive and the GTP-bound form is active in downstream signaling. The transitions between the GTP-bound and GDP-bound forms are usually facilitated by two types of proteins: the guanine nucleotide exchange factors (GEFs), which switch GTPases on by catalyzing the exchange of GDP for GTP, and GTPase activating proteins (GAPs), which switch GTPases off by speeding up the endogenous GTPase activity, converting GTP to GDP (Figure 3–32; Movie 3–4). As will be discussed in Section 3.22, proper signal termination is an important aspect of signaling.

In the context of trimeric G protein signaling discussed in Section 3.18, ligand-activated GPCRs act as GEFs for the trimeric G proteins. By stabilizing the transition state of the nucleotide-free conformation of Ga (Figure 3–31B), GPCRs catalyze the exchange of GDP for GTP on Ga (Step 2 of Figure 3–31C). The reaction is driven in the direction of Ga–GTP production by the dissociation of Ga–GTP from the GPCR and from Gβγ. We will learn more about GPs and GAPs in GPCR signaling in the context of visual transduction in Chapter 4.

(see Section 3.11), epinephrine is produced primarily by chromaffin cells in the adrenal gland, circulates through the blood, and acts as a hormone to mediate systemic responses to extreme conditions, the so-called ‘flight, fight, and flight’ responses. (A small number of CNS neurons also produce epinephrine as a modulatory neurotransmitter.) Classic biochemical studies demonstrated that epinephrine activates β-adrenergic receptors to produce an intracellular second messenger called cyclic AMP (cAMP). cAMP is synthesized from ATP by the action of a membrane-associated enzyme called adenylate cyclase (Figure 3–33). In fact, studies of mechanisms by which β-adrenergic receptors activate adenylate cyclase, together with parallel investigations of the signal transduction pathways downstream from rhodopsin activation (to be discussed in Section 4.4), first led to the discovery that trimeric G proteins act as essential intermediates in GPCR signaling.

Originally identified as a second messenger in the context of epinephrine action, cAMP is a common downstream signal for many GPCRs. In Chapters 4 and 6, we will learn that cAMP and its cousin cyclic GMP (cGMP) can directly gate ion channels in visual and olfactory systems. However, the most widely used cAMP effector is the cAMP-dependent protein kinase (also called A-kinase, protein kinase A, or PKA). PKA is a serine/threonine kinase, which means that it adds phosphate onto specific serine or threonine residues of target proteins and...
Figure 3-33 Norepinephrine speeds up heart rate: GPCR signaling through cyclic AMP (cAMP) and protein kinase A (PKA). From left, norepinephrine binding to the β-adrenergic receptor activates $G_{\beta\gamma}$, a Ga variant, in the cardiac muscle cell. $G_{\beta\gamma}$GTP associates with and activates the membrane-bound adenylate cyclase (AC). AC catalyzes the production of cAMP from ATP; cAMP activates PKA. Each PKA consists of two regulatory (R) and two catalytic (C) subunits. Each regulatory subunit contains two cAMP binding sites, and is associated with the A-kinase anchoring protein (AKAP). When all four cAMP binding sites on the regulatory subunits are occupied, the catalytic subunits are released from the complex, become active (C*), and phosphorylate their substrates.

In the cardiac muscle cell, a key PKA substrate is a voltage-gated Ca$^{2+}$ channel. PKA phosphorylation of the Ca$^{2+}$ channel increases its open probability, facilitating Ca$^{2+}$ influx. A rise in intracellular Ca$^{2+}$ increases cardiac muscle contraction and heart rate. The pathway from $G_{\beta\gamma}$ to PKA activation is used in many other cellular contexts.

3.20 $\alpha$ and $\beta\gamma$ G protein subunits trigger diverse signaling pathways that alter membrane conductance

The human genome encodes twenty Ga, six Gb, and three Gy variants. Their different combinations give rise to a myriad of trimeric G proteins that are coupled to different GPCRs and can trigger diverse signaling pathways. For example, in addition to the $G_{\beta\gamma}$ we just discussed, a variant of Ga called $G_{i}$ (for inhibitory G protein) also binds to adenylate cyclase but inhibits its activity, resulting in a decrease of intracellular cAMP concentration. Different Ga variants are associated with different receptors and regulate distinct downstream signaling pathways. With regards to regulating postsynaptic neuronal function, the ultimate effectors are usually ion channels that regulate membrane potential or neurotransmitter release, most notably K$^{+}$ and Ca$^{2+}$ channels (see Box 2-4). In the previous section, we discussed a classic example of how norepinephrine activates a Ca$^{2+}$ channel via cAMP and PKA to increase cardiac muscle contraction. In the next two sections, we examine more examples to highlight the diverse outcomes of GPCR signaling.
How do neurotransmitters act on postsynaptic neurons?

ACh, glutamate, or serotonin.

**Figure 3-34 GPCR signaling through phospholipase C (PLC) and Ca²⁺.** From left, activation of metabotropic receptors in response to a variety of neurotransmitters (for example, ACh, glutamate, serotonin) activates Gq, a variant of Gα. Gq-GTP in turn activates PLC, which catalyzes the conversion from PIP₂ to DAG and IP₃. IP₃ activates the IP₃ receptor (IP₃R, an IP₃-gated Ca²⁺ channel) on the ER membrane, allowing Ca²⁺ to be released from ER to the cytosol. DAG and Ca²⁺ coactivate PKC. Ca²⁺ also binds to calmodulin (CaM), and the resulting complex activates CaMKII and other CaM kinases. Asterisks represent activated components.

An important G protein effector of many metabotropic receptors (for example, receptors for ACh, glutamate, serotonin) is a membrane-associated enzyme called **phospholipase C (PLC)** (Figure 3-34; Movie 3-6). PLC is activated by Gq, a Gα variant. Activated PLC cleaves a membrane-bound phospholipid called PIP₂ (phosphatidyl 4,5-bisphosphate) to produce two important second messengers: **diacylglycerol (DAG)** and **inositol 1,4,5-triphosphate (IP₃)**. DAG binds to and activates **protein kinase C (PKC)**, a serine/threonine kinase. PKC activation also requires a rise of intracellular Ca²⁺ concentration. This is achieved by IP₃, which binds to an IP₃-gated Ca²⁺ channel (the IP₃ receptor) on the membrane of the endoplasmic reticulum (ER) and triggers the release of ER-stored Ca²⁺ into the cytosol. In addition to activating PKC, Ca²⁺ interacts with many additional effectors. A key effector is a protein called **calmodulin**. The Ca²⁺/calmodulin complex can regulate diverse signaling pathways, including the activation of Ca²⁺/calmodulin-dependent protein kinases (CaM kinases), another important group of serine/threonine kinases. Like PKA, both PKC and CaM kinases phosphorylate many downstream target proteins, including ion channels and receptors, to modulate their activity. A specific subtype of CaM kinases, **CaM kinase II (CaMKII)**, is one of the most abundant proteins in the postsynaptic density (see Section 3.16). Ca²⁺ can also directly increase the open probability of Ca²⁺-dependent K⁺ channels (see Box 2-4). Thus, activation of PLC activates PKC and at the same time causes a rise of intracellular Ca²⁺ concentration, both of which can alter neuronal excitability (Figure 3-34).

Historically, Ga was identified first as the signaling intermediate between the GPCR and the effector. However, Gβγ can also mediate signaling. This was first shown in the case of ACh regulation of heartbeat. In fact, the concept of a chemical neurotransmitter was first established in this context in a classic experiment conducted in 1921 by Otto Loewi. It was known that stimulating the **vagus nerve**, a cranial nerve that connects the brainstem with internal organs, would slow the heartbeat. Loewi collected the fluid from a frog heart that had been stimulated by the vagus nerve, added it to an unstimulated heart, and found that beating of the second heart also was slowed. This experiment showed that vagus nerve stimulation released a chemical transmitter, identified afterwards as ACh, to slow the heartbeat. (According to Loewi, the initial idea of this experiment came from a dream in the middle of the night. He wrote it down on a piece of paper and went back to sleep. The next morning he remembered dreaming about something...
important but could not remember what it was or decipher what he had written. After spending a desperate day, he remembered what the dream was when he woke up in the middle of the next night. This time he got up immediately and went to the lab to perform the experiment.

Subsequent work has shown that ACh binds to a specific muscarinic AChR and triggers the dissociation of the trimeric G protein complex. \( \beta \gamma \) subunits then bind to and activate a class of \( K^+ \) channels called GIRKs (G-protein-coupled inward-rectifier \( K^+ \)) channels, resulting in \( K^+ \) efflux, hyperpolarization of cardiac muscle cells, and a slowing of the heartbeat (Figure 3-35).

We have seen that two different neurotransmitters, norepinephrine and ACh, act on different receptors, G proteins, and effectors to speed up or slow down the heart rate, respectively (compare Figures 3-33 and 3-35). In fact, the \( G_\alpha \) that is activated by the muscarinic AChR is a \( G_\text{i} \) variant, which inhibits adeny late cyclase and counteracts the effect of \( \beta \)-adrenergic receptors. These neurotransmitters are used in the two opposing branches of the autonomic nervous system. The sympathetic branch, which uses norepinephrine as a neurotransmitter, and the parasympathetic branch, which uses ACh as a neurotransmitter, usually have antagonistic functions (see Section 8.12 for more details).

**3.21 Metabotropic receptors can act on the presynaptic terminal to modulate neurotransmitter release**

In addition to acting on the dendrites and cell bodies, metabotropic receptors can also act on the presynaptic terminals of their postsynaptic target neurons to modulate neurotransmitter release. In the simplest form, neurons can use metabotropic receptors to modulate their own neurotransmitter release, as in the case of sympathetic neurons that release norepinephrine (Figure 3-36A). The presynaptic terminals of these neurons express \( \alpha \)-adrenergic receptors that can bind norepinephrine released into the synaptic cleft. Activation of these presynaptic \( \alpha \)-adrenergic receptors rapidly inhibits the voltage-gated \( Ca^{2+} \) channel at the active zone, which reduces the depolarization-induced \( Ca^{2+} \) entry that is essential for triggering neurotransmitter release. This negative feedback loop results in diminishing levels of neurotransmitter release, leading to presynaptic depression. As noted in Section 3.10, many such mechanisms of short-term plasticity act on the presynaptic cell by altering the probability of neurotransmitter release.

To provide further insight into the mechanism by which norepinephrine inhibits presynaptic \( Ca^{2+} \) channels, cell-attached patch clamp recordings were performed under different conditions. In the control condition where no norepinephrine was applied, a depolarizing voltage step induced an inward current through the presumed voltage-gated \( Ca^{2+} \) channels within the membrane patch (Figure 3-36B, left). When norepinephrine was included only in the patch pipette, depolarization-induced inward current was greatly reduced (Figure 3-36B, middle). When norepinephrine was applied in the media but not in the pipette, depolarization-induced inward current was similar to the control (Figure 3-36B, right).Because activation of \( \alpha \)-adrenergic receptors outside the patch did not inhibit
How do neurotransmitters act on postsynaptic neurons?

Figure 3–36 Local action of norepinephrine on a presynaptic Ca$^{2+}$ channel. (A) From left, released norepinephrine (NE, blue) binds and activates a presynaptic $\alpha$-adrenergic receptor ($\alpha$AR). Activated G$\beta$y binds and inhibits the presynaptic voltage-gated Ca$^{2+}$ channel, which reduces Ca$^{2+}$ influx and thereby inhibits neurotransmitter release. (B) A patch pipette was used to record the current flow across a patch of plasma membrane from a frog sympathetic neuron in culture in the cell-attached mode (see Figure 2–30A). A depolarizing voltage step (from −80 mV to −10 mV) was applied to activate voltage-gated Ca$^{2+}$ channels in the membrane patch underneath the electrode (Na$^{+}$ and K$^{+}$ channels were inhibited by blockers included in the pipette solution). Left, in the absence of norepinephrine (NE), the depolarization step induced the opening of the Ca$^{2+}$ channel within the patch, as seen by the inward current. Middle, when the patch pipette was filled with NE, the depolarization-induced Ca$^{2+}$ current was largely eliminated. Right, if NE was excluded from the patch pipette but was applied everywhere else, depolarization still induced the inward Ca$^{2+}$ current. Together, these experiments indicated that the action of norepinephrine on the Ca$^{2+}$ channel was confined to the patch of membrane underneath the pipette. (B, adapted from Lipscombe D, Kongsamut S & Tsien RW [1989] Nature 340:639–642. With permission from Macmillan Publishers Ltd.)

 voltage-gated Ca$^{2+}$ channels within the patch, these data argued against involvement of an intracellular second messenger that can diffuse in the cytoplasm, and for a mechanism by which $\alpha$-adrenergic receptors act locally on the voltage-gated Ca$^{2+}$ channels within the membrane patch. Indeed, subsequent experiments determined that this inhibition is mediated by direct binding of G$\beta$y to the Ca$^{2+}$ channel (Figure 3–36A), as in the case of ACh activation of GIRK.

A presynaptic terminal of a given neuron can also contain metabotropic receptors for neurotransmitters produced by other neurons. In this case, the presynaptic terminal of a neuron acts as the postsynaptic site for these other neurons (Figure 3–37). Depending on the nature of the neurotransmitter, the type of the receptor, the signaling pathway, and final effector, the net effect could either be facilitation or inhibition of neurotransmitter release. Accordingly, these effects are termed presynaptic facilitation or presynaptic inhibition. Presynaptic facilitation can be achieved by closing K$^{+}$ channels, which depolarizes the presynaptic membrane potential and makes it easier to activate voltage-gated Ca$^{2+}$ channels so that Ca$^{2+}$ entry can trigger neurotransmitter release; we will see an example of this in Chapter 10 where serotonin mediates presynaptic facilitation in the sea slug Aplysia to enhance the magnitude of a reflex to a noxious stimulus. Presynaptic inhibition can be achieved by opening K$^{+}$ channels or by closing voltage-gated Ca$^{2+}$ channels, both of which inhibit neurotransmitter release. For example, in Chapter 6, we will learn that Drosophila olfactory receptor neurons (ORNs) activate GABAergic local interneurons, which synapse onto ORN axon terminals to provide negative feedback control of ORN neurotransmitter release through the GABA$_{A}$ receptors. (Presynaptic inhibition can also be achieved through GABA acting on ionotropic GABA$_{A}$ receptors that are present on the presynaptic terminals.
of some neurons.) Presynaptic facilitation and inhibition are also widely used in the vertebrate nervous systems.

3.22 GPCR signaling features multiple mechanisms of signal amplification and termination

As we have seen in previous sections, metabotropic neurotransmitter receptors have diverse functions that depend on their locations and their coupling to different G proteins, signaling pathways, and effectors. Their effects unfold more slowly than the rapid ion conduction of ionotropic receptors. However, second messenger systems contribute an important property: signal amplification. For example, activation of a single adrenergic receptor can trigger multiple rounds of G protein activation; each activated adenylate cyclase can produce many cAMP molecules; and each activated PKA can phosphorylate many substrate molecules.

Signals need to be properly terminated in order for cells to respond to future stimuli. Indeed, all signaling events we have discussed so far are associated with built-in termination mechanisms. For example, the GPCR is deactivated when its ligand dissociates; Go-GTP is deactivated by its intrinsic GTPase activity, often facilitated by GTPase-activating proteins (GAPs); Gßy is deactivated by re-association with Go-GDP; adenylate cyclase is deactivated in the absence of Go-GTP; the cAMP produced by adenylate cyclase is metabolized into AMP by an enzyme called phosphodiesterase; the catalytic subunits of PKA re-associate with regulatory subunits and become inactive when cAMP concentration declines; and protein phosphatases remove phosphates from phosphorylated proteins, thus counteracting the actions of kinases. While some of these termination mechanisms are constitutive, others are regulated by signals.

Signal amplification and termination apply generally to signal transduction pathways (Box 3-4). In Chapter 4, we will see a salient example of signal amplification and termination in the context of studying how photons are converted to electrical signals in vision.

3.23 Postsynaptic depolarization can induce new gene expression

In addition to changing the membrane potentials and excitability of postsynaptic neurons on timescales of milliseconds (through ionotropic receptors) or tens of milliseconds to seconds (through metabotropic receptors), neurotransmitters can also trigger long-term (hours to days) changes in the physiological state of
In response to extracellular signals, cells utilize many pathways to relay such signals to varied effectors and produce specific biological effects; this process is generally referred to as **signal transduction**. In the context of synaptic transmission, we have focused on the actions of ionotropic and metabotropic receptors that change the membrane potential of the postsynaptic cell. In this box, we expand the scope by placing neurotransmitter receptor signaling in the general framework of signal transduction, and by discussing receptor tyrosine kinase signaling pathways, which are crucial for both nervous system development and function.

In a typical signal transduction pathway (Figure 3-38A), an extracellular signal (a ligand) is detected by a **cell-surface receptor** in the recipient cell. (We will learn an exception to this in Chapter 9: steroid hormones diffuse across the cell membrane to bind receptors within the cell.) The signal is then relayed through one or a series of intracellular signaling proteins to reach their effector(s), producing cellular responses to the extracellular signal. The final effectors are diverse, but usually fall into one of the following categories: (1) enzymes that alter the metabolism of the cell; (2) regulators of gene expression that change chromatin structure, gene transcription, mRNA metabolism, or protein translation and degradation; (3) cytoskeletal proteins that regulate cell shape, cell movement, and intracellular transport; (4) ion channels that alter the cell's membrane potential and excitability. Indeed, we can map what we have learned about metabotropic and ionotropic receptor signaling onto this general scheme of signal transduction (Figure 3-38B).

The extracellular signal can come from different sources. If the signal is produced by the recipient cell itself (as is the case of presynaptic norepinephrine receptor signaling; see Figure 3-36), it is termed an **autocrine** signal. If the signal comes from nearby cells, it is termed a **paracrine** signal; neurotransmitters can be considered as specialized paracrine signals where the target cells are restricted to postsynaptic partners. If the signal comes from a remote cell through circulating blood, it is called an **endocrine** signal or a hormone (as is the case of epinephrine). When the signal comes from a neighboring cell, it can either be a diffusible molecule such as a neurotransmitter or a secreted protein,
Box 3–4: Signal transduction and receptor tyrosine kinase signaling

or a membrane-bound protein that requires cell–cell contact in order to send the signal. Secreted and membrane-bound protein ligands are widely used in cell–cell communication during development, which will be discussed in detail in Chapters 5 and 7.

In addition to the ionotrophic receptors and metabotropic receptors (GPCRs), a major class of cell-surface receptors used in the nervous system is enzyme-coupled receptors, where the receptor has an enzymatic activity in its intracellular domain. As an example, we discuss here a widely used class of enzyme-coupled receptors called receptor tyrosine kinases (RTKs), which are transmembrane proteins with an N-terminal extracellular ligand-binding portion and a C-terminal intracellular portion possessing a tyrosine kinase domain as well as tyrosine phosphorylation sites (Figure 3–39A). About 60 genes in the mammalian genome encode RTKs. We focus below on RTK signaling involving the neurotrophin receptors, but the general principles apply to other RTK signaling. Neurotrophins are a family of secreted proteins that regulate the survival, morphology, and physiology of target neurons (we will discuss the biological effects of these proteins in Section 7.15). They bind to and activate a family of RTKs called the Trk receptors.

How does neurotrophin binding to Trk activate signaling? Neurotrophins naturally form dimers. When each neurotrophin binds a Trk receptor, the neurotrophin dimer brings two Trk receptors in close proximity, such that the tyrosine kinase of one Trk can phosphorylate the tyrosine residues on the other Trk. Phosphorylation of key tyrosine residues creates binding sites for specific adaptor proteins. These adaptor proteins contain either an SH2 (src homology 2) domain or a PTB (phosphotyrosine binding) domain, which enables the adaptors to bind phosphorylated tyrosine in the context of specific amino acid sequences and thereby initiate downstream signaling. In the Trk receptors, for instance, two key tyrosine residues recruit the binding of several specific adaptor proteins, eliciting separate transduction pathways that also cross-talk with each other (Figure 3–39A).

One such signaling pathway is initiated by binding of the adaptor Shc (Figure 3–39B), which binds tyrosine-phosphorylated Trk via its PTB domain and becomes tyrosine phosphorylated by Trk. This recruits the binding of Grb2, an SH2-domain-containing adaptor protein. Grb2 is associated with Sos, a guanine nucleotide exchange factor for the small GTPase Ras (see Box 3–3). Ras is normally associated with the membrane because of lipid modification analogous to Go. Thus, Trk activation recruits Sos to the plasma membrane to catalyze the exchange of GDP for GTP on Ras. Ras-GTP binds a downstream effector called Raf, a serine/threonine protein kinase. Raf phosphorylates and activates another serine/threonine protein kinase Mek, which in turn phosphorylates and activates a third serine/threonine kinase Erk. Activated Erk

Figure 3–39 Neurotrophin receptor as an example of receptor tyrosine kinase (RTK) signaling. (A) In the absence of neurotrophin, Trk receptors are present as monomers and tyrosine residues (Y) are not phosphorylated. Binding of the neurotrophin dimer brings two Trk receptors in close proximity, allowing the kinase domain of each Trk to phosphorylate the tyrosine residues on the other Trk. Tyrosine phosphorylation recruits binding of specific adaptor proteins, each eliciting a downstream signaling event. Different adaptors can bind the same phosphorylated tyrosine (as in the case of adaptor 1 and adaptor 2). (B) Details of one adaptor pathway. Shc binds to a membrane-proximal phosphorylated tyrosine on Trk, leading to tyrosine phosphorylation of Shc. This helps recruit the binding of the Grb2-Sos complex. Sos acts as a guanine nucleotide exchange factor that catalyzes the conversion of Ras-GDP to Ras-GTP (red zigzag lines indicate lipid modification of Ras). Ras-GTP binds to Raf, which phosphorylates and activates Mek, which in turn phosphorylates and activates Erk. Activated Erk can directly and indirectly phosphorylate a number of transcription factors, which activate or repress transcription of target genes. Raf, Mek, and Erk (also called MAP kinase) constitute the MAP kinase cascade.
Box 3–4: Signal transduction and receptor tyrosine kinase signaling

phosphorylates and activates a number of transcription factors (DNA-binding proteins that activate or repress transcription of target genes), which leads to transcription of specific genes that promote neuronal survival and differentiation, two major biological effects of neurotrophin signaling during development.

Erk is also called MAP kinase (for mitogen-activated protein kinase), and therefore Mek is a MAP kinase kinase (since it phosphorylates MAP kinase). Erk-Mek-Erk kinase cascade is often referred to as a MAP kinase cascade, which acts downstream of Ras and a number of other signaling molecules. The Ras-MAP kinase cascade is a widely used signaling pathway that serves many functions, including cell survival and differentiation discussed above, cell fate determination (see Section 5.17), as well as cell proliferation. It is also used in activity-dependent transcription (see Section 3.23).

postsynaptic neurons by inducing expression of new genes. As a specific example, transcription of Fos was induced by ionotropic AChR activation within 5 minutes of nicotine application (Figure 3–40). Fos encodes a transcription factor, and its transient activation can change the expression of many downstream target genes.

Fos is the prototype of a class of genes called immediate early genes (IEGs), whose transcription is rapidly induced by external stimuli in the presence of protein synthesis inhibitors; this means that no new protein synthesis is required to turn on IEGs. In neurons, IEGs can be rapidly induced by neuronal activity in postsynaptic neurons in response to presynaptic neurotransmitter release. Some IEGs, such as Fos or Egr1 (early growth response-1), encode transcription factors that regulate the expression of other genes. Other IEGs encode regulators of neuronal communication more directly. Among them, brain-derived neurotrophic factor (BDNF) is a secreted neurotrophin that regulates the morphology and physiology of target neurons (see Box 3–4). Arc (activity-regulated cytoskeleton-associated protein) is a cytoskeletal protein present at the postsynaptic density that regulates trafficking of glutamate receptors, thus contributing to synaptic plasticity. As will be discussed in later chapters of this book, activity-dependent transcription (that is, regulation of gene expression by neuronal activity) plays a prominent role in the maturation of synapses and neural circuits during development and in their modulation by experience in adulthood. Because of their rapid induction by neuronal activity, expression of IEGs has also been widely used as a means to identify which neurons in the brain are activated by specific experiences or behavioral episodes (see Box 13–3).

Many signaling pathways have been identified that link neurotransmitter receptor to transcription. A rise in intracellular Ca2+ concentration ([Ca2+]i) is often a key step. [Ca2+]i rise can be accomplished by several means: through the NMDA receptor at the postsynaptic density in the dendritic spines (see Figure 3–24), through voltage-gated Ca2+ channels enriched on the dendritic trunk and the cell body, and through the IP3 receptors (see Figure 3–34) or the related ryanodine receptors on the ER membrane. Instead of being activated by IP3, ryanodine receptors are activated by a rise in [Ca2+]i, and thus amplify the Ca2+ signal; ryanodine is a plant-derived agonist of this ER-resident Ca2+ channel. Although free Ca2+ ions usually do not diffuse far from the source of entry into the cytosol, they can associate with various Ca2+-binding proteins, most notably calmodulin (CaM) (see Figure 3–34), and initiate signals that can be transduced to the nucleus (Figure 3–41). For example, Ca2+/CaM can activate a number of CaM kinases, including CaMKII enriched in postsynaptic density and CaMKIV enriched in the nucleus. A specific isoform of CaMKII, γCaMKII, has recently been shown to act as a shuttle that transports Ca2+/CaM from the plasma membrane near the voltage-gated Ca2+ channel to the nucleus so that Ca2+/CaM can activate nuclear effectors such as CaMKIV. In addition, Ca2+/CaM can activate several subtypes of adenylate cyclase, leading to the production of cAMP and activation of PKA. The Ras-MAP kinase cascade (see Box 3–4) is yet another signaling pathway that can be activated by Ca2+/CaM.
As a specific example, we discuss how these pathways lead to activation of a transcription factor called CREB. CREB was originally identified because it binds to a DNA element (CRE) in the promoter of the gene that produces a neuropeptide somatostatin, rendering somatostatin’s transcription responsive to cAMP regulation. (CRE stands for cAMP response element; CREB for CRE binding protein.) CRE was subsequently found in the promoter of many IEGs including Fos. Phosphorylation at amino acid serine-133 is crucial for the activity of CREB as a transcriptional activator. Biochemical experiments have shown that serine-133 can be phosphorylated by a number of kinases, including PKA, CaMKIV, and a protein kinase called Rsk (ribosomal protein S6 kinase); Rsk is in turn activated by MAP kinase phosphorylation. Although all of these kinases can be activated by Ca²⁺ (Figure 3-41), each pathway has unique properties. For example, the CaM kinase-mediated pathway is more rapid, resulting in CREB phosphorylation that peaks within minutes after a transient neuronal depolarization, whereas the MAP kinase pathway mediates a gradual increase of CREB phosphorylation over 60 minutes following a transient neuronal depolarization.

In addition to CREB, other Ca²⁺-responsive transcription factors are known to bind to different IEG promoters. Thus, neuronal activity has many routes to access the nucleus and change the transcriptional program of postsynaptic cells. Furthermore, neuronal activity and Ca²⁺ can also affect chromatin structures through enzymes that control the methylation of DNA and the post-translational modification (for example, methylation, demethylation, acetylation, and deacetylation) of histones, the protein component of the chromatin. These epigenetic modifications also alter gene expression patterns through regulation of chromatin structures and accessibility of promoters to specific transcription factors. As will be discussed in Chapter 11, mutations in many components of the synapse-to-nucleus signaling pathways have been found to cause human brain disorders, highlighting the important role of activity-dependent transcription in human mental health.

3.24 Dendrites are sophisticated integrative devices

Regulating gene expression aside, the primary function of synaptic transmission is to influence the firing patterns of postsynaptic neurons. This is the means by which information is propagated from one layer of neurons to the next within
neural circuits. As a way of integrating what we've learned about neuronal communication in Chapter 2 and this chapter, in the final two sections we discuss how synaptic inputs are integrated in the postsynaptic neuron to produce its firing pattern, thus completing a full round of neuronal communication (see Figure 1-18). We start our discussion with excitatory inputs in this section.

Most excitatory inputs to a neuron are provided on the dendrites via transient changes of conductance (for example, opening of ionotropic glutamate receptor channels), producing EPSCs and consequently EPSPs (see Figure 3-23). In order to influence the firing pattern, these electrical signals need to travel to the axon initial segment to contribute to the depolarization there. As we learned in Section 2.8, electrical signals evolve over time and decay across distance, specified by the passive (cable) properties of neuronal fibers such as the time constant (\( \tau \)) and length constant (\( \lambda \)). Theoricians have used model neurons to calculate EPSPs at the soma produced by synaptic input at different locations in dendrites. In the model neuron shown in Figure 3-42A, for example, the complex dendritic tree is simplified to 10 compartments, each varying distances from the soma in order to calculate somatic EPSPs in response to dendritic input. A transient increase of synaptic conductance, equivalent to a transient opening of excitatory neurotransmitter receptor channels, produces somatic EPSPs with different shapes and amplitudes when applied to different locations in the dendrites (Figure 3-42B). The further distant the synaptic input is, the slower is the rise of the somatic EPSP and the broader the EPSP spreads temporally. Furthermore, the further away the synaptic input, the smaller is the amplitude of somatic EPSP. This is because EPSPs produced from more distant synapses decay more substantially as they need to travel longer distances to reach the soma. In this model neuron, a synaptic input given at compartment 4 or 8 produces a peak somatic EPSP amplitude that is only 29% or 10% of the peak somatic EPSP amplitude when the same input is given at the soma (Figure 3-42B).

A mammalian CNS neuron receives on average thousands of excitatory synaptic inputs along its dendritic tree. A single EPSP at one synapse is usually insufficient to depolarize the postsynaptic neuron above the threshold for firing action potentials, due to the small size of an individual EPSP when it arrives at the axon initial segment. Indeed, at any given time, the postsynaptic neuron integrates many excitatory inputs in order to reach the firing threshold. Such integration takes two forms. In spatial integration, nearly simultaneously activated synapses at different spatial locations sum their excitatory postsynaptic currents when they converge along the path to the soma, producing a larger EPSP (Figure 3-43A). In temporal integration, synapses activated within a specific window (including successive activation at the same synapse) sum their postsynaptic currents to produce a larger EPSP (Figure 3-43B).

**Figure 3-42** Somatic EPSPs from dendritic inputs in a model neuron.

(A) The soma and dendritic tree of this neuron are simplified to 10 compartments for the purpose of mathematical modeling. Compartment 1 represents the soma, and compartments 2–10 represent dendritic segments with increasing distance from the soma, with the length constant (\( \lambda \)) as the unit. Dotted lines illustrate divisions between every two compartments.

(B) When a transient excitatory input of the same size and shape (dotted curve, with y axis to the right) is provided at compartment 1, 4, or 8, the shapes of EPSPs at the soma show distinct profiles. The somatic EPSP produced by the input given at the soma (compartment 1) has the largest amplitude and fastest rising and decay time, the somatic EPSP produced by the input given at compartment 8 has the smallest amplitude and slowest rising and decay time, and the somatic EPSP produced by the input given at compartment 8 has the intermediate amplitude and temporal spread. Time is represented in the unit of the time constant \( \tau \). (Adapted from Rall W [1967] J Neurophysiol 30:1138–1168.)
As we see from the model neuron in Figure 3-42, inputs from proximal synapses contribute more to the firing of the neuron because they are less attenuated. In some mammalian neurons, distal synapses are stronger in order to compensate for such distance-dependent attenuation. Importantly, inputs from distal synapses also have a longer window during which to contribute to temporal integration (Figure 3-43B). On the basis of excitatory inputs alone, we can already see that individual dendrites act as sophisticated integrative devices, given the complex morphology and abundant excitatory synapses of typical mammalian CNS neurons. On a moment-by-moment basis, a spiking neuron converts analog signals from the many inputs it receives into a digital signal of whether or not to fire an action potential.

While the passive properties of neuronal membranes we have discussed thus far provide a foundation for understanding how synaptic inputs contribute to firing of postsynaptic neurons, recent studies indicate that dendritic integration is more complex and nuanced. Voltage-gated Na⁺, Ca²⁺, and K⁺ channels are present on the dendrites of many mammalian CNS neurons, which endow active properties to the dendritic membrane (that is, voltage-dependent conductance changes). For example, opening of voltage-gated Na⁺ or Ca²⁺ channels by EPSPs causes further depolarization in dendrites, and thereby amplifies the EPSP signal. Co-activation of nearby excitatory synapses in dendritic branches can produce dendritic spikes that actively propagate across dendritic segments, conceptually similar to the action potentials we studied in Chapter 2. Although these dendritic spikes may not propagate all the way to the soma (because of the lower density of voltage-gated channels in dendrites compared to axons), they nevertheless amplify synaptic input and propagate membrane potential changes across a large distance with smaller attenuation compared to passive decay. Finally, action potentials produced in the axon initial segment can back-propagate in neurons with voltage-gated channels in dendrites, and these back-propagated action potentials can interact with EPSPs in interesting ways.

As a specific example, we study an experiment in which a cortical pyramidal neuron in an in vitro brain slice was subjected to dual patch clamp recording at the soma and at the apical dendrites (Figure 3-44A). Electrical stimulation of its input axons produced a synaptic potential, but it was significantly attenuated in the soma, below the threshold of firing an action potential (Figure 3-44B). However, if the recorded neuron was induced to fire an action potential by current injection 5 ms prior to input stimulation, the back-propagated action potential synergized with the dendritic synaptic potential to reach the threshold of firing a dendritic spike, which greatly amplified the synaptic potential, allowing it to trigger two additional action potentials that were propagated to distant target neurons (Figure 3-44C). Assuming that under physiological conditions, the
pyramidal neuron fires action potentials in response to proximal dendritic inputs, this integration mechanism can enable the neuron to amplify near-synchronous input at the proximal and distal dendrites by producing a burst of action potentials that could not be generated by either the distal or the proximal input alone.

It appears that many mammalian CNS neurons have active properties, which often differ in different neurons or even in different compartments of the same neuron because of differential distribution and density of voltage-gated ion channels. We are far from a complete understanding of how synaptic potentials are integrated in light of these active properties; indeed this is a highly active area of research.

3.25 Synapses are strategically placed at specific locations in postsynaptic neurons

In addition to excitatory inputs discussed in the previous section, each neuron also receives inputs from inhibitory neurons and modulatory neurons. How these inputs shape the output of the postsynaptic neuron depends on which subcellular compartments of the postsynaptic neuron these inputs synapse onto.

In general, most excitatory synapses are located on dendritic spines distributed throughout the dendritic tree (Figure 3-45). The various presynaptic terminals that target a given postsynaptic neuron may originate from many different presynaptic partner neurons, but each dendritic spine typically receives synaptic input from a single excitatory presynaptic terminal. The thin spine neck creates chemical and electrical compartments for each synapse such that it can be modulated independently from neighboring synapses. These largely independent compartments enable neurons to encode information in the strengths of individual synapses with different input neurons. A neuron can thus modulate its connection strengths with different input neurons independently according to prior experience; this property is crucial for memory, as will be discussed in more detail in Chapter 10.

In contrast to excitatory synapses that are most enriched on spines, inhibitory synapses form broadly across the postsynaptic membrane at dendritic shafts, dendritic spines, the cell body, and the axon initial segment. These distributions allow inhibitory synapses to generate IPSPs at strategic places to oppose the action of EPSPs as they pass by (see Figure 3-29). Let's use a typical pyramidal neuron in the cerebral cortex to illustrate inhibitory inputs it receives from three...
CHAPTER 3  Signaling across synapses

Figure 3-47  Subcellular distribution of synaptic input. For a typical mammalian neuron, excitatory inputs are received mostly at the dendritic spines (and along the dendrites for neurons that lack dendritic spines). Inhibitory inputs are received at the dendritic spines and shaft, cell body, axon initial segment, and presynaptic terminals. Modulatory inputs are received at dendrites, cell bodies, and presynaptic terminals.

types of GABAergic neurons (Figure 3-46A). The Martinotti cell targets its presynaptic terminals on distal dendrites of the pyramidal cell, and thereby affects the integration of synaptic potentials along specific dendritic segments. For instance, activation of the Martinotti cells can inhibit the production or propagation of the dendritic spikes discussed in previous sections. The basket cell targets its presynaptic terminals around the cell body of the pyramidal neuron, and thereby influences the overall integration of synaptic input from all dendritic branches. The chandelier cell targets its presynaptic terminals specifically to the initial axon segment of many pyramidal cells (Figure 3-46B), such that its synaptic inputs to the pyramidal cell have the most direct impact on the production of action potentials.

A postsynaptic neuron can receive synaptic input at its own presynaptic terminals, as discussed in Section 3.21. Here, inputs do not control the action potential firing rate, but rather the efficacy with which action potentials in the postsynaptic neuron lead it to release neurotransmitters. The presynaptic partners in these cases are mostly modulatory neurons that use transmitters such as acetylcholine, dopamine, serotonin, and norepinephrine. Some GABAergic neurons also exert their action on the presynaptic terminals of their target neurons (see Figure 3-37).

In summary, individual neurons are complex and highly organized integrators. Each neuron receives inputs from its numerous presynaptic partners at its complex dendritic tree, its cell body, its axon initial segment, and its presynaptic terminals (Figure 3-47). The interactions of excitatory, inhibitory, and modulatory inputs together shape the neuron's output patterns, which are communicated to its own postsynaptic target neurons by the frequency and timing of action potentials and the probability of neurotransmitter release induced by each action potential. Some neurons also receive input (and send output) through electrical synapses (Box 3-5). At a higher level, individual neurons are parts of complex neural circuits that perform diverse information-processing functions, from sensory perception to behavioral control. Having studied the basic concepts and principles of neuronal communication, we are now ready to apply them to fascinating neurobiological problems in the following chapters.
Box 3-5: Electrical synapses

Although chemical synapses are the predominant form of interneuronal communication, electrical synapses are also prevalent in both vertebrate and invertebrate nervous systems. The morphological correlate of the electrical synapses is the gap junction, which usually contains hundreds of closely clustered channels that bring the plasma membranes of two neighboring cells together (see Figure 1-14B) and allow passage of ions and small molecules between the two cells. In mammalian neurons, electrical synapses usually occur at the somatodendritic compartments of two partner neurons.

In vertebrates, gap junctions are made predominantly by a family of connexin proteins, encoded by about 20 genes in the mammalian genome. Each gap junction channel is composed of 12 connexin subunits, with 6 subunits on each apposing plasma membrane forming a hemi-channel. Each connexin subunit has four transmembrane domains with an additional N-terminal domain embedded in the membrane. As revealed by the crystal structure for connexin-26 (Figure 3-48), extensive interactions between the extracellular loops of the hemi-channels bring the two apposing membranes from neighboring cells within 4 nm of each other, and align the two hemi-channels to form a pore with an innermost diameter of 1.4 nm. Invertebrate gap junctions are made by a different family of proteins called innexins (invertebrate connexin). A third family of proteins called pannexins may contribute to gap junctions in both vertebrates and invertebrates.

Electrical synapses differ from chemical synapses in a number of important ways. First, whereas chemical synapses transmit signals with a delay on the order of 1 ms between depolarization in the presynaptic terminal and synaptic potential generation in the postsynaptic cell, electrical synapses transmit electrical signals with virtually no delay. Second, whereas chemical synapses transmit only depolarizing signals (and, in spiking neurons, only supra-threshold signals that produce action potentials), electrical synapses transmit both depolarizing and hyperpolarizing signals. Third, whereas chemical synapses are asymmetrical—membrane potential changes in the presynaptic neuron produce membrane potential changes in the postsynaptic neuron, but not vice versa—electrical signals can flow in either direction across electrical synapses. Exceptions exist to this rule, however; some electrical synapses prefer one direction over the opposite direction, and are called rectifying electrical synapses. Finally, many electrical synapses allow small molecules such as peptides and second messengers to pass through; indeed, the diffusion of small-molecule dye from one cell to another, called dye-coupling, is often used as a criterion to identify the presence of gap junctions between two cells. The conductance of electrical synapses can be modulated by a number of factors such as the membrane potential, the transjunctional voltage (the difference between membrane potentials across the electrical synapse), and chemical factors such as phosphorylation, pH, and Ca²⁺ concentration.

The special properties of electrical synapses discussed above are utilized in many circuits in invertebrates and vertebrates. For instance, electrical synapses are found in circuits where rapid transmission is essential, such as in the vertebrate retina for processing motion signals (where they transmit analog signals between non-s spiked neurons), and in escape circuits to avoid predators. Indeed, electrical synapses were first characterized between the giant axon and motor neuron of the crayfish escape circuit in the 1950s. Another utility of electrical synapses is to facilitate synchronized firing between electrically coupled neurons (another term for neurons that form electrical synapses with each other).

As a specific example, we study below electrical synapses in the mammalian cerebral cortex.

Using whole-cell patch recording techniques (see Box 13-2 and Section 13.21 for details) in a cortical slice preparation, researchers found that when two fast-spiking (FS) inhibitory neurons (corresponding mostly to basket cells in Figure 3-46A) were recorded simultaneously with patch electrodes, current injection into one

(Continued)
Box 3-5: Electrical synapses

cell caused nearly synchronous membrane potential changes in both cells; both depolarization and hyperpolarization could result depending on the sign of the injected current (Figure 3-49A), indicating that these two cells formed electrical synapses. Paired recording of many cell types indicated that electrical synapses form with exquisite cell-type specificity. For example, low-threshold-spiking (LTS) inhibitory neurons (corresponding mostly to Martinotti cells in Figure 3-46A) also formed electrical synapses with each other at a high probability, but they rarely formed electrical synapses with FS neurons. Excitatory pyramidal neurons, which could be identified as regular-spiking (RS) cells by their electrophysiological properties, did not form any electrical synapses with themselves or with FS or LTS neurons (Figure 3-49B). Furthermore, whereas injecting sub-threshold depolarizing currents into one of the two electrically coupled FS cells did not elicit action potentials, injecting the same sub-threshold depolarizing currents into both FS cells elicited synchronous action potentials (Figure 3-49C). This suggested that a network of FS cells can act as detectors for synchronous activities, and their synchronous firing can further strengthen synchronous cortical activities. Subsequent work indicated that in addition to FS and LTS cells, other specific types of inhibitory neurons also form type-specific electrical synapse networks, thus providing rich substrates for coordinating electrical activities in the cerebral cortex.

Figure 3-49 Electrical synapses between inhibitory neurons in the rat cerebral cortex. (A) Top, image of a rat cortical slice with two cells and two patch electrodes, taken with differential interference contrast microscopy. Bottom left, when positive (blue) or negative (red) current \( i_{inj} \) was injected into cell 1, it depolarized or hyperpolarized the membrane potential \( V_m \) of cell 1 (top or bottom traces of \( V_m \)). In addition, cell 2 was also depolarized or hyperpolarized at the same time (top or bottom traces of \( V_m \)). Note the reduced amplitude and slower rising time of \( V_m \) compared to \( V_m \), due to attenuation across the gap junction and the time taken to charge the membrane capacitance of cell 2. Bottom right, positive or negative current injected into cell 2 \( i_{inj} \) also caused depolarization or hyperpolarization of both cells. Thus, these two cells form electrical synapses. (B) Quantification of electrical synapses between specific types of cells based on paired recording in panel A. Cells were classified based on their firing patterns into fast spiking (FS), low-threshold spiking (LTS), or regular spiking (RS), corresponding roughly to basket cells, Martinotti cells, and pyramidal cells, respectively. Arrows indicate the directionality of electrical synapse tested in paired recording. (C) The top and bottom traces show the injection of small depolarizing currents into cell 2 (top) or cell 2 (bottom). Injection into a single cell (open arrowheads) did not cause firing of either cell. Injecting the same size current into both cells simultaneously (filled arrowheads) caused both cells to fire action potentials. (Adapted from Galarreta M & Hestrin S [1999] Nature 402:72–75. With permission from Macmillan Publishers Ltd. See also Gibson JR, Beierlein M & Connors BW [1999] Nature 402:75–79.)

**SUMMARY**

Neurons communicate with each other using electrical and chemical synapses. Electrical synapses allow rapid and bidirectional transmission of electrical signals between neurons via the gap junction channels. Although less prevalent than chemical synapses, electrical synapses are widely used in both invertebrates and vertebrates—for example in neural circuits that require rapid information
propagation or synchronization. Chemical synapses are unidirectional: electrical signal in the presynaptic neuron is transmitted to the postsynaptic neuron or muscle via the release of a chemical intermediate, the neurotransmitter.

At the presynaptic terminal, neurotransmitter release is mediated by fusion of the synaptic vesicle with the presynaptic plasma membrane. Action potential arrival depolarizes the presynaptic terminal, causing the opening of voltage-gated \( \text{Ca}^{2+} \) channels at the active zone. \( \text{Ca}^{2+} \) influx, acting through a synaptic vesicle-associated \( \text{Ca}^{2+} \) sensor synaptotagmin, releases the molecular clamp on a partially assembled SNARE complex. The full assembly of the SNARE complex, consisting of one v-SNARE on the synaptic vesicle and two t-SNAREs on presynaptic plasma membrane, provides the force that drives membrane fusion and release of transmitter from within the synaptic vesicle to the synaptic cleft. Excess neurotransmitter molecules are rapidly degraded or recycled through reuptake mechanisms. After neurotransmitter release, synaptic vesicles are rapidly recycled and refilled with neurotransmitters, enabling continual synaptic transmission in response to future action potentials.

Nervous systems across the animal kingdom utilize a common set of neurotransmitters. In the vertebrate CNS, glutamate is the main excitatory neurotransmitter, whereas GABA and glycine are the main inhibitory neurotransmitters. Acetylcholine is the excitatory neurotransmitter in the vertebrate neuromuscular junction (and some CNS neurons), but can also act as a modulatory neurotransmitter in the CNS. Other neuropeptides include dopamine, serotonin, norepinephrine, and neuropeptides. The specific actions of neurotransmitters are determined by the properties of their receptors on the postsynaptic neurons.

Neurotransmitter receptors are either ionotropic or metabotropic. Ionotropic receptors are ion channels that are gated by neurotransmitter binding, and act rapidly to produce synaptic potentials within a few milliseconds of presynaptic action potential arrival. The ionotropic acetylcholine and glutamate receptors are nonselective cation channels with reversal potentials around 0 mV; upon neurotransmitter binding these receptors produce depolarization in the form of excitatory postsynaptic potentials. The NMDA receptor acts as a coincidence detector as its channel opening depends both on presynaptic glutamate release and a depolarized state of the postsynaptic neuron. Ionotropic GABA and glycine receptors are \( \text{Cl}^- \) channels, with reversal potentials near or below the resting potential. Their opening usually produces \( \text{Cl}^- \) influx, which counteracts excitatory postsynaptic potentials and inhibits postsynaptic neurons from reaching the threshold at which they fire action potentials.

Metabotropic receptors for acetylcholine, glutamate, GABA, monoamines, and neuropeptides are all G-protein-coupled receptors. Neurotransmitter binding leads to the association of the metabotropic receptor with the trimeric G protein, exchange of GDP for GTP, and dissociation of \( \text{G}_\alpha \)-GTP and \( \text{G}_\beta \gamma \) subunits from each other and from the receptor. \( \text{G}_\alpha \)-GTP and \( \text{G}_\beta \gamma \) each can activate different effectors depending on specific G protein variants and cellular context. \( \text{G}_\beta \gamma \) can act on \( \text{K}^+ \) and \( \text{Ca}^{2+} \) channels directly, whereas \( \text{G}_\alpha \) usually acts via second messengers such as \( \text{cAMP} \) and \( \text{Ca}^{2+} \) to activate protein kinases that phosphorylate ion channels to change membrane potentials and excitability of the postsynaptic neurons. Metabotropic receptor activation usually causes membrane potential changes within tens of milliseconds to seconds. Longer-term changes of postsynaptic neurons in response to neurotransmitter release and neuronal activity involve synapse-to-nucleus signaling and alterations of gene expression.

Chemical synapses are highly organized structurally. At the presynaptic terminal, the active zone protein complexes bring synaptic vesicles to the immediate vicinity of voltage-gated \( \text{Ca}^{2+} \) channels such that \( \text{Ca}^{2+} \) influx rapidly triggers neurotransmitter release. Trans-synaptic cell adhesion proteins, by interacting with scaffolding proteins in both the presynaptic active zone and the postsynaptic density, help align active zone and high-density neurotransmitter receptors across the synaptic cleft. Postsynaptic density scaffolding proteins further link neurotransmitter receptors to their regulators and effectors for efficient synaptic transmission and for regulating synaptic plasticity.
Integration of excitatory, inhibitory, and modulatory inputs at the dendrites, cell bodies, and axon initial segments of postsynaptic neurons collectively determine their own action potential firing patterns. Synaptic input to the axon terminals of postsynaptic neurons further modulates the efficacy with which postsynaptic action potentials lead to neurotransmitter release. All of these mechanisms are amply used in the nervous system to produce functions from sensation to action that we will study in the following chapters.

FURTHER READING

Books and reviews

Presynaptic mechanisms

Postsynaptic mechanisms


