Neurotransmitters and Receptor and Transport Proteins in Signal Transmission at Synapses

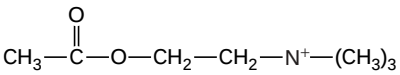
As noted earlier, synapses are the junctions where neurons release a chemical neurotransmitter that acts on a postsynaptic target cell, which can be another neuron or a muscle or gland cell (see Figure 7-31). In this section, we focus on several key issues related to impulse transmission:

- How neurotransmitters are packaged in membrane-bounded *synaptic vesicles* in the axon terminus
- How arrival of an action potential at axon termini in presynaptic cells triggers secretion of neurotransmitters
- How binding of neurotransmitters by receptors on postsynaptic cells leads to changes in their membrane potential
- How neurotransmitters are removed from the synaptic cleft after stimulating postsynaptic cells

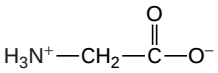
Neurotransmitter receptors fall into two broad classes: ligand-gated ion channels, which open immediately upon neurotransmitter binding, and G protein–coupled receptors. Neurotransmitter binding to a G protein–coupled receptor induces the opening or closing of a *separate* ion channel protein over a period of seconds to minutes. These "slow" neurotransmitter receptors are discussed ~~in Chapter 13~~ along with G protein–coupled receptors that bind different types of ligands and modulate the activity of cytosolic proteins other than ion channels. Here we examine the structure and operation of the *nicotinic acetylcholine receptor* found at many nerve-muscle synapses. The first ligand-gated ion channel to be purified, cloned, and characterized at the molecular level, this receptor provides a paradigm for other neurotransmitter-gated ion channels.

Neurotransmitters Are Transported into Synaptic Vesicles by H^+-Linked Antiport Proteins

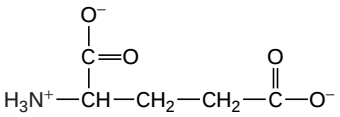
Numerous small molecules function as neurotransmitters at various synapses. With the exception of **acetylcholine,** the



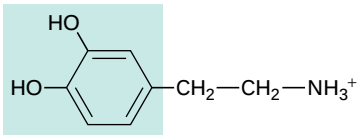
Acetylcholine



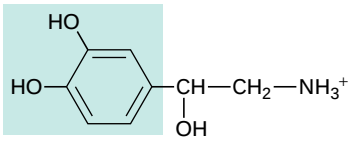
Glycine



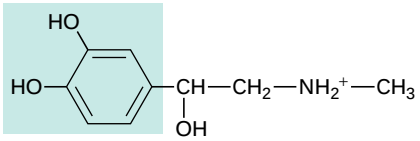
Glutamate



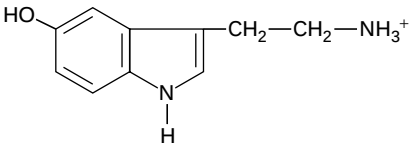
Dopamine
(derived from tyrosine)



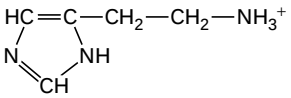
Norepinephrine
(derived from tyrosine)



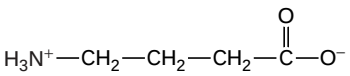
Epinephrine
(derived from tyrosine)



Serotonin, or **5-hydroxytryptamine**
(derived from tryptophan)



Histamine
(derived from histidine)

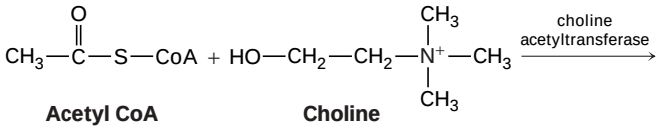


γ-Aminobutyric acid, or **GABA**
(derived from glutamate)

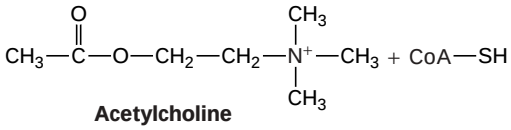
neurotransmitters shown in Figure 7-41 are amino acids or derivatives of amino acids. Nucleotides such as ATP and the corresponding nucleosides, which lack phosphate groups, also function as neurotransmitters. Each neuron generally produces just one type of neurotransmitter.

All the "classic" neurotransmitters are synthesized in the cytosol and imported into membrane-bound synaptic vesicles within axon terminals, where they are stored. These vesicles are 40–50 nm in diameter, and their lumen has a low pH, generated by operation of a V-class proton pump in the vesicle membrane. Similar to the accumulation of metabolites in plant vacuoles (see Figure 7-23), this proton concentration gradient (vesicle lumen > cytosol) powers neurotransmitter import by ligand-specific H^+-linked antiporters in the vesicle membrane.

For example, acetylcholine is synthesized from acetyl coenzyme A (acetyl CoA), an intermediate in the degradation of glucose and fatty acids, and choline in a reaction catalyzed by choline acetyltransferase:



Acetyl CoA **Choline**



Acetylcholine

Synaptic vesicles take up and concentrate acetylcholine from the cytosol against a steep concentration gradient, using an H^+/acetylcholine antiporter in the vesicle membrane. Curiously, the gene encoding this antiporter is contained entirely within the first intron of the gene encoding choline acetyltransferase, a mechanism conserved throughout evolution for ensuring coordinate expression of these two proteins. Different H^+/neurotransmitter antiport proteins are used for import of other neurotransmitters into synaptic vesicles.

Influx of Ca^{2+} Through Voltage-Gated Ca^{2+} Channels Triggers Release of Neurotransmitters

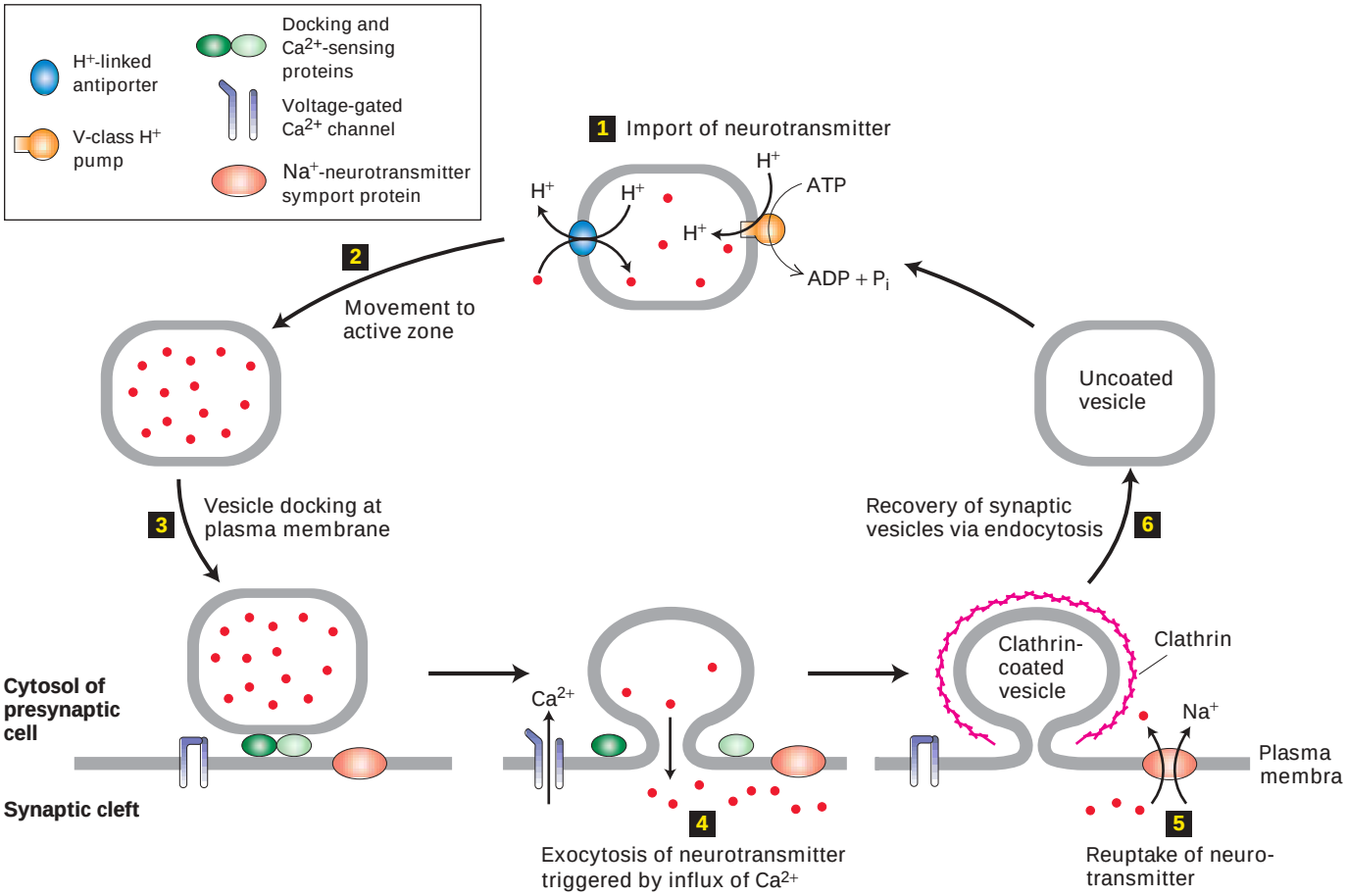
Neurotransmitters are released by **exocytosis,** a process in which neurotransmitter-filled synaptic vesicles fuse with the axonal membrane, releasing their contents into the synaptic cleft. The exocytosis of neurotransmitters from synaptic vesicles involves vesicle-targeting and fusion events similar to those that occur during the intracellular transport of secreted and plasma-membrane proteins (Chapter 17). Two features

◀ FIGURE 7-41 **Structures of several small molecules that function as neurotransmitters.** Except for acetylcholine, all these are amino acids (glycine and glutamate) or derived from the indicated amino acids. The three transmitters synthesized from tyrosine, which contain the catechol moiety (blue highlight), are referred to as catecholamines.

critical to synapse function differ from other secretory pathways: (a) secretion is tightly coupled to arrival of an action potential at the axon terminus, and (b) synaptic vesicles are recycled locally to the axon terminus after fusion with the plasma membrane. Figure 7-42 shows the entire cycle whereby synaptic vesicles are filled with neurotransmitter, release their contents, and are recycled.

Depolarization of the plasma membrane cannot, by itself, cause synaptic vesicles to fuse with the plasma membrane. In order to trigger vesicle fusion, an action potential must be converted into a chemical signal—namely, a localized rise in the cytosolic Ca^{2+} concentration. The transducers of the electric signals are *voltage-gated Ca^{2+} channels* localized to the region of the plasma membrane adjacent to the synaptic vesicles. The membrane depolarization due to arrival of an action potential opens these channels, permitting an influx of Ca^{2+} ions from the extracellular medium into the axon terminal. This ion flux raises the local cytosolic Ca^{2+} concentration near the synaptic vesicles from <0.1 μM, characteristic of the resting state, to 1–100 μM. Binding of Ca^{2+} ions to proteins that connect the synaptic vesicle with the plasma membrane induces membrane fusion and thus exocytosis of the neurotransmitter. The subsequent export of extra Ca^{2+} ions by ATP-powered Ca^{2+} pumps in the plasma membrane rapidly lowers the cytosolic Ca^{2+} level to that of the resting state, enabling the axon terminus to respond to the arrival of another action potential.

A simple experiment demonstrates the importance of voltage-gated Ca^{2+} channels in release of neurotransmitters. A preparation of neurons in a Ca^{2+}-containing medium is treated with tetrodotoxin, a drug that blocks voltage-gated Na^+ channels and thus prevents conduction of action



▲ FIGURE 7-42 **Cycling of neurotransmitters and of synaptic vesicles in axon terminals.** The entire cycle depicted here typically takes about 60 seconds. Note that several transport proteins participate in the filling of synaptic vesicles with neurotransmitter (red circles), its release by exocytosis, and subsequent reuptake from the synaptic cleft. Once synaptic-vesicle membrane proteins (e.g., pumps, antiporters, and fusion proteins needed for exocytosis) are specifically recovered by endocytosis in clathrin-coated vesicles, the clathrin coat is depolymerized, yielding vesicles that can be filled with neurotransmitter. Unlike most neurotransmitters, acetylcholine is not recycled. See text for details. [See T. Südhof and R. Jahn, 1991, *Neuron* **6**:665; K. Takei et al., 1996, *J. Cell. Biol.* **133**:1237; and V. Murthy and C. Stevens, 1998, *Nature* **392**:497.]

potentials. As expected, no neurotransmitters are secreted into the culture medium. If the axonal membrane then is artificially depolarized by making the medium ≈100 mM KCl, neurotransmitters are released from the cells because of the influx of Ca^{2+} through open voltage-gated Ca^{2+} channels. Indeed, patch-clamping experiments show that voltage-gated Ca^{2+}channels, like voltage-gated Na^+ channels, open transiently upon depolarization of the membrane.

Two pools of neurotransmitter-filled synaptic vesicles are present in axon terminals: those "docked" at the plasma membrane, which can be readily exocytosed, and those in reserve in the *active zone* near the plasma membrane. Each rise in Ca^{2+} triggers exocytosis of about 10 percent of the docked vesicles. Membrane proteins unique to synaptic vesicles then are specifically internalized by **endocytosis,** usually via the same types of clathrin-coated vesicles used to recover other plasma-membrane proteins by other types of cells. After the endocytosed vesicles lose their clathrin coat, they are rapidly refilled with neurotransmitter. The ability of many neurons to fire 50 times a second is clear evidence that the recycling of vesicle membrane proteins occurs quite rapidly.

Signaling at Synapses Usually Is Terminated by Degradation or Reuptake of Neurotransmitters

Following their release from a presynaptic cell, neurotransmitters must be removed or destroyed to prevent continued stimulation of the postsynaptic cell. Signaling can be terminated by diffusion of a transmitter away from the synaptic cleft, but this is a slow process. Instead, one of two more rapid mechanisms terminates the action of neurotransmitters at most synapses.

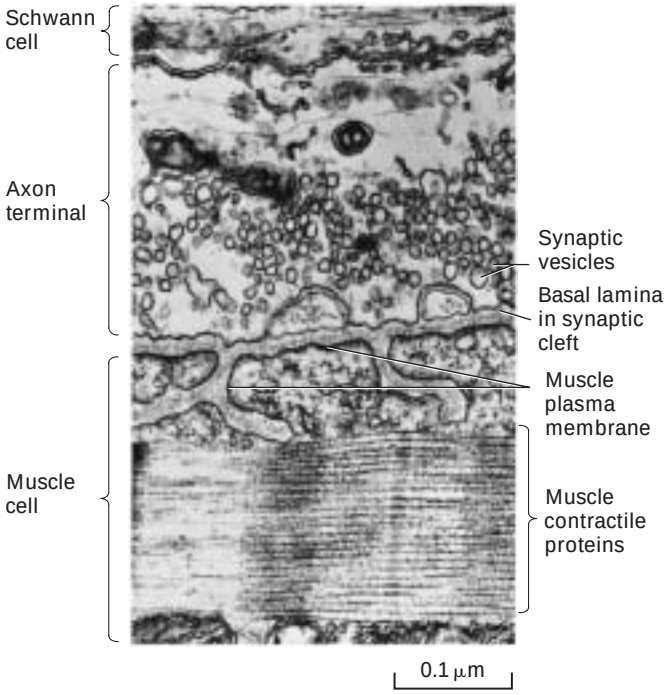
Signaling by acetylcholine is terminated when it is hydrolyzed to acetate and choline by *acetylcholinesterase,* an enzyme localized to the synaptic cleft. Choline released in this reaction is transported back into the presynaptic axon terminal by a Na^+/choline symporter and used in synthesis of more acetylcholine. The operation of this transporter is similar to that of the Na^+-linked symporters used to transport glucose into cells against a concentration gradient (see Figure 7-21).

With the exception of acetylcholine, all the neurotransmitters shown in Figure 7-41 are removed from the synaptic cleft by transport into the axon terminals that released them. Thus these transmitters are recycled intact, as depicted in Figure 7-42 (step 5). Transporters for GABA, norepinephrine, dopamine, and serotonin were the first to be cloned and studied. These four transport proteins are all Na^+-linked symporters. They are 60–70 percent identical in their amino acid sequences, and each is thought to contain 12 transmembrane α helices. As with other Na^+ symporters, the movement of Na^+ into the cell down its electrochemical gradient provides the energy for uptake of the neurotransmitter. To maintain electroneutrality, Cl^- often is transported via an ion channel along with the Na^+ and neurotransmitter.

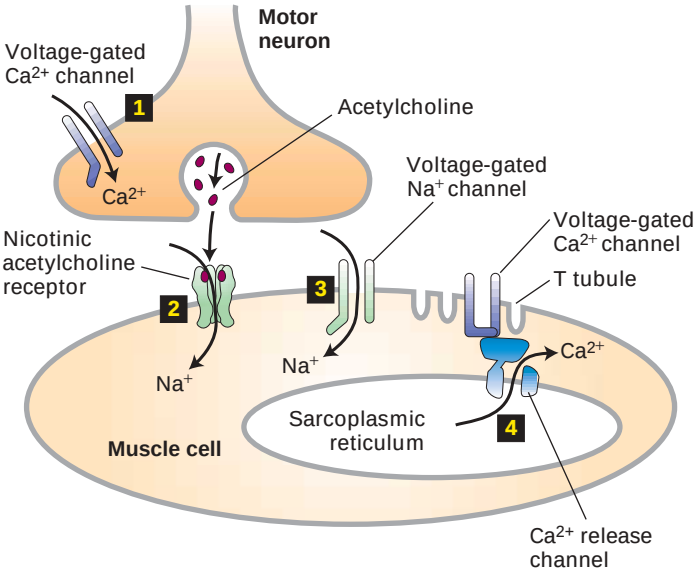
Cocaine inhibits the transporters for norepinephrine, serotonin, and dopamine. Binding of cocaine to the dopamine transporter inhibits reuptake of dopamine, thus prolonging signaling at key brain synapses; indeed, the dopamine transporter is the principal brain "cocaine receptor." Therapeutic agents such as the antidepressant drugs fluoxetine (Prozac) and imipramine block serotonin uptake, and the tricyclic antidepressant desipramine blocks norepinephrine uptake. ▮

Opening of Acetylcholine-Gated Cation Channels Leads to Muscle Contraction

Acetylcholine is the neurotransmitter at synapses between motor neurons and muscle cells, often called *neuromuscular junctions.* A single axon terminus of a frog motor neuron may contain a million or more synaptic vesicles, each containing 1000–10,000 molecules of acetylcholine; these vesicles often accumulate in rows in the active zone (Figure 7-43). Such a neuron can form synapses with a single skeletal muscle cell at several hundred points.



▲ FIGURE 7-43 **Synaptic vesicles in the axon terminal near the region where neurotransmitter is released.** In this longitudinal section through a neuromuscular junction, the basal lamina lies in the synaptic cleft separating the neuron from the muscle membrane, which is extensively folded. Acetylcholine receptors are concentrated in the postsynaptic muscle membrane at the top and part way down the sides of the folds in the membrane. A Schwann cell surrounds the axon terminal. [From J. E. Heuser and T. Reese, 1977, in E. R. Kandel, ed., *The Nervous System,* vol. 1, *Handbook of Physiology,* Williams & Wilkins, p. 266.]



▲ FIGURE 7-44 **Sequential activation of gated ion channels at a neuromuscular junction.** Arrival of an action potential at the terminus of a presynaptic motor neuron induces opening of voltage-gated Ca^{2+} channels (step 1) and subsequent release of acetylcholine, which triggers opening of the ligand-gated acetylcholine receptors in the muscle plasma membrane (step 2). The resulting influx of Na^+ produces a localized depolarization of the membrane, leading to opening of voltage-gated Na^+ channels and generation of an action potential (step 3). When the spreading depolarization reaches T tubules, it is sensed by voltage-gated Ca^{2+} channels in the plasma membrane. This leads to opening of Ca^{2+}-release channels in the sarcoplasmic reticulum membrane, releasing stored Ca^{2+} into the cytosol (step 4). The resulting rise in cytosolic Ca^{2+} causes muscle contraction by mechanisms discussed in Chapter 19.

The nicotinic acetylcholine receptor, which is expressed in muscle cells, is a ligand-gated channel that admits both K^+ and Na^+. The effect of acetylcholine on this receptor can be determined by patch-clamping studies on isolated outside-out patches of muscle plasma membranes (see Figure 7-17c). Such measurements have shown that acetylcholine causes opening of a cation channel in the receptor capable of transmitting 15,000–30,000 Na^+ or K^+ ions per millisecond. However, since the resting potential of the muscle plasma membrane is near E_K, the potassium equilibrium potential, opening of acetylcholine receptor channels causes little increase in the efflux of K^+ ions; Na^+ ions, on the other hand, flow into the muscle cell driven by the Na^+ electrochemical gradient.

The simultaneous increase in permeability to Na^+ and K^+ ions following binding of acetylcholine produces a net depolarization to about −15 mV from the muscle resting potential of −85 to −90 mV. As shown in Figure 7-44, this localized depolarization of the muscle plasma membrane triggers opening of voltage-gated Na^+ channels, leading to generation and conduction of an action potential in the muscle cell surface membrane by the same mechanisms described previously for neurons. When the membrane depolarization reaches T tubules, specialized invaginations of the plasma membrane, it affects Ca^{2+} channels in the plasma membrane apparently without causing them to open. Somehow this causes opening of adjacent Ca^{2+}-release channels in the sarcoplasmic reticulum membrane. The subsequent flow of stored Ca^{2+} ions from the sarcoplasmic reticulum into the cytosol raises the cytosolic Ca^{2+} concentration sufficiently to induce muscle contraction.

Careful monitoring of the membrane potential of the muscle membrane at a synapse with a cholinergic motor neuron has demonstrated spontaneous, intermittent, and random ≈2-ms depolarizations of about 0.5–1.0 mV in the absence of stimulation of the motor neuron. Each of these depolarizations is caused by the spontaneous release of acetylcholine from a single synaptic vesicle. Indeed, demonstration of such spontaneous small depolarizations led to the notion of the quantal release of acetylcholine (later applied to other neurotransmitters) and thereby led to the hypothesis of vesicle exocytosis at synapses. The release of one acetylcholine-containing synaptic vesicle results in the opening of about 3000 ion channels in the postsynaptic membrane, far short of the number needed to reach the threshold depolarization that induces an action potential. Clearly, stimulation of muscle contraction by a motor neuron requires the nearly simultaneous release of acetylcholine from numerous synaptic vesicles.