Chapter 17

Neurons and Synaptic Signaling

Animals are comprised of a vast number of cells whose activities must be precisely coordinated with each other. Because the cells that need to interact are often separated by long distances, animals have developed a nervous system that allows virtually instantaneous communication among the various regions of the body. Evolution of the nervous system has reached its zenith in higher vertebrates, where billions of nerve cells form trillions of connections with one another. These elaborate cellular networks receive sensory information from both inside and outside the body, integrate and analyze the information, and then respond by triggering appropriate responses. As the chapter unfolds, we will see that in spite of its inherent complexity, the nervous system communicates using two cellular phenomena that are well understood: changes in the electrical properties of the plasma membrane, and the release of chemical neurotransmitters that transmit signals from one cell to the next. As background for this chapter, the descriptions of membrane potentials, action potentials, and plasma membrane receptors included in Chapter 6 (pages 195–221) should first be reviewed.

NEURON STRUCTURE

To facilitate communication between distant regions of the body that is both rapid and precisely targeted, the cells of the nervous system have evolved a highly specialized morphology that makes them look quite different from any other cell type. We will therefore begin our discussion of neural communication by describing the architectural organization of nervous tissue, emphasizing the relationship between nerve cell structure and the mechanism by which nerve cells communicate.

The Discovery of Neurons Was Hindered by Their Unusual Morphology

Shortly after Schleiden and Schwann first formulated the cell theory in 1839 (page 4), microscopic examination of a variety of different tissues provided growing support for their contention that all living tissues are composed of cells. But a notable exception was encountered in the nervous system, where little evidence for the presence of individual cells was initially detected. Instead, early microscopic studies of nervous tissue revealed only hazy outlines that looked like a network of connecting channels running between spherical cell bodies. Biologists therefore tended to view the nervous system as a physically continuous network of cell bodies and cytoplasmic extensions without boundaries that would define individual cells.

A major impediment to understanding the true organization of nervous tissue no doubt lay in the unusual size and shape of typical nerve cells, whose long, branching cytoplasmic extensions make individual cells difficult to visualize in their entirety. This obstacle was finally overcome in 1873 by an impoverished Italian physician, Camillo Golgi, who was experimenting with different fixing and staining methods for nervous tissue. Working by candlelight in his kitchen, Golgi discovered that exposing chromate-hardened brain tissue to silver nitrate reveals the complete outline of individual nerve cells, including their branched cytoplasmic extensions (Figure 17-1). But the significance of this remarkable advance was not fully appreciated until several years later, when the Spanish neurobiologist Santiago Ramón y Cajal used Golgi's technique to trace the cytoplasmic extensions of individual nerve cells. These studies led Ramón y Cajal to conclude that each nerve cell body has its own set of cytoplasmic extensions that are not continuous with those of other cell bodies. In other words, it appeared as if each cell body and its associated cytoplasmic extensions represented a single nerve cell, or **neuron.**

Ramón y Cajal's conclusion that nervous tissue is comprised of individual neurons did not go unchallenged, however. Golgi and many others still believed that the cytoplasmic extensions of nerve cell bodies are all continuous with one another, and a heated controversy developed that was exacerbated when the two major protagonists, Golgi and Ramón y Cajal, shared the Nobel Prize in 1906. Although the theory that nervous tissue is composed of individual neurons gradually gained prominence, it took almost 50 years before the advent of electron microscopy finally allowed cell biologists to detect the membrane boundaries that separate the cytoplasmic extensions of one neuron from those of its neighbors. Thus more than a hundred years intervened between Schleiden and Schwann's formulation of the cell theory and definitive proof for the idea that nervous tissue is composed of individual cells, each bounded by its own plasma membrane.

Axons and Dendrites Are Cytoplasmic Extensions of the Cell Body That Send and Receive Electrical Signals

A typical neuron is composed of two principal regions: a *cell body* and a series of thin cytoplasmic extensions

Figure 17-1 *A Nerve Cell (Neuron) Stained by the Golgi Method* *The cell body, numerous dendrites, an axon, and the axon hillock are clearly visible. Courtesy of A. Peters.*

emerging from the cell body called *dendrites* and *axons* (Figure 17-2). The **cell body** contains a centrally placed nucleus that often exhibits a prominent nucleolus. Under the light microscope, the cytoplasm of the cell body appears to be filled with large clumps of material that stain intensely with basic dyes. For many years this material, named *Nissl substance* after its discoverer Franz Nissl, was believed to be an artifact generated during sample preparation. However, electron microscopic studies eventually revealed that the Nissl substance corresponds to dense masses of ribosomes and endoplasmic reticulum that occur in high concentration in the cell body. In addition, the cell body usually contains an extensive Golgi complex and large numbers of mitochondria and lysosomes.

The **dendrites** and **axons** that project from the cell body can be distinguished from each other in several ways (Table 17-1). The most important difference is functional: dendrites are specialized for receiving signals from other cells, whereas axons are involved primarily in sending signals. Dendrites are highly branched, relatively short (less than a millimeter in length), emerge in large numbers from a single cell body, and exhibit small protuberances called dendritic *spines* that are specialized for receiving signals from other cells. Axons often have relatively few branches, are longer than dendrites (up to a meter or more in length), and only one or two axons is present per cell. The enormous length of some axons allows neurons in the central nervous system to make direct contact with cells located at the end of a person's arms or legs. Axons emerge from a spherical region of the cell body called the *axon hillock*, which is characterized by a reduced content of free and membrane-bound ribosomes.

The cytoplasm of dendrites and axons often contains numerous vesicles and mitochondria, but the most prevalent components are cytoskeletal filaments that provide mechanical support and facilitate the movement of proteins and organelles. The cytoskeletal elements involved in these activities include microtubules, neurofilaments, and actin filaments (Figure 17-3). **Microtubules** (also called *neurotubules*) are present in high concentration in both axons and dendrites; in axons they are oriented with their plus ends (the end where microtubule assembly occurs) pointing away from the cell body, whereas in dendrites they are oriented in both directions—that is, with their plus ends pointing both away from and toward the cell body (see Figure 13-66). **Neurofilaments**, which are a type of intermediate filament (page 619), occur in highest concentration in axons, where they are organized into longitudinal crosslinked arrays that provide mechanical support and help keep the axon from breaking. Finally, **actin filaments** form a network beneath the plasma membrane of the entire neuron.

Glial Cells Play a Supporting Role and Produce Myelin Sheaths

Supporting cells called **glial cells** occur in large numbers in the nervous system, filling the spaces between neuron cell bodies and surrounding the axons and dendrites. In the mammalian brain, glia outnumber neurons by a ratio of at least ten to one. Two types of glial cells predominate in the *central nervous system* (brain and spinal cord). The first, called an **astrocyte**, is a star-shaped cell possessing long cytoplasmic extensions that contain large numbers of intermediate filaments composed of *glial acidic fibrillary protein*. The great mechanical strength of intermediate filaments suggests that astrocytes provide structural support for the nervous system.

The other type of glial cell found in the central nervous system is the **oligodendrocyte**, which produces insulating sheaths that allow axons to transmit electrical signals quickly and efficiently. The presence of such sheaths was first noted in the 1860s by Rudolph Virchow while he was investigating the microscopic organization of different regions of the brain. When viewed with the naked eye, the vertebrate brain and spinal cord

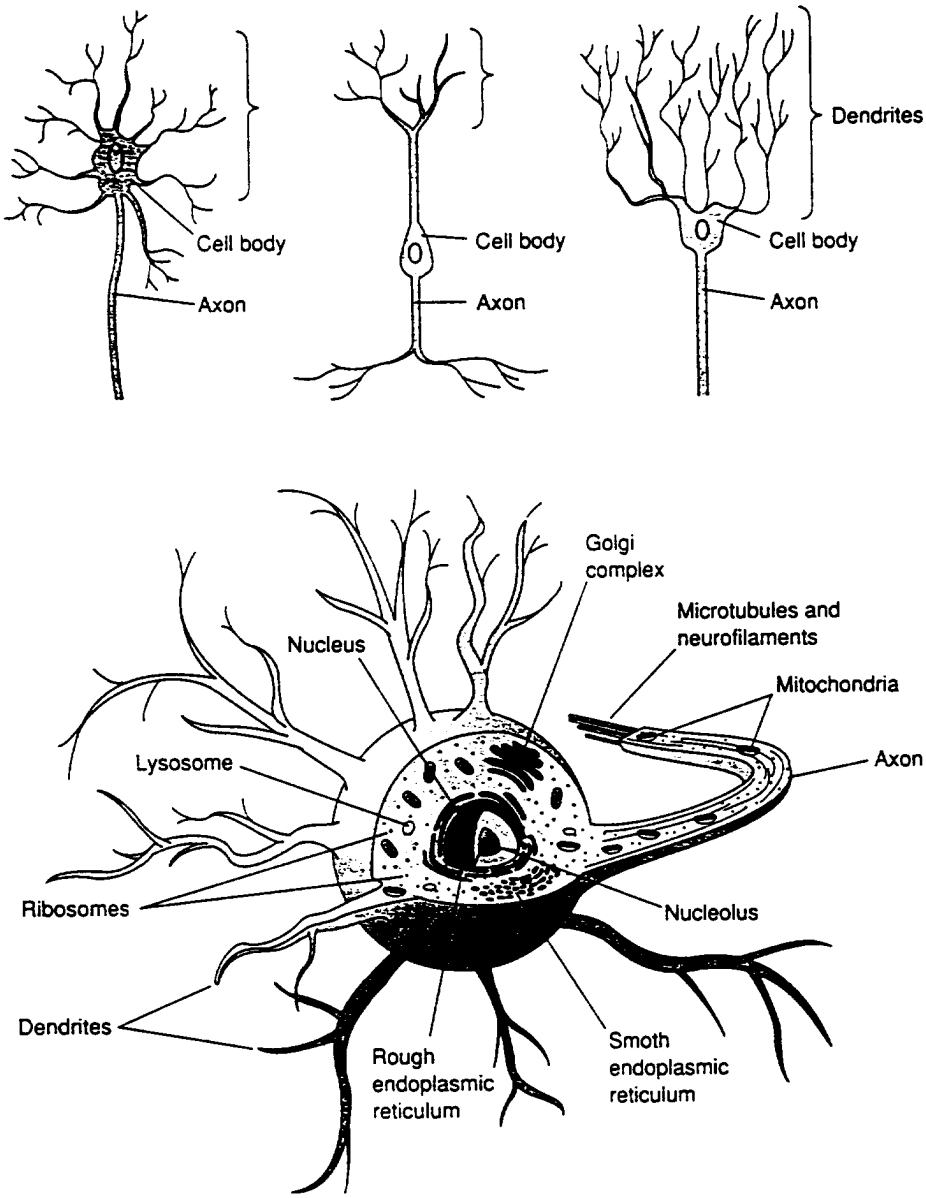


Figure 17-2 *Organization of the Neuron* (Top) *Schematic diagram showing several arrangements of the cell body, axon, and dendrites.* (Bottom) *Diagram of a single neuron showing the various organelles located in the cell body.*

Table 17-1 Distinguishing Features of Typical Dendrites and Axons

Dendrites	Axons
Many per cell	Never more than one or two
Relatively short (< 1 mm)	May be very long (1 meter or more)
Diameter lessens with distance from the cell body	Diameter relatively constant
Many branches	Few branches
Contain spines	Smooth surface
Never myelinated	Often myelinated
Microtubules usually outnumber neurofilaments	Neurofilaments usually outnumber microtubules
Microtubules oriented both toward and away from cell body	Microtubules oriented with minus end toward cell body
Ribosomes present	Ribosomes absent
Receive signals	Send signals

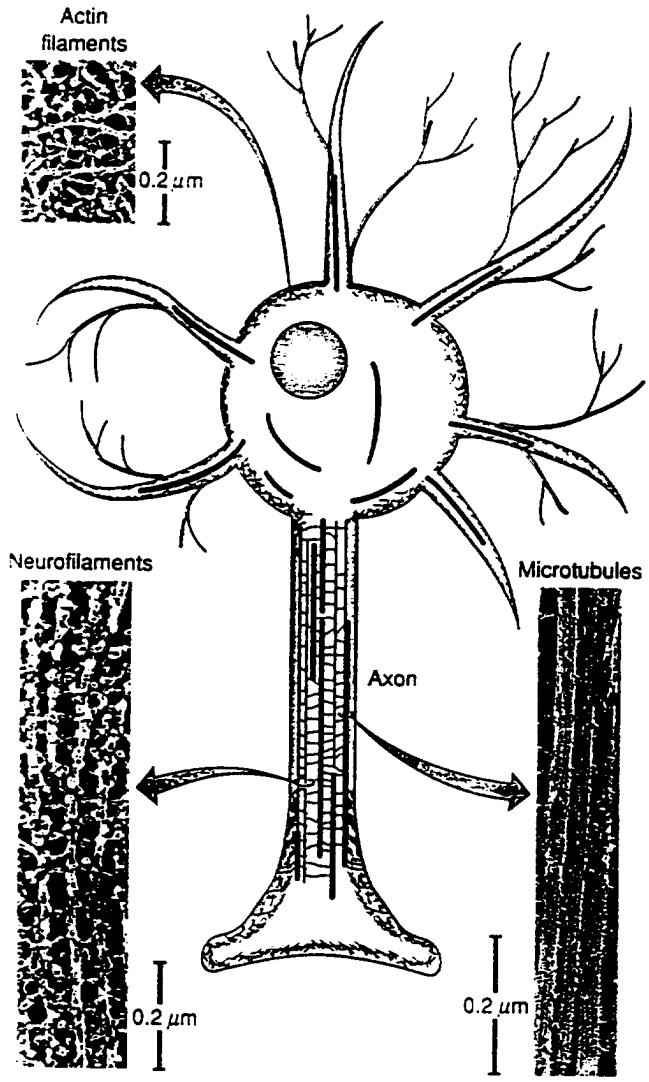


Figure 17-3 *The Neuronal Cytoskeleton* *Microtubules and neurofilaments are concentrated in axons and dendrites, where they are oriented in parallel. Actin filaments are arranged in a meshlike network beneath the plasma membrane of the entire neuron. Micrographs courtesy of N. Hirokawa.*

exhibit differently colored regions referred to as *gray* and *white matter*, respectively. Upon microscopic examination, Virchow discovered that white matter contains a closely packed array of axons covered by a white material which he named **myelin** (from the Greek word for "marrow") because it is concentrated in the core, or marrow, of the brain. Subsequent studies revealed that myelin consists of sheaths of stacked membranes that surround and insulate the axons of many (but not all) neurons, increasing the speed at which electrical signals are transmitted. In the central nervous system, myelin is produced by oligodendrocytes, which wrap their plasma membranes around axons to produce a multilayered membrane sheath; in peripheral nerves, the comparable function is carried out by another kind of glial cell, the **Schwann cell** (see Figure 5-3). The final thickness of a myelin sheath is directly related to axon diameter: the largest axons have myelin sheaths with the greatest number of membrane layers, whereas small axons have fewer layers, or even none at all.

In the late 1870s the French pathologist Louis-Antoine Ranvier first noted that the myelin sheath is periodically interrupted, creating **nodes of Ranvier** where one glial cell terminates and the next one begins. As each node is approached, the layers of myelin terminate one by one until the plasma membrane of the axon is exposed (Figure 17-4). We will see later in the chapter that these bare areas of plasma membrane are directly involved in the mechanism by which electrical signals are propagated along an axon.

Axonal Transport Moves Materials Back and Forth along the Axon

Because the nucleus and biosynthetic machinery of the neuron are localized in the cell body, far removed from distant regions of the axon, the question arises as to how the axon's supply of essential molecules and organelles is maintained. Simple diffusion is not an adequate explanation, because even small molecules such as glucose would take months or years to diffuse the length of a typical axon. To solve this problem, neurons have developed mechanisms for transporting materials down the axon at rates that vary with the substance being transported. The transport process was first detected in 1948 by Paul Weiss and Helen Hiscoe, who found that constricting an axon causes a swelling to appear just before the point of obstruction (Figure 17-5, *top*). When the constriction is removed, the accumulated material can be seen migrating down the axon at a rate of about 1–5 mm per day. Weiss initially proposed that the flow of material is caused by bulk cytoplasmic movements propelled by contractile waves moving down the axon surface. Although the postulated contractile waves have been detected in neurons growing in culture (where bulk cytoplasmic flow may contribute to axon lengthening), comparable evidence for bulk cytoplasmic flow in the nongrowing axons of mature neurons has not been forthcoming. Indeed, it would be hard to explain what happens to the flowing cytoplasm that would continually be arriving at the axon tip in mature cells.

It has subsequently been shown that movement of materials down nongrowing axons involves the transport of substances through the cytoplasm rather than bulk movement of the cytoplasm itself. Two broad classes of transport have been detected. The first, called **slow axonal transport**, moves proteins and cytoskeletal filaments down the axon at rates of about 1–5 mm per day. Although microtubule sliding has been implicated in this type of transport, the force-generating mechanism has not been clearly identified.

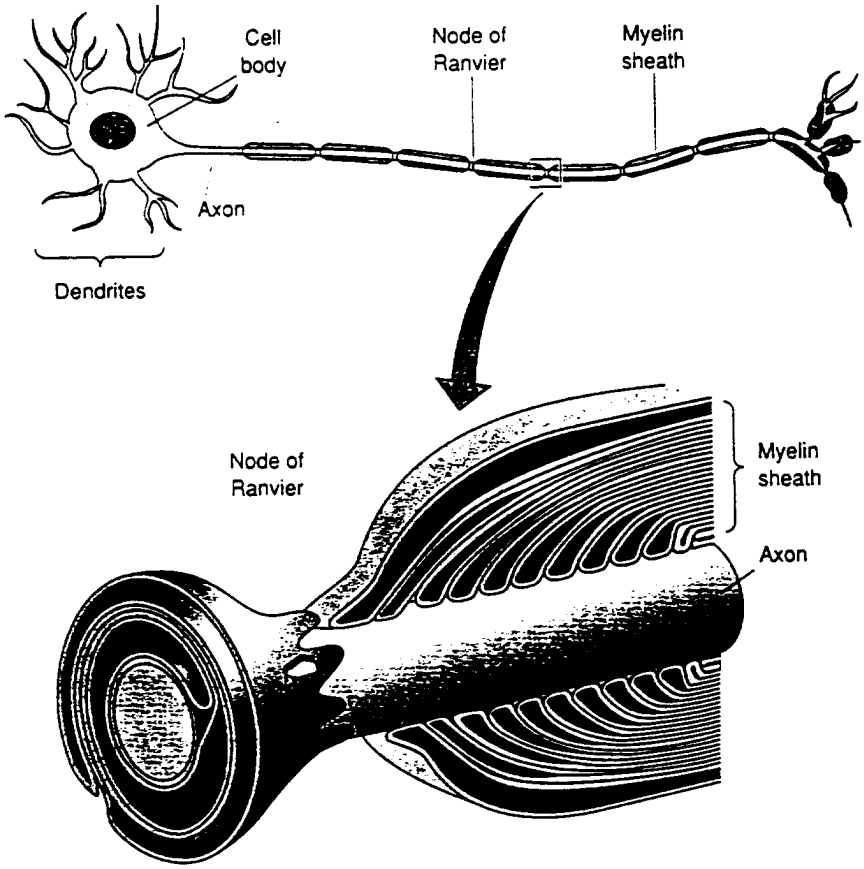


Figure 17-4 *Structure of the Node of Ranvier* *The diagram of the entire neuron* (top) *shows how the nodes of Ranvier interrupt the myelin sheath. In the enlarged diagram at the bottom, the cutaway portion* (right side) *shows how the layers of myelin terminate one by one as the node is approached, exposing the plasma membrane of the axon.*

The other type of transport is called **fast axonal transport** because it moves materials up to a hundred times faster than slow axonal transport. Fast transport was first detected by injecting radioactive substances into the cell body and then monitoring the appearance of radioactivity at various points along the axon (see Figure 17-5, *bottom*). Such experiments revealed that whereas slow axonal transport moves certain molecules down the axon at rates of about 1-5 mm per day, other materials are simultaneously propelled down the same axon by fast transport at rates of 10-500 mm per day (Figure 17-6). At these rates, it would take about a day for fast transport and a year for slow transport to move a cellular component from the cell body to the tip of an axon 35 cm long.

In addition to differing in speed, fast and slow transport can be distinguished by differences in the materials being transported. Slow transport mediates the movement of proteins and cytoskeletal filaments, whereas fast transport propels membrane-bound organelles such as mitochondria and membrane vesicles. Another difference is that slow transport moves substances only toward the axon tip (the *anterograde* direction), but fast transport can move materials toward the axon tip as well as back toward the cell body (the *retrograde* direction). Fast transport in the anterograde direction moves mitochondria and neurotransmitter-filled vesicles toward the axon tip; fast transport in the retrograde transport moves other membrane-bound vesicles back to the cell body. As we learned in Chapter 13, fast transport is mediated by the motor proteins *kinesin* and *dynein* (pages 616-617). Vesicles linked to axonal microtubules by kinesin move in the anterograde direction, whereas vesicles attached to microtubules by dynein migrate in the retrograde direction.

TRANSMISSION OF NERVE IMPULSES

Neurons transmit signals by changing the electrical potential across their plasma membranes. We explained in Chapter 6 that the main type of electrical signal used by nerve and muscle cells is the **action potential** (pages 199-202). An action potential is caused by stimuli that trigger a transient increase in membrane permeability to Na^+, thereby allowing a tiny amount of Na^+ to diffuse

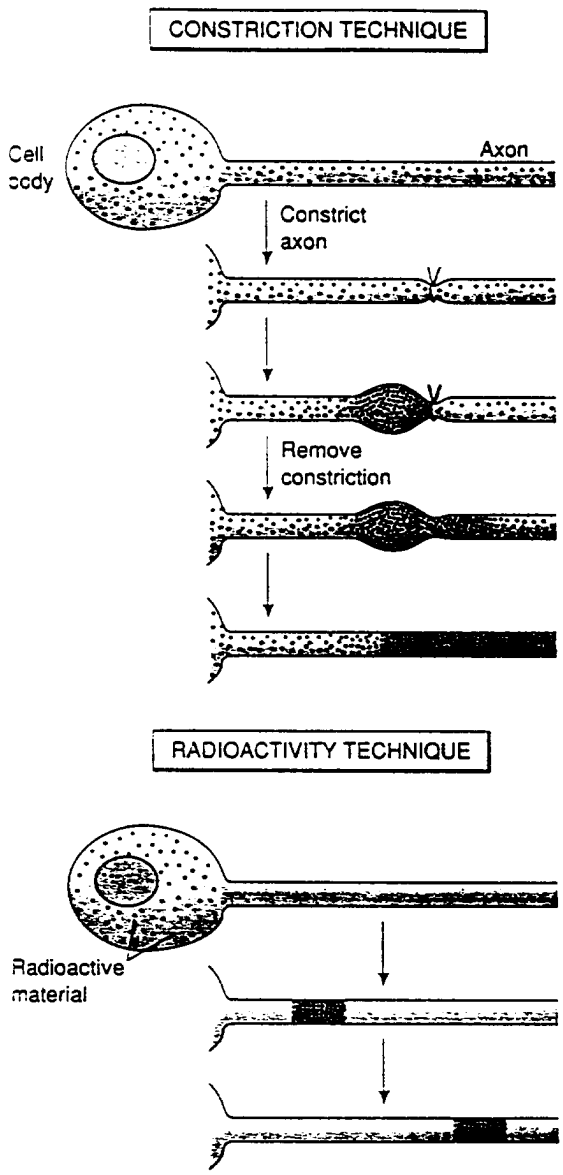


Figure 17-5 ***Two Methods for Detecting Axonal Transport*** (Top) *Placing a constriction in an axon causes material to accumulate just before the constriction point. When the constriction is removed, the mass of accumulated material can be observed migrating down the axon.* (Bottom) *Radioactive substances injected into a cell body are incorporated into cellular molecules that gradually migrate down the axon. The moving radioactivity can be detected either by autoradiography or by cutting the axon into segments and directly measuring the radioactivity in each segment (see Figure 17-6).*

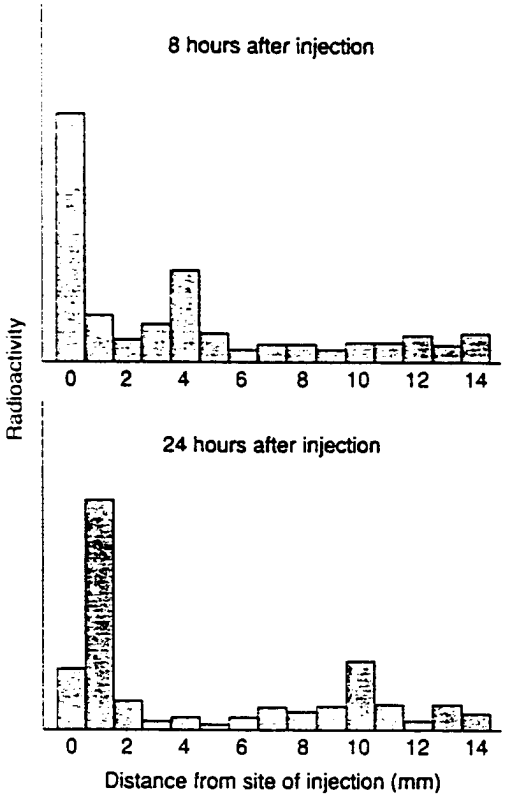


Figure 17-6 ***Evidence for the Existence of Both Slow and Fast Axonal Transport in the Same Axon*** *After injecting radioactive amino acids into the cell bodies of crayfish neurons, the axons were cut into 1-mm sections and the radioactivity in each segment was measured. After 24 hours, one peak of radioactivity has migrated about 10 mm down the axon (fast axonal transport) while a second, slower-moving peak of radioactivity has migrated only 1 mm from the cell body (slow axonal transport).*

into the cell. The resulting small depolarization causes a few voltage-gated Na^+ channels to open, which in turn permits an additional influx of Na^+ that depolarizes the membrane even further and thus causes more Na^+ channels to open. As this cycle repeats, the rapidly increasing depolarization of the plasma membrane eventually triggers the opening of voltage-gated K^+ channels; K^+ therefore diffuses out of the cell, reestablishing the normal membrane potential.

Although action potentials play a prominent role in neural communication, they are only one aspect of the mechanism by which electrical signals are transmitted through the nervous system. In this segment of the chapter, we will see how action potentials and other types of electrical signals are propagated along the neuron surface and then transmitted from one neuron to another.

Axon Diameter and Myelination Influence the Rate at Which Action Potentials Are Propagated

Once an action potential has been triggered at a given site on a nerve cell plasma membrane, the signal must be propagated to the end of the axon so that it can be transmitted to another cell. The way in which the electrical signal is propagated along the plasma membrane varies among different types of neurons. Let us begin by considering the behavior of neurons with long axons (>1 mm). To initiate an action potential, a stimulus must

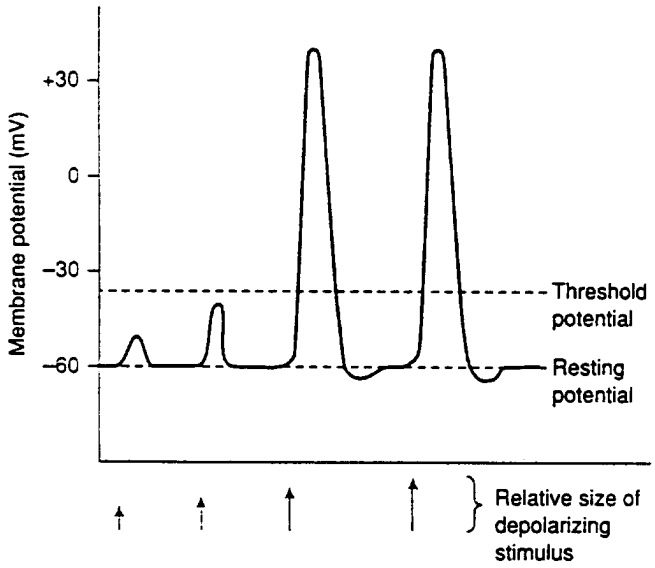


Figure 17-7 *Evidence for the Existence of a Threshold Potential* *If a small electric current is applied to a neuron to trigger a tiny depolarization, an action potential is not elicited. An action potential is triggered only after the depolarizing stimulus has exceeded a critical value called the threshold potential. Once the threshold potential has been reached, further increases in the magnitude of the depolarizing stimulus do not affect the size of the action potential.*

first depolarize a site on the neuron plasma membrane to a critical value termed the **threshold potential** (Figure 17-7). An increase in stimulus strength beyond this point has no further effect on the magnitude of the action potential. The reason subthreshold stimuli do not bring about action potentials is that the Na^+ influx caused by small depolarizations is less than the normal rate of K^+ efflux, allowing the latter to return the membrane potential to its resting value. Hence an action potential can only be triggered by an initial depolarization that is large enough to produce a rate of Na^+ influx that is greater than the initial rate of K^+ efflux.

Once an initial action potential has been triggered, a local flow of electric current is induced that alters the membrane potential in surrounding areas of the plasma membrane; during this process, positive ions associated with the inner surface of the plasma membrane at the site of the action potential diffuse to adjacent, negatively charged regions of the inner membrane surface, creating a flow of current that depolarizes the surrounding membrane and triggers new action potentials (Figure 17-8). This cycle is repeated again and again along the membrane surface, creating a series of action potentials that travel away from the original site of excitation. Since most action potentials are initiated at or near the cell body, impulses tend to travel away from this region and toward the end of the axon. Each newly initiated action potential is equivalent in magnitude to the original one, so the signal that eventually reaches the tip of the axon is virtually identical to the originating signal.

The velocity of action potential propagation is influenced by two principal factors: axon diameter and myelination. Large-diameter axons provide less electrical resistance to the flow of current, so action potentials move along the plasma membrane more rapidly. In quantitative terms, this phenomenon yields a propagation velocity that is roughly proportional to the square root of the axon diameter (Figure 17-9, *inset*).

Myelination, on the other hand, influences propagation velocity by virtue of its insulating effect. In unmyelinated axons, the ions carrying the local currents that trigger new action potentials can leak back across the plasma membrane, thereby reducing the flow of current to adjacent membrane regions and hence decreasing the effectiveness of action potential propagation. By reducing ion leakage, the presence of a myelin sheath increases the speed at which areas adjacent to the initial action potential are brought to threshold. Because action potentials can only occur where the plasma membrane is exposed to extracellular fluid (which contains the Na^+ needed for an action potential), the presence of a myelin sheath also influences propagation velocity by preventing action potentials from occurring anywhere except at the nodes of Ranvier, where myelin is absent. Action potentials in myelinated axons therefore jump from node to node, a phenomenon known as *saltatory conduction*. The time required for a depolarizing current to flow from node to node is small compared to the time needed to generate a new action potential at each node, so propagation velocity increases with increasing distance between nodes. The distance between nodes, which are spread out along the axon, increases with axon diameter, leading to faster propagation in larger axons (see Figure 17-9). Myelination permits large axons to propagate action potentials at velocities in excess of 100 meters per second, which is an order of magnitude faster than the velocity of impulse conduction in unmyelinated nerve axons of similar diameter. The importance of myelination for normal signal transmission is vividly demonstrated by the disease *multiple sclerosis*, an autoimmune disorder in which destruction of myelin in various regions of the brain causes speech and vision defects, tremors, and paralysis (Figure 17-10).

Although the propagation of action potentials is an efficient means of transmitting electrical signals in neurons with long axons, most neurons in the central nervous system have axons measuring less than a millimeter in length and do not utilize (nor can they generate) action potentials for signal transmission. If a neuron of this type is partially depolarized, either by an external stimulus or by a chemical signal released from another neuron, the localized change in membrane potential is passively conducted across the rest of the plasma membrane by local current flow without ever triggering an action potential. However, the leakage of ions through the plasma membrane causes local currents to dissipate rapidly and hence signal transmission is limited to distances of about a millimeter. But in

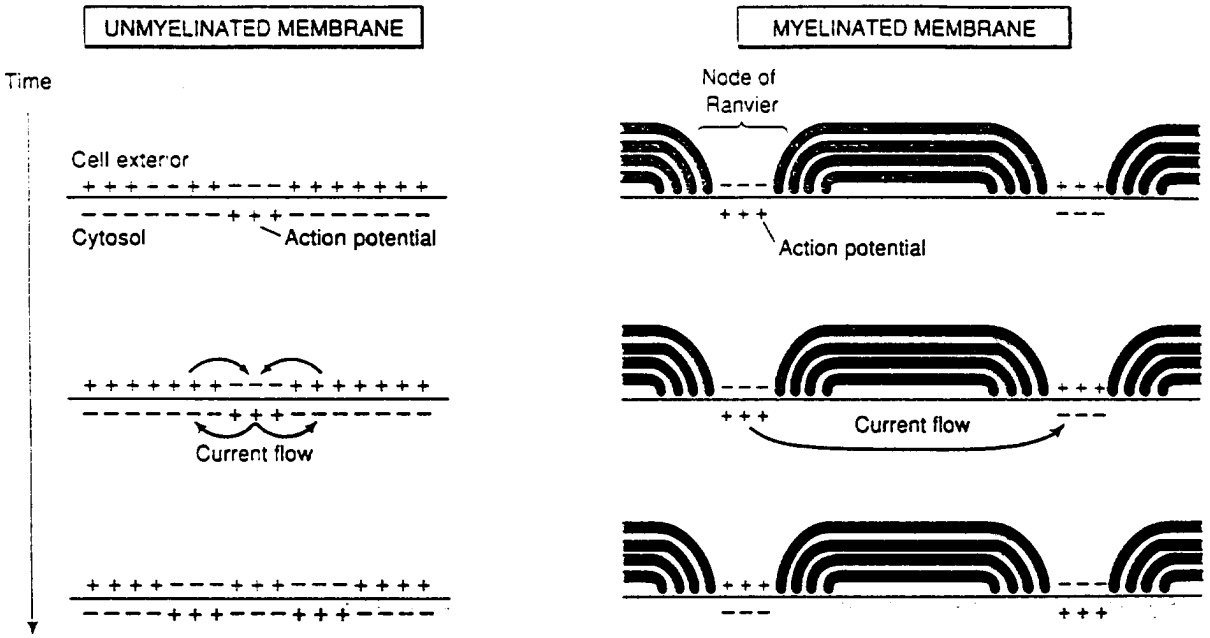


Figure 17-8 *Propagation of Action Potentials* (Left) *After an initial action potential has been triggered in an unmyelinated axon, a flow of current is induced that depolarizes the surrounding membrane, causing action potentials to spread along the axon.* (Right) *In myelinated axons, action potentials can only occur where the plasma membrane is exposed to the extracellular fluid. Therefore action potentials jump from one node of Ranvier to the next.*

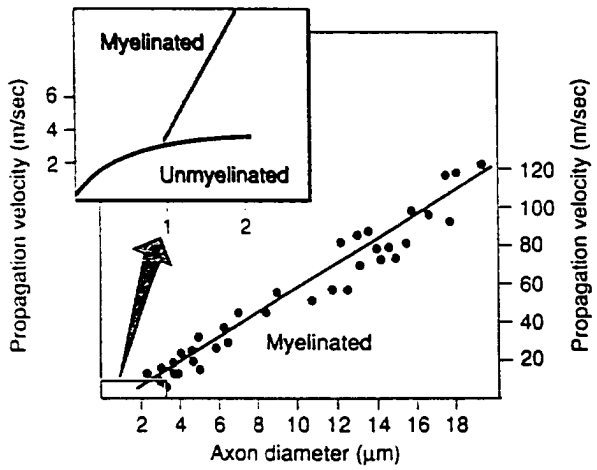


Figure 17-9 *Relationship between Axon Diameter and the Velocity at Which Nerve Impulses Are Propagated* *In unmyelinated axons, velocity increases with the square root of axon diameter* (inset). *Myelinated axons propagate impulses much faster because the action potential jumps from one node of Ranvier to the next. Since the spacing between nodes increases linearly with axon diameter, velocity in myelinated nerves increases linearly with diameter.*

spite of this limitation, the transmission of local currents has the advantage of being equally suitable for propagating membrane hyperpolarizations and depolarizations. The existence of this mechanism for transmitting signals means that a **nerve impulse** can be any change in membrane potential that is transmitted along nerve cells, including, but not restricted to, action potentials.

Neurons Communicate with Each Other at Specialized Junctions Called Synapses

Electrical impulses that travel along nerve cells have an inherent directionality that is imposed by the organization of the cell's dendrites and axons. Dendrites are designed to receive signals from sensory cells or the axons of other neurons, whereas axons transmit signals to other neurons or to appropriate target cells. The directionality of signal transmission is imposed by cell-cell junctions called **synapses**, which are specialized for transmitting nerve impulses from one cell to another. Most synapses transmit signals in one direction only, from the *presynaptic* cell to the *postsynaptic cell.* An individual neuron may be involved in hundreds or even thousands of different synapses, functioning as the presynaptic cell at some synapses and the postsynaptic cell at others. While many synapses transmit signals from the axon of one neuron to the dendrite of a second neuron, others may transmit signals from axon to cell body, axon to axon, or even dendrite to dendrite. Synapses also transmit signals from nerve axons to other cell types, such as secretory cells and muscle cells. The synapses that link nerve axons to muscle cells, known as *neuromuscular junctions*, have already been described in Chapter 13 (page 573).

Synapses are of two fundamentally different types: *chemical* and *electrical* (Figure 17-11). **Chemical synapses**, which are the most common, transmit signals by releasing chemical *neurotransmitters* that diffuse from the presynaptic cell to the postsynaptic cell. A space of 20 to 30 nm, known as the **synaptic cleft**, separates the plasma membranes of the presynaptic

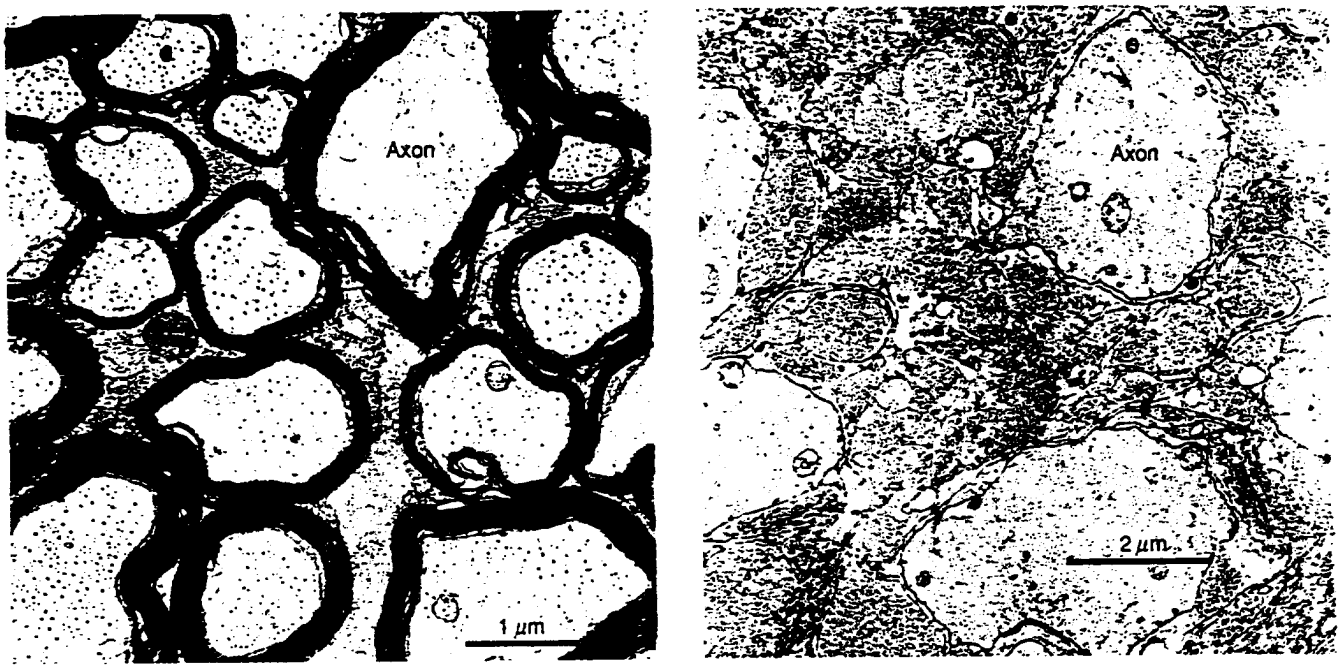


Figure 17-10 *Disruption of the Myelin Sheath in Multiple Sclerosis* (Left) *Thin-section electron micrograph showing myelinated axons in a normal individual.* (Right) *Electron micrograph of nervous tissue from an individual with multiple sclerosis, showing axons lacking their normal myelin sheath. Courtesy of C. S. Raines.*

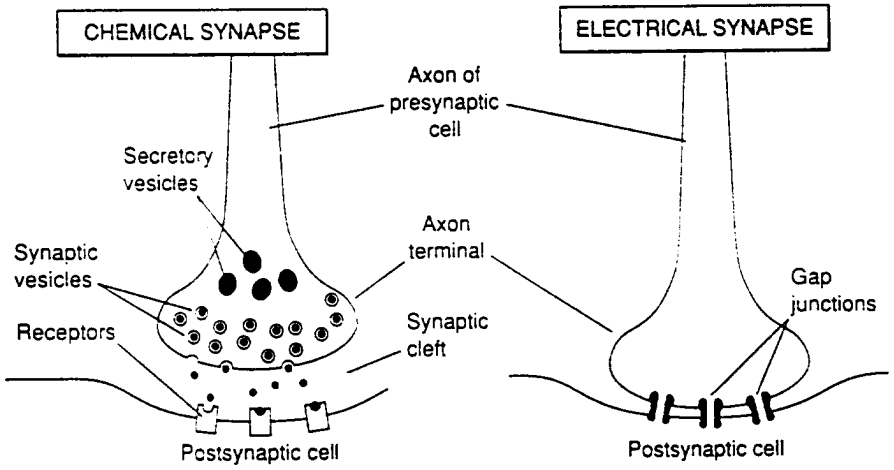


Figure 17-11 *Structure of Chemical and Electrical Synapses* *In chemical synapses, signals are transmitted by chemical neurotransmitters that are discharged by exocytosis from the presynaptic cell, diffuse across the synaptic cleft, and bind to receptors on the surface of the postsynaptic cell. In electrical synapses, signals are transmitted by ions that flow directly through gap junctions that link the presynaptic and postsynaptic cells.*

and postsynaptic cells in the region of a chemical synapse. The tip of the axon is expanded near the synapse to form a bulblike structure called an **axon terminal.** Axon terminals are characterized by the absence of a myelin sheath and the presence of numerous vesicles that contain neurotransmitters destined to be released from the axon upon arrival of an electrical impulse (Figure 17-12). These neurotransmitter-filled vesicles are of two general types: small **synaptic vesicles** about 50 nm in diameter that are localized directly beneath the plasma membrane at the axon tip, and larger **secretory vesicles** measuring 90–250 nm that are more diffusely distributed in the axon terminal.

In **electrical synapses,** which are relatively rare, the presynaptic and postsynaptic cells are physically joined by *gap junctions* (page 240) that allow direct electrical communication between the cytoplasms of the two cells. At such locations, the plasma membranes of the presynaptic and postsynaptic cells are separated

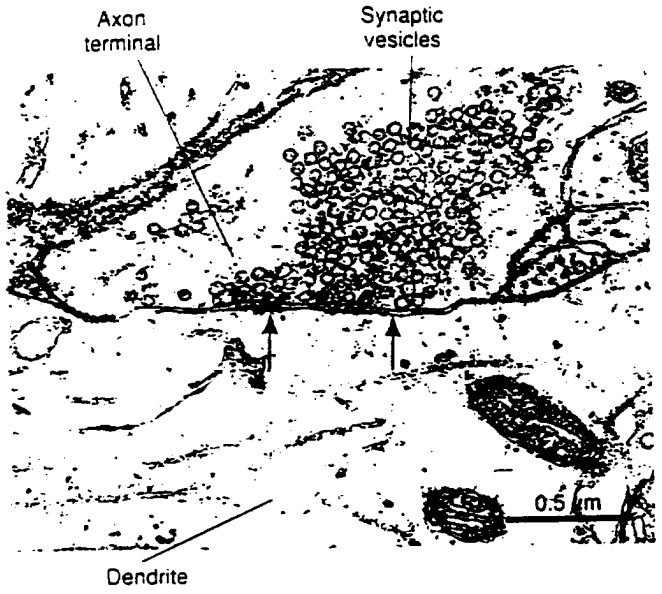


Figure 17-12 *Electron Micrograph of a Chemical Synapse* *The axon terminal contains numerous small synaptic vesicles. The arrows point to the synaptic cleft. Courtesy of S. G. Waxman.*

Figure 17-13 *Electron Micrograph of an Electrical Synapse* *This micrograph shows an axon and dendrite whose plasma membranes are very closely apposed where the two cells are joined by an electrical synapse (arrows). Numerous gap junctions, too small to be seen at this magnification, link the two cells in the region of the synapse. Courtesy of S. G. Waxman.*

by a space of about 2 nm, which is roughly one-tenth the distance of the synaptic cleft in chemical synapses (Figure 17-13). The flow of ions through the gap junctions of electrical synapses allows membrane depolarization to pass directly from the presynaptic to the postsynaptic cell. This direct electrical coupling permits signals to be transmitted across the synapse more rapidly than at chemical synapses, where time is required for the diffusion of chemical neurotransmitters across the synaptic cleft.

Neurons Employ a Variety of Different Chemicals as Neurotransmitters

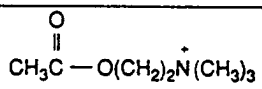
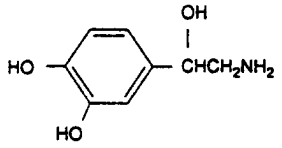
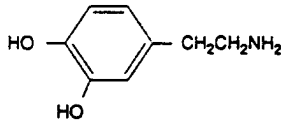
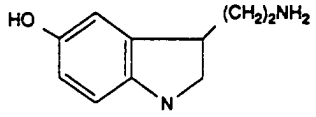
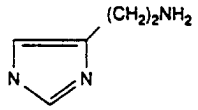
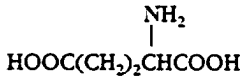
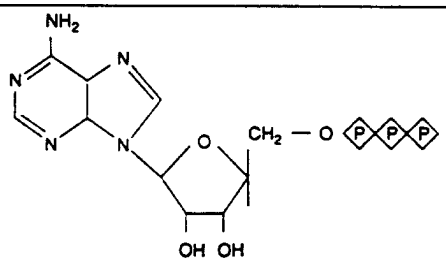
When a nerve impulse reaches a synapse, the signal must be transmitted to the adjacent neuron. Signals move across synapses so rapidly that for many years synaptic transmission was thought to be a purely electrical phenomenon. Although this explanation has turned out to be correct for electrical synapses, most synapses are of the chemical type, where signals are carried across the synaptic cleft by the diffusion of molecules called **neurotransmitters.** Two criteria must be met before a substance can qualify as a neurotransmitter. First, the molecule in question must be released from nerve endings upon stimulation, and second, it must be shown that administering the molecule to the postsynaptic cell evokes the same response as a normal nerve impulse. Based on these criteria, several dozen molecules have been identified as neurotransmitters, including the amines *acetylcholine, norepinephrine, dopamine, serotonin,* and *histamine;* the amino acids *glutamate, γ-aminobutyric acid (GABA),* and *glycine;* the peptides *enkephalin, β-endorphin, neurotensin, somatostatin,* and *substance P;* and the purine nucleotide, *ATP* (Table 17-2).

To determine which type of neurotransmitter is released at any given synapse, cells can be stained with fluorescent antibodies designed to detect the presence of particular compounds. A surprising conclusion to emerge from such studies is that many neurons utilize more than one kind of neurotransmitter. In numerous cases, a single axon terminal has been found to store both a small molecule neurotransmitter—such as acetylcholine, dopamine, serotonin, norepinephrine, GABA, or glycine—along with a peptide neurotransmitter like enkephalin, neurotensin, somatostatin, or substance P. Another common arrangement is the presence of two or more peptide neurotransmitters in the same axon terminal, and in a few cases, two small molecule neurotransmitters (e.g., serotonin and GABA) have been detected together.

Neurotransmitters Are Stored in Vesicles That Discharge Their Contents into the Synaptic Cleft

During the transmission of signals across a chemical synapse, neurotransmitters are released from the axon terminal. An early clue to the mechanism underlying

Table 17-2 Examples of Neurotransmitters

Neurotransmitter	Structure	Major Site of Action
Amines		
Acetylcholine		Neuromuscular junction and brain
Norepinephrine		Central nervous system and sympathetic nervous system
Dopamine		Brain and sympathetic nervous system
Serotonin		Brain
Histamine		Hypothalamus
Amino Acids		
γ-Aminobutyric acid (GABA)	$HOOC(CH_2)_3NH_2$	Central nervous system
Glycine	$HOOCCH_2NH_2$	Spinal cord
Glutamate		Central nervous system
Peptides		
Thyrotropin releasing factor (TRF)	3 amino acids	Hypothalamus
Leu-enkephalin	5 amino acids	Brain
Met-enkephalin	5 amino acids	Brain
Gonadotropin releasing factor (GnRF)	10 amino acids	Hypothalamus
Somatostatin	14 amino acids	Hypothalamus
β-Endorphin	31 amino acids	Pituitary
Substance P	13 amino acids	Brain
Neurotensin	10 amino acids	Brain
Calcitonin gene-related peptide (CGRP)	37 amino acids	Brain
Vasoactive intestinal peptide (VIP)	28 amino acids	Brain, intestine
Purines		
ATP		Brain

Note: This table is only a partial listing of neurotransmitters. The number of peptides released from nerve cells and implicated in chemical signaling in the nervous system is significantly longer than the list presented here.

neurotransmitter release was provided in the 1950s by Bernhard Katz, who discovered that the postsynaptic membrane of resting frog muscle undergoes continual spontaneous depolarizations measuring about 0.5 mV. These **miniature postsynaptic potentials** are orders of magnitude smaller than the larger depolarizations, called **excitatory postsynaptic potentials**, that occur in the postsynaptic membrane when the presynaptic neuron is actually stimulated. But careful examination of the size of the depolarizations produced by normal nerve stimulation revealed them to be exact multiples of the spontaneous miniature potentials, leading Katz to conclude that neurotransmitter is released from the presynaptic neuron in discrete units or *quanta*, rather than in continuously graded amounts.

At about the same time as the quantal release theory was being formulated, electron microscopists discovered the presence of membrane vesicles in axon terminals, and subcellular fractionation studies revealed the presence of neurotransmitters in these vesicles. The preceding observations led to the speculation that each vesicle contains a "quantum" of neurotransmitter that is released from the cell upon nerve stimulation. According to this theory, miniature postsynaptic potentials are caused by the spontaneous expulsion of the contents of a few vesicles from the axon terminal, while the excitatory postsynaptic potentials that occur during normal nerve stimulation are produced by the release of hundreds of vesicles triggered by the arrival of an action potential. As would be predicted by this hypothesis, excessive stimulation of nerve cells has been shown to deplete the vesicles that normally reside in the axon terminal. Presynaptic vesicles have even been observed in the process of fusing with the plasma membrane, providing direct support for the conclusion that the neurotransmitters stored in these vesicles are discharged into the synaptic cleft (Figure 17-14).

Figure 17-14 *Electron Micrograph Showing Fusion of Synaptic Vesicles with the Presynaptic Plasma Membrane* *The arrows point to two synaptic vesicles in the axon terminal* (top) *discharging their contents into the synaptic cleft. Courtesy of J. E. Heuser.*

Neurotransmitter Release Is Regulated by the Entry of Calcium Ions into the Axon Terminal

How does an action potential arriving at an axon terminal trigger the discharge of neurotransmitter-filled vesicles? Like exocytosis in other cell types (page 284), neurotransmitter discharge is regulated by calcium ions. The plasma membrane of an unstimulated neuron is relatively impermeable to Ca^{2+}, whose concentration is higher outside the cell (about $10^{-3}M$) than inside (about $10^{-7}M$) When an action potential reaches the axon terminal, the membrane depolarization causes voltage-gated Ca^{2+} channels in the presynaptic plasma membrane to open. Calcium ions then diffuse into the cytosol and trigger the exocytosis of neurotransmitter-filled vesicles. Experimental support for the preceding scenario has come from studies using *aequorin*, a protein that emits light when exposed to Ca^{2+}. If aequorin is introduced into nerve cells, light is emitted at the axon terminal each time the cell transmits a nerve impulse, indicating an increase in the cytosolic Ca^{2+} concentration. The proposed role of these calcium ions in triggering the release of neurotransmitter has been verified by showing that neurotransmitter discharge can be induced by injecting Ca^{2+} directly into axon terminals.

Compared to exocytosis in other cell types, Ca^{2+}-induced neurotransmitter release into the synaptic cleft is extremely rapid. The speed of the process is based on the existence of two populations of synaptic vesicles: (1) a *releasable pool* of vesicles bound to the inner surface of the plasma membrane and poised for neurotransmitter release, and (2) a *reserve pool* of vesicles attached to the cytoskeleton by the protein **synapsin**. To explain the speed of neurotransmitter release, it has been proposed that the releasable vesicles are bound to the plasma membrane in a way that aligns a channel-forming protein in the vesicle membrane with a comparable protein in the plasma membrane (Figure 17-15). According to this model, the channel formed by the two proteins would normally be closed, but an increase in Ca^{2+} concentration triggers a conformational change that allows the channel to open and release neurotransmitter directly into the synaptic cleft.

The vesicles that make up the releasable pool represent a small fraction of the total synaptic vesicle population. It has therefore been suggested that controlling

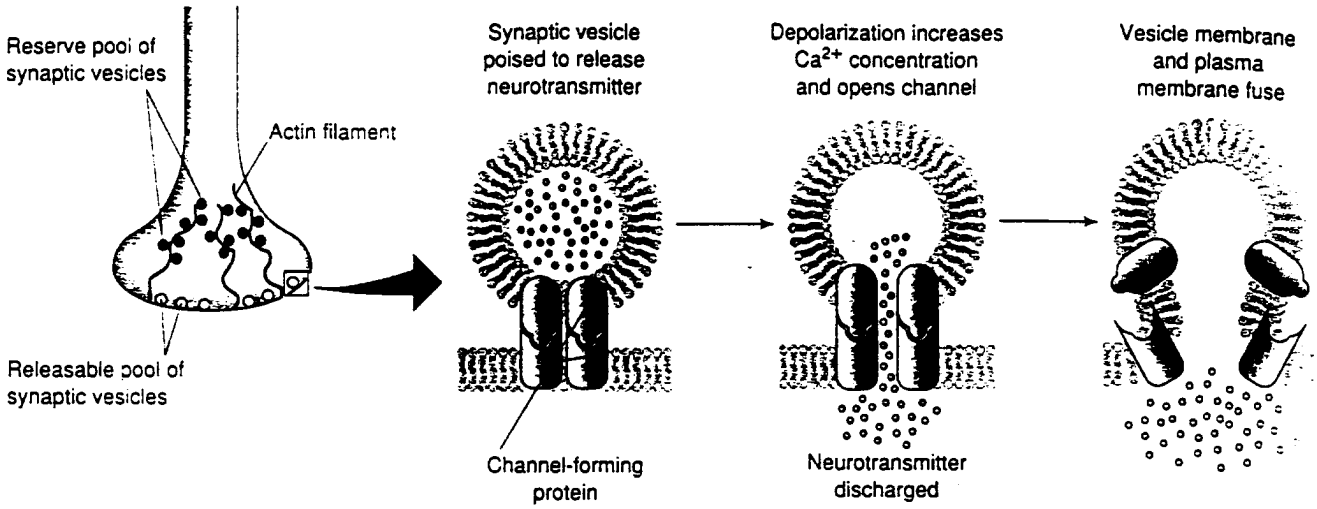


Figure 17-15 *A Model That Has Been Proposed to Explain the Speed of Neurotransmitter Release* *Synaptic vesicles are divided into two populations: a reserve pool of vesicles bound to the actin cytoskeleton and a releasable pool of vesicles bound to the presynaptic plasma membrane. It has been suggested that the releasable vesicles are bound to the plasma membrane in a way that aligns a channel-forming protein in the vesicle membrane with a comparable protein in the plasma membrane: calcium ions that enter the axon upon membrane depolarization trigger a conformational change in the channel-forming protein that opens the channel and allows neurotransmitter to diffuse into the synaptic cleft.*

the relative number of vesicles in the releasable pool might be employed by nerve cells as a mechanism for regulating the efficiency of synaptic communication. It is known that the entry of calcium ions into the axon terminal during synaptic transmission stimulates a Ca^{2+}-calmodulin-dependent protein kinase which in turn catalyzes the phosphorylation of *synapsin*. The phosphorylation of synapsin disrupts its ability to link synaptic vesicles to the cytoskeleton, allowing more vesicles to join the releasable pool. Hence the number of vesicles that can discharge neurotransmitter into the synaptic cleft is elevated in neurons that have been repeatedly stimulated, making synaptic transmission more efficient.

Synaptic Vesicles Are Recycled after Fusing with the Presynaptic Membrane

Since neurotransmitter release is accompanied by the fusion of synaptic vesicles with the presynaptic membrane, a mechanism must exist for preventing uncontrolled expansion of the plasma membrane during synaptic transmission. The discovery that the number of vesicles present in the axon terminal does not decrease significantly during normal stimulation of nerve cells suggests that the membrane material being added to the plasma membrane is continually being recycled to form more vesicles. The recycling process can be observed microscopically by incubating neurons in a medium containing *horseradish peroxidase*, a tracer molecule that is easily visualized. Nerves stimulated in the presence of horseradish peroxidase collect the tracer in new vesicles that form by invagination of the plasma membrane (Figure 17-16). These vesicles are of a special type called *coated vesicles*, which contain a protein coat made of *clathrin* (page 296). The coated vesicles coalesce into larger structures, which are then converted back into new synaptic vesicles (Figure 17-17). This recycling process does not reutilize vesicle membranes indefinitely, however. In axon terminals where neurotransmitter discharge is actively taking place, some of the horseradish peroxidase appears in lysosomes that are returned to the cell body by retrograde

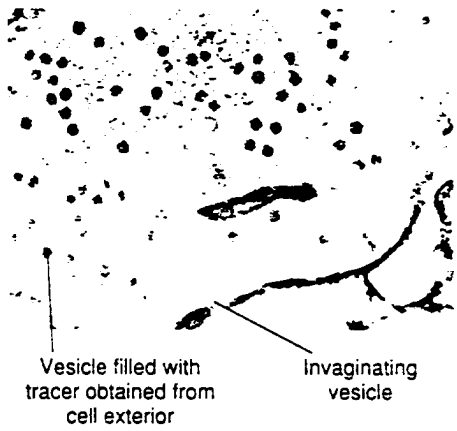


Figure 17-16 *Evidence for Recycling of Synaptic Vesicles* *Neurons were incubated in a medium containing horseradish peroxidase, a tracer molecule that forms an electron-opaque reaction product. The dark synaptic vesicles are filled with the tracer, indicating that these vesicles must have incorporated the horseradish peroxidase when they invaginated from the plasma membrane. Courtesy of A. B. Harris.*

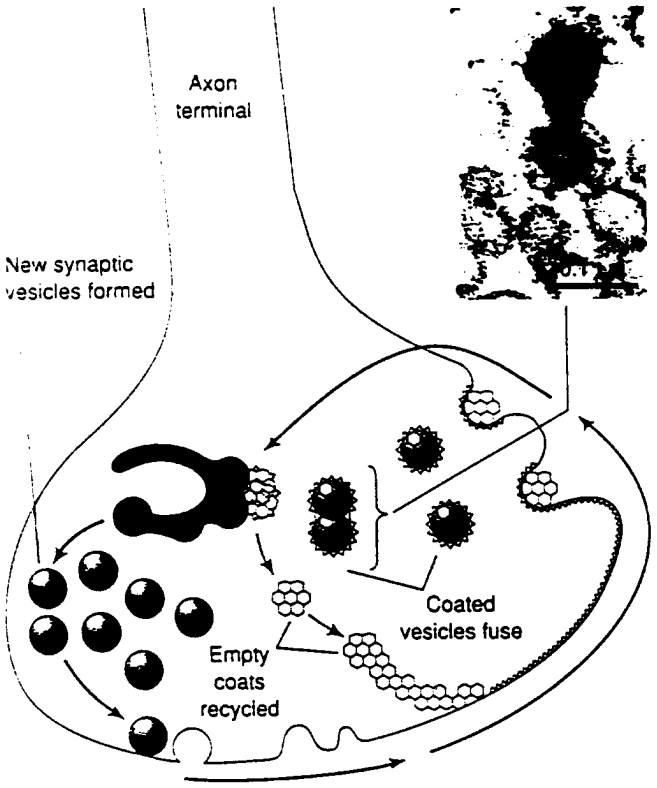


Figure 17-17 ***Synaptic Vesicle Recycling*** *Membrane material added to the plasma membrane during exocytosis of synaptic vesicles is retrieved by endocytosis of coated vesicles that then fuse together, lose their coats, and ultimately subdivide into new synaptic vesicles. The electron micrograph shows the fusion of two coated vesicles with each other. Micrograph courtesy of J. E. Heuser.*

transport. Such vesicles are replaced by new synaptic vesicles that are delivered to the axon terminal by anterograde transport.

Neurotransmitters Bind to Postsynaptic Receptors That Mediate Either Fast or Slow Chemical Transmission

Neurotransmitters that have been released from an axon terminal diffuse across the synaptic cleft in a few tenths of a millisecond and bind to specific receptors located in the postsynaptic plasma membrane. Binding of a neurotransmitter to its corresponding receptor usually brings about a change in the permeability and hence electrical potential of the postsynaptic membrane. *Excitatory* neurotransmitters trigger depolarization of the postsynaptic membrane, whereas *inhibitory* neurotransmitters induce membrane hyperpolarization.

Chemical neurotransmission can be divided into two principal categories based on differences in the type of receptor involved (Figure 17-18). The more familiar category is **fast chemical transmission,** which utilizes receptors that function as *neurotransmitter-gated ion channels.* The binding of a neurotransmitter molecule to such an ion channel receptor alters the permeability of the channel, triggering an immediate change in ion flow across the postsynaptic membrane and a resulting alteration in membrane potential. Because the neurotransmitter receptor alters membrane permeability directly, fast chemical transmission requires only a few milliseconds to transmit a signal from one cell to another. The neurotransmitters most commonly employed for fast transmission are *acetylcholine* and *glutamate* for excitatory signals, and *glycine* and *GABA* for inhibitory signals.

Many synapses also utilize a second signaling mechanism called **slow chemical transmission** because it is slower in both the onset of the response, which requires hundreds of milliseconds, and in the duration of the response, which may last for seconds, minutes, or hours. The receptors employed for slow chemical transmission utilize **G proteins** (page 211) to exert their effects on the postsynaptic cell. G protein-linked receptors themselves are not ion channels, but they can influence ion channels or alter the levels of intracellular second messengers through the action of intermediary G proteins. Most of the *amine* and *peptide* neurotransmitters exert their effects on postsynaptic cells through G protein-linked receptors.

In addition to differing in the type of receptor employed and the speed of the response, slow and fast transmission also differ in the way the neurotransmitters are stored and released in the axon terminal. Fast transmitters are synthesized in the axon terminal and packaged into small *synaptic vesicles* that are secreted from sites specialized for rapid release. In contrast, slow neurotransmitters are usually synthesized and packaged into larger *secretory vesicles* in the cell body; the vesicles are then transported to the axon terminal, where their contents are secreted from many sites. Some neurotransmitters are stored in both kinds of vesicles. Many amines, for example, are stored in both secretory and synaptic vesicles, but they function mainly in slow transmission because their main targets are G protein-linked receptors.

Acetylcholine Is Involved in Both Fast Excitatory Transmission and Slow Inhibitory Transmission

Fast excitatory transmission is most frequently mediated by either acetylcholine or glutamate. Acetylcholine is employed at the neuromuscular junction to trigger the contraction of skeletal muscle (page 573), while glutamate is the main excitatory neurotransmitter in the central nervous system. Acetylcholine and glutamate both mediate fast excitatory transmission by binding to and opening neurotransmitter-gated cation channels in the postsynaptic membrane. Since the electrochemical gradient is steeper for Na^+ than for K^+, the influx of Na^+ exceeds the efflux of K^+, leading to an inward flow of

sodium ions. This depolarizes the postsynaptic membrane to its threshold, triggering an action potential. The best characterized neurotransmitter-gated ion channel is the **nicotinic acetylcholine receptor,** which occurs in the plasma membrane of vertebrate skeletal muscle cells and in the electric organs of certain fish. The three-dimensional structure of this ion channel receptor and the mechanism by which its permeability properties are influenced by acetylcholine were described in Chapter 6 (pages 208-211).

While the interaction of acetylcholine with nicotinic receptors is a classic example of fast excitatory transmission, this is not the only way that acetylcholine acts. Acetylcholine released by certain neurons that innervate heart muscle triggers a slow inhibitory transmission event that leads to a decrease in the rate at which the heart beats. The effects of acetylcholine on skeletal and heart muscle differ because the two tissues have different kinds of acetylcholine receptors. Instead of nicotinic receptors, heart muscle utilizes **muscarinic acetylcholine receptors.** The binding of acetylcholine to muscarinic receptors activates a G protein that causes K^+ channels to open. Since the concentration of K^+ is higher inside the cell than outside, K^+ diffuses out of the muscle cell and the membrane hyperpolarizes (the membrane potential becomes more negative). This hyperpolarization of the plasma membrane inhibits muscle contraction, which is normally triggered by membrane *depolarization.*

Once a neurotransmitter has bound to its receptor and triggered an alteration in the postsynaptic cell, its action must be terminated to prevent the target cell from remaining in a continued state of excitation (or inhibition). In the case of acetylcholine, the enzyme *acetylcholinesterase* residing in the synaptic cleft quickly degrades the neurotransmitter to choline and acetate. Because acetylcholine was the first neurotransmitter to be studied in detail, inactivation by enzymatic degradation was once thought to terminate the action of other neurotransmitters as well. Subsequent research has revealed, however, that enzymatic inactivation is the exception rather than the rule. The most common mechanism for terminating the action of a neurotransmitter is to transport it back into the axon terminal from which it was released, a phenomenon known as *neurotransmitter reuptake.* The reuptake process is highly selective; for example, norepinephrine-releasing neurons take up only norepinephrine, serotonin-releasing neurons take up only serotonin, and so forth.

GABA and Glycine Mediate Fast Inhibitory Transmission

The binding of acetylcholine to muscarinic receptors illustrates the phenomenon of *slow* inhibitory transmission because membrane hyperpolarization is induced by a G protein rather than by the direct action of the neurotransmitter itself. A different type of inhibitory mechanism is employed by the amino acid neurotransmitters *γ-aminobutyric acid (GABA)* and *glycine,* which are the main inhibitory neurotransmitters of the nervous system. Instead of binding to receptors that ac-

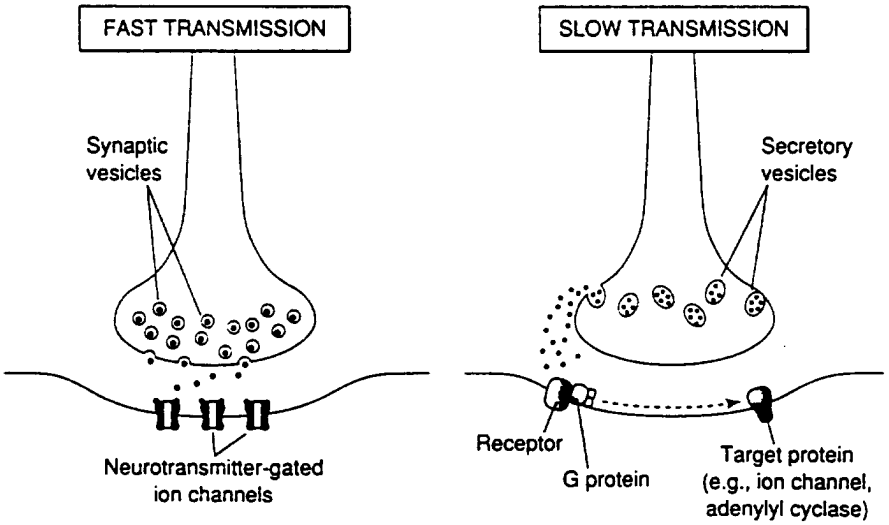


Figure 17-18 *Fast and Slow Chemical Transmission* (Left) *Fast transmission utilizes small-molecule neurotransmitters that are released from synaptic vesicles, diffuse across the synaptic cleft, and act directly on ion channels in the postsynaptic membrane.* (Right) *During slow transmission other neurotransmitters, including peptides and most amines, are released from large secretory vesicles and then act on G protein-linked receptors in the postsynaptic membrane. Both types of vesicles, and hence both types of transmission, can occur in the same axon terminal.*

tivate G proteins. GABA and glycine function by directly opening Cl^- channels. Since Cl^- is present in higher concentration outside the cell than inside, negatively charged chloride ions flow into the neuron and hyperpolarize the plasma membrane. The net result is a *fast* inhibitory response because the neurotransmitters are acting directly on a neurotransmitter-gated ion channel. Inhibitory transmitters are commonly employed by one neuron to control the release of neurotransmitters by another neuron. In this phenomenon, called *presynaptic inhibition*, the axons of inhibitory neurons terminate on the axons of excitatory neurons (Figure 17-19). When an inhibitory neuron is stimulated, it hyperpolarizes the axon of the excitatory neuron by releasing an inhibitory neurotransmitter such as GABA or glycine. After the excitatory axon has been hyperpolarized, action potentials that reach the axon terminal are less effective at depolarizing the membrane and triggering neurotransmitter release.

The neurotransmitter-gated Cl^- channels that function as receptors for glycine and GABA exhibit a structural resemblance both to each other and to the nicotinic acetylcholine receptor. All three receptors are constructed from five subunits that surround a central hydrophilic channel. Since the glycine and GABA receptors function as channels for negative ions and the acetylcholine receptor acts as a channel for positive ions, one might expect differences in the charged amino acids lining the three kinds of ion channels. The important differences appear to be located at the two ends of each channel (Figure 17-20). Negatively charged amino acids surround both ends of the channel in the acetylcholine receptor, whereas positively charged amino acids surround both ends of the glycine and GABA receptors. Presumably the negatively charged amino acids in the acetylcholine receptor facilitate the movement of positively charged sodium and potassium ions through the channel, and the positively charged amino acids in the GABA and glycine receptors facilitate the movement of negatively charged chloride ions through the channel.

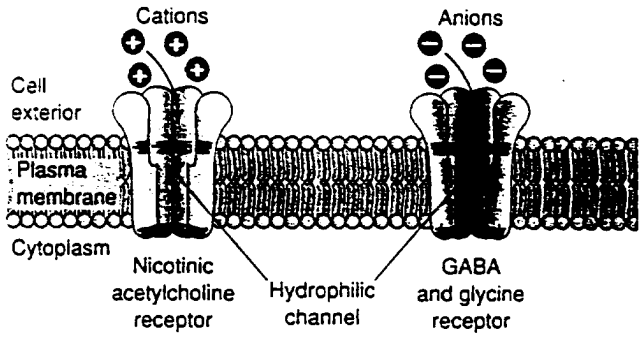


Figure 17-20 *Schematic Representation of Neurotransmitter-Gated Ion Channels* *In order to visualize the central hydrophilic channel, only four of the five subunits comprising each of these ion channels are shown. Negatively charged amino acids at both ends of the channel facilitate the movement of positive ions through the nicotinic acetylcholine receptor, whereas positively charged amino acids at both ends of the channel facilitate the movement of negative ions through the GABA and glycine receptors.*

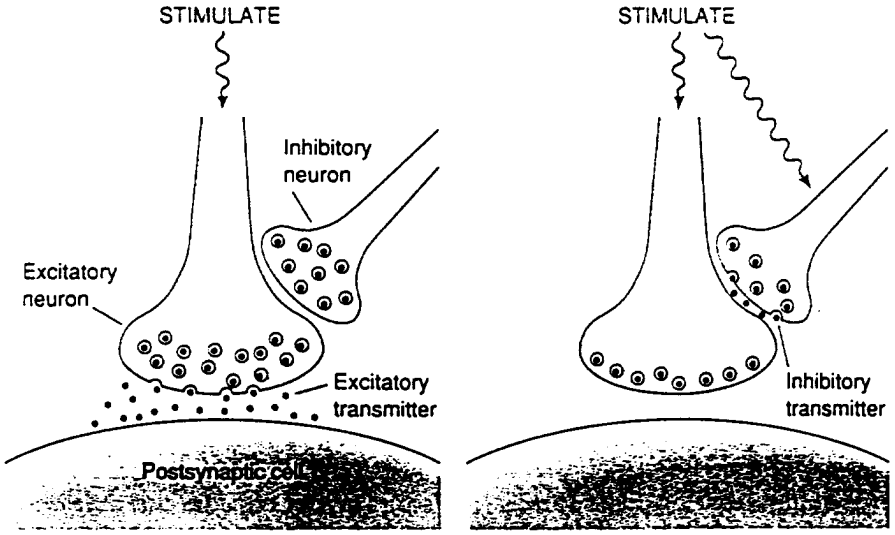


Figure 17-19 *Presynaptic Inhibition* *This phenomenon occurs in situations where the axon of an inhibitory neuron terminates on the axon of an excitatory neuron.* (Left) *When only the excitatory neuron is stimulated, excitatory neurotransmitter is released from its axon terminal and the postsynaptic cell is activated.* (Right) *When the excitatory and inhibitory neurons are both stimulated, the inhibitory transmitter opens Cl^- (or K^+) channels in the presynaptic membrane of the excitatory neuron, hyperpolarizing the membrane and thereby reducing the ability of the excitatory neuron to release neurotransmitter.*

Several Neurotransmitters Bind to Receptors That Influence Adenylyl Cyclase

Slow synaptic transmission exhibits a number of similarities to hormonal communication by non-neural cells. As we learned in Chapter 6, the binding of hormones to plasma membrane receptors often leads to the formation of intracellular second messengers such as cyclic AMP (page 211). Several lines of evidence point to the conclusion that cyclic AMP also mediates some of the actions of certain neurotransmitters, including dopamine, serotonin, and norepinephrine. First, subcellular fractionation experiments involving homogenates of brain tissue have shown that the *synaptosome fraction*, which is enriched in synaptic membranes, contains large amounts of *adenylyl cyclase*, the enzyme that catalyzes cyclic AMP formation. Second, electrical stimulation of nervous tissue has been found to elevate cyclic AMP levels in postsynaptic cells, as does the direct application of neurotransmitters like dopamine, norepinephrine, and serotonin. And finally, direct administration of cyclic AMP to neurons elicits changes in membrane potential similar to those produced by these neurotransmitters alone.

One of the first neurotransmitter-controlled adenylyl cyclase systems to be detected in neurons is associated with the action of **dopamine.** Dopamine is a particularly interesting neurotransmitter because it has been implicated in a variety of medical problems. For example, the tremors and uncontrollable body movements that occur in Parkinson's disease are associated with the degeneration of neurons that release dopamine. This association has fostered the idea that individuals afflicted with Parkinson's disease might improve if the amount of dopamine in the brain could be restored to normal. Since dopamine cannot enter the brain from the circulation, patients have been treated instead with a dopamine precursor called *levo-dihydrophenylalanine* (*L-DOPA*). Although administration of L-DOPA does relieve some of the symptoms of Parkinson's disease, progression of the illness unfortunately continues. In contrast to Parkinson's disease, which involves the underproduction of dopamine, patients with certain types of schizophrenia produce too much dopamine. Therefore schizophrenia is often treated with such drugs as chlorpromazine (Thorazine), which blocks dopamine receptors. Another type of dopamine abnormality is associated with the use of cocaine, which blocks the uptake system responsible for transporting dopamine back into the axon terminal from which it was released. When the uptake system is blocked, dopamine persists in the synaptic cleft for an abnormally long period of time and continues to act upon the postsynaptic cell.

The medical importance of dopamine has led to considerable interest in the mechanism of action of dopamine receptors. Using brain regions rich in dopamine-secreting neurons as starting material, Paul Greengard was the first investigator to isolate adenylyl cyclase preparations whose activity is stimulated by dopamine. The ability of dopamine to activate adenylyl cyclase is inhibited by antischizophrenic drugs known to block dopamine receptors, whereas drugs that mimic the actions of dopamine are found to stimulate adenylyl cyclase activity. Such observations suggest that at least some of the effects of dopamine on nerve cells are mediated by the binding of dopamine to receptors that trigger the activation of adenylyl cyclase, leading to an enhanced production of cyclic AMP.

Cyclic AMP-Dependent Phosphorylation of a Potassium Channel Is Associated with a Simple Type of Learning

The role of cyclic AMP in synaptic transmission has been extensively studied in *Aplysia californica*, a marine mollusk possessing a relatively simple nervous system that is capable of simple types of learning. In response to a gentle stimulus, such as a mild electric shock to the tail, *Aplysia* reflexively withdraws its gill. Repeating the stimulus several times in succession causes the gill-withdrawal reflex to become less intense as the organism becomes accustomed (*habituated*) to the stimulus. However, if the habituated organism is then subjected to a strong noxious stimulus, such as a sharp blow to the head or tail, it responds to the next gentle electric shock by vigorously withdrawing its gill. This suddenly increased sensitivity, called *sensitization*, represents a simple type of learning—that is, a change in an organism's behavior based on previous experience.

The sensitization of *Aplysia* to electric shock is mediated by a special group of regulatory neurons, called **facilitator neurons,** whose axons make synaptic connections with the axons of the sensory cells that trigger the gill-withdrawal reflex (Figure 17-21). Electric shock causes the facilitator neurons to release serotonin, which activates adenylyl cyclase and stimulates cyclic AMP formation in the axon of the sensory neuron. Cyclic AMP in turn activates protein kinase A, which phosphorylates a K^+ channel and thereby causes it to close. Since the repolarization phase at the end of a normal action potential depends on an outward flow of K^+, the reduced efflux of K^+ prolongs the action potential by keeping the membrane depolarized for a longer period of time. Therefore Ca^{2+} channels activated by the action potential remain open longer and more neurotransmitter is released. The net effect is that release of serotonin from the facilitator neurons causes the signal transmitted by the sensory neurons to become stronger with time.

Although the gill-withdrawal reflex is an extremely simple type of learning, it illustrates an important general principle: Learning is associated with changes in the efficiency of synaptic communication between

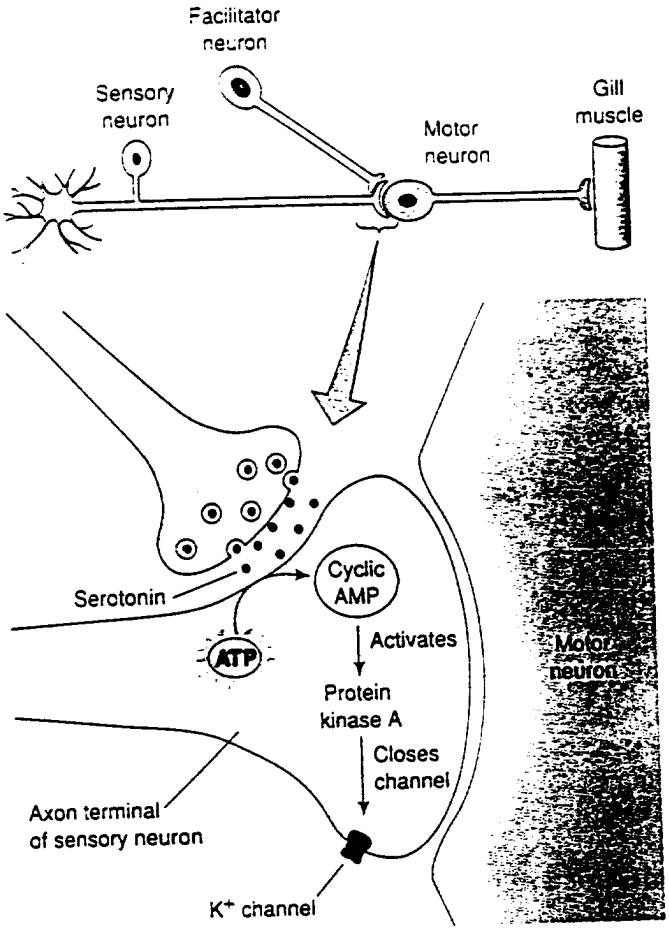


Figure 17-21 ***Mechanism by Which Serotonin Released from Facilitator Neurons Enhances the Gill-Withdrawal Reflex in*** **Aplysia** *Serotonin released by the facilitator neuron stimulates the production of cyclic AMP in the sensory neuron, triggering the closing of K^+ channels. When the sensory neuron is subsequently stimulated and an action potential arrives at its axon terminal, the closed K^+ channels prolong the action potential, thereby causing the sensory cell to discharge more neurotransmitter and hence activate the motor neuron more effectively.*

nerve cells. The phosphorylation of K^+ channels is but one of many cellular changes that appear to be involved in changing the efficiency of synaptic communication during learning.

Enkephalins and Endorphins Inhibit Pain-Signaling Neurons by Binding to Opiate Receptors

The milky fluid derived from seedpods of the opium poppy has been known since ancient Greek times to relieve pain and induce a sense of well-being. *Morphine*, the major active ingredient in this fluid, was first isolated in 1803 and gained widespread medical use before its toxic and addictive properties came to be appreciated. Many attempts have since been made to produce painkillers with the potency, but not the addictive properties, of morphine. For example, two methyl groups were added to morphine in the laboratories of the Bayer company in the 1890s, producing a derivative called *heroin* that was initially touted as a nonaddictive painkiller. In spite of this failure and others like it, the fact that *opiates* (morphine and its derivatives) are the most effective drugs for the treatment of severe chronic pain has motivated continued interest in such substances.

It has long been suspected that opiates produce their effects by binding to specific receptors in nerve cell membranes. Historically this belief was based on the structural specificity of opiates. Not only do small changes in the chemical structure of opiates lead to dramatic changes in potency, but only one of the two possible stereoisomers (mirror-image forms) of any given opiate is pharmacologically active. The existence of **opiate receptors** was finally demonstrated in the early 1970s, when Avram Goldstein, Solomon Snyder, Eric Simon, and Lars Terenius independently demonstrated that radioactive opiates bind to opiate receptors present in membrane fragments derived from brain tissue. The biological relevance of this binding was demonstrated by showing that (1) antagonists that block the pharmacological effects of opiates inhibit the binding of opiates to their receptors, and (2) the ability of various opiates to bind to opiate receptors correlates with the physiological potency of each drug (Figure 17-22).

Studies on the distribution of opiate binding sites in the nervous system has revealed that large numbers of opiate receptors reside in the areas of the spinal cord

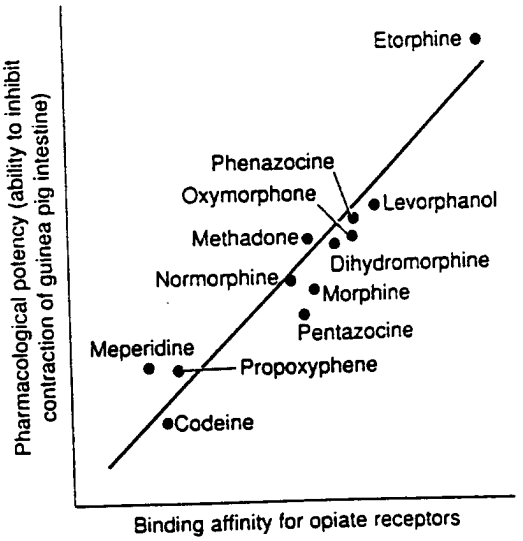


Figure 17-22 ***Relationship between the Pharmacological Potencies of Various Opiates and Their Ability to Bind to Opiate Receptors*** *Opiates that bind most strongly to opiate receptors also exhibit the greatest pharmacological potency, thereby suggesting that opiates act by binding to receptors.*

and brain that are associated with the perception of dull chronic pain. The brain's limbic system, which mediates emotional behavior, also contains many opiate receptors. Hence it is not surprising that drugs that selectively interact with such pathways relieve pain and induce euphoria. But why are opiate receptors present in the first place? Since they are clearly not designed to interact with morphine, the existence of opiate receptors suggests that the body must manufacture its own morphinelike substances that normally bind to the receptors. This possibility was confirmed in 1975 when John Hughes and Hans Kosterlitz isolated two small peptides from brain tissue that mimic the ability of morphine to inhibit the contraction of intestinal muscle, and whose activities are blocked by antagonists that interfere with the action of morphine. The two peptides, each consisting of five amino acids, were named *Met-enkephalin* (Tyr-Gly-Gly-Phe-Met) and *Leu-enkephalin* (Tyr-Gly-Gly-Phe-Leu). Both **enkephalins** were found to bind to opiate receptors. In addition, more than a dozen larger peptide hormones called **endorphins** have been isolated from nervous tissue and shown to bind to opiate receptors.

Analysis of the enkephalin content of various regions of the nervous system has revealed that the distribution of enkephalins closely parallels that of opiate receptors. Moreover, fluorescent antibodies directed against enkephalins selectively stain nerve endings (Figure 17-23), suggesting that enkephalins function as neurotransmitters. Support for this conclusion has come from the discovery that applying enkephalins to certain neurons slows down synaptic transmission, as would be expected for an inhibitory neurotransmitter. Although the mechanism by which enkephalins inhibit synaptic transmission is not completely understood, opiate receptors are known to be G protein-linked receptors.

Figure 17-23 *Nervous Tissue Stained with Fluorescent Antibodies That Bind to Enkephalin* *The antibodies stain nerve endings located in discrete areas of the brain, suggesting that enkephalins function as neurotransmitters. Courtesy of S. H. Snyder.*

One of the first effects of enkephalin binding to an opiate receptor is the activation of a G protein that opens a plasma membrane K^+ channel; since the K^+ concentration is higher inside the cell than outside, K^+ diffuses out of the cell and the membrane hyperpolarizes. Opiate receptors can also close plasma membrane Ca^{2+} channels and inhibit adenylyl cyclase. Closing of Ca^{2+} channels is thought to inhibit neurotransmitter release, but the significance of adenylyl cyclase inhibition is not well understood.

Nitric Oxide Is a Novel Type of Neurotransmitter That Acts by Stimulating Cyclic GMP Formation

Nitric oxide (NO) is a toxic, short-lived gas molecule that at first glance appears to be an unlikely candidate for a neurotransmitter. (Nitric oxide should not be confused with nitrous oxide, or N_2O, which is the "laughing gas" used for anesthesia.) Nitric oxide is produced by the enzyme *NO synthase*, which converts the amino acid arginine to nitric oxide and citrulline. The first clearly defined role of nitric oxide in neural signaling involves the control of blood vessel dilation. It has been known for many years that acetylcholine dilates blood vessels by causing the smooth muscle layers of the vessels to relax. But the acetylcholine receptors that mediate this effect do not reside in the muscle cells themselves; instead, acetylcholine binds to receptors located in the thin layer of *endothelial cells* that lines the inner surface of blood vessels. If the endothelial cells are removed, the vessels no longer relax when acetylcholine is added.

Figure 17-24 (*top*) illustrates how the binding of acetylcholine to endothelial cells triggers the relaxation of the adjacent muscle cells. This complex multistep pathway can be divided into six stages. (1) Acetylcholine binds to G protein-linked receptors that activate the phosphoinositide signaling pathway, causing inositol trisphosphate (IP_3) to be produced by the endothelial cells. (2) IP_3 triggers the release of stored calcium ions from the endoplasmic reticulum, raising the Ca^{2+} concentration in the cytosol. (3) The calcium ions bind to calmodulin, forming a Ca^{2+}-calmodulin complex that stimulates NO synthase to produce nitric oxide. (4) Nitric oxide is a gas that readily diffuses through biological membranes, allowing it to pass from the endothelial cells into the adjacent smooth muscle cells. (5) Upon entering the smooth muscle cells, nitric oxide activates the enzyme *guanylyl cyclase*, which catalyzes the formation of cyclic GMP. (6) The increased cyclic GMP concentration activates protein kinase G, which induces muscle relaxation by catalyzing the phosphorylation of appropriate muscle proteins.

In addition to its role in dilating blood vessels, nitric oxide has been implicated in the mechanism of neural signaling by glutamate, the most commonly employed

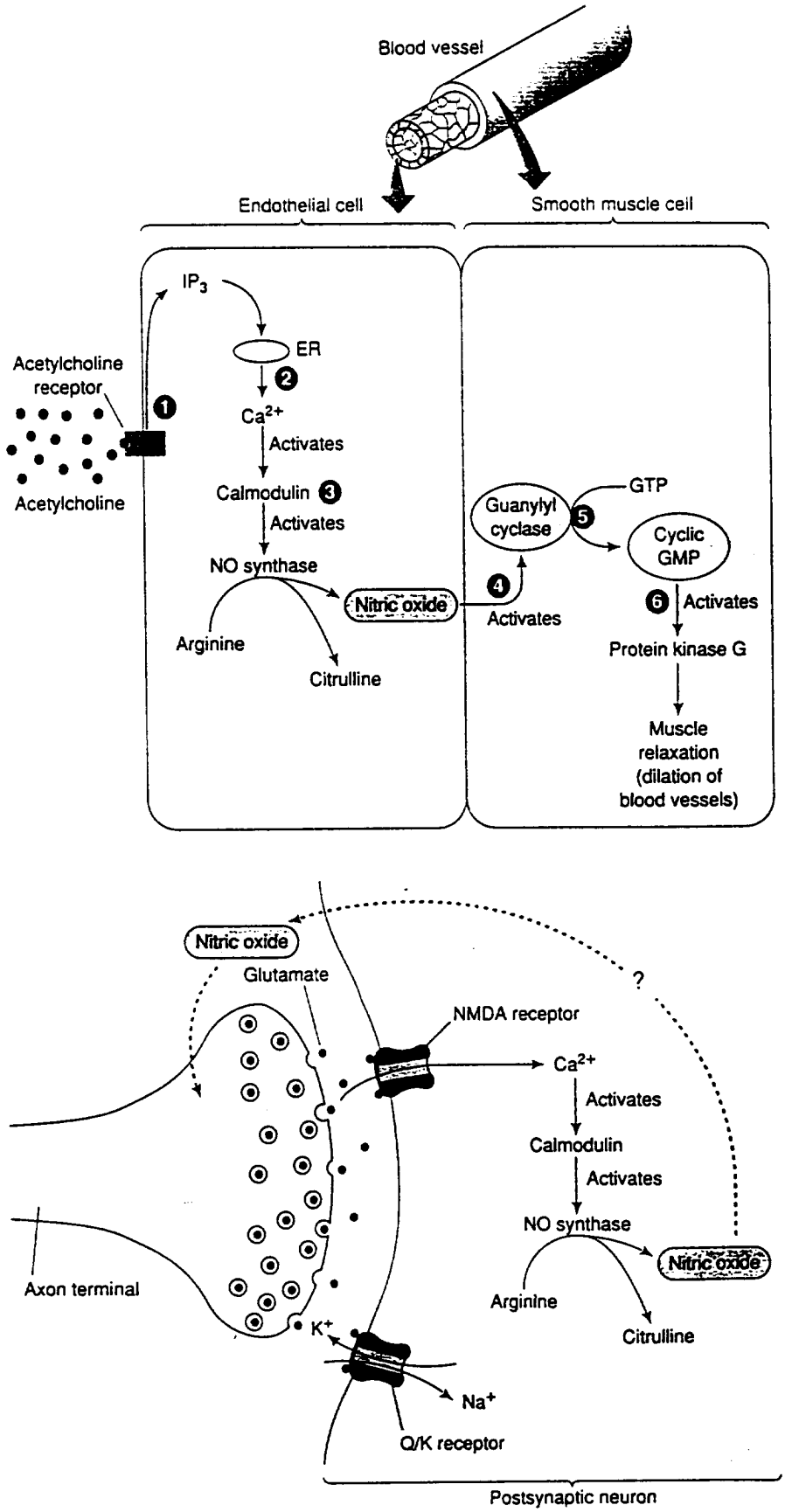


Figure 17-24 *Examples of Neural Signaling Pathways Involving Nitric Oxide* (Top) *The binding of acetylcholine to endothelial cells triggers the production of nitric oxide, which diffuses into the adjacent smooth muscle cells and stimulates guanylyl cyclase, thereby leading to muscle relaxation.* (Bottom) *The binding of glutamate to NMDA receptors during synaptic transmission triggers the production of nitric oxide in the postsynaptic neuron. It has been postulated that the nitric oxide diffuses back into the presynaptic axon terminal and signals the axon to increase its output of neurotransmitters.*

neurotransmitter in the brain. Glutamate interacts with several different classes of receptors, some functioning as ion channels and others linked to G proteins. One type of ion channel, called the *NMDA receptor*, is highly permeable to Ca^{2+} as well as to Na^+ and K^+. Hence the binding of glutamate to NMDA receptors allows calcium ions to enter the postsynaptic cell and bind to calmodulin, forming a Ca^{2+}-calmodulin complex that stimulates NO synthase to produce nitric oxide (see Figure 17-24, *bottom*).

Recent evidence suggests that the nitric oxide formed by this pathway may be involved in the process of **long-term potentiation**, or LTP, which is thought to be one of the primary means for storing memories. LTP refers to the increase in the efficiency of synaptic transmission that occurs when repeated stimulation of a presynaptic neuron leads to the repetitive transmission of signals across a synapse to a postsynaptic neuron. Such repetition causes the synaptic connection between the two cells to become strengthened or "potentiated"; that is, a bigger response occurs in the postsynaptic cell the next time signals are sent. Recent experiments have shown that inhibitors of NO synthase prevent LTP from occurring in slices of rat brain, and that rats injected with inhibitors of NO synthase lose the ability to learn certain types of behavior. Such observations suggest that nitric oxide may be involved in establishing LTP. One possible role is that the nitric oxide formed in the postsynaptic neuron during synaptic transmission may diffuse back into the presynaptic axon terminal, signaling the axon to increase its output of neurotransmitters.

The realization that nitric oxide functions in neural signaling raises the question of whether any other gases are similarly involved. One potential candidate is *carbon monoxide*, a gas that shares with nitric oxide the ability to dilate blood vessels by stimulating guanylyl cyclase. Several regions of the brain contain the enzyme *heme oxygenase*, which catalyzes the formation of carbon monoxide during the breakdown of heme. The production of carbon monoxide by brain cells and its ability to stimulate cyclic GMP formation indicates that carbon monoxide might play a signaling role comparable to that of nitric oxide.

DETECTING STIMULI AND TRIGGERING RESPONSES

We have now seen that the transmission of nerve impulses involves changes in membrane potential that are passed from neuron to neuron using electrical and chemical synapses. But if nerve impulses are to carry information that is useful to the organism as a whole, neurons must be able to do more than just communicate with one another. The nervous system must also receive sensory information from both inside and outside the body and use the information that has been received to trigger appropriate responses. In this segment of the chapter we will briefly discuss a few examples that illustrate how the nervous system detects stimuli and triggers responses.

Vertebrate Photoreceptors Detect Light Using a Mechanism That Involves Cyclic GMP

The ability of the nervous system to perceive its internal and external environments depends on specialized **sensory cells** that can be grouped into four principal types: *photoreceptors* that are sensitive to light, *mechanoreceptors* that respond to physical pressure or movement (e.g., sound and touch), *chemoreceptors* that detect chemicals (e.g., odor and taste), and *thermoreceptors* that monitor temperature. In each case the sensory cell transforms the stimulus being perceived into a common form—namely, a change in membrane potential.

To illustrate how sensory cells convert external stimuli into changes in membrane potential, let us consider photoreceptors as an example. The vertebrate retina is comprised of two kinds of photoreceptor cells called **rods** and **cones** because of the distinctive shapes of their light-sensitive tips (Figure 17-25). Rod cells are more sensitive to dim light but cannot distinguish colors; cone cells are responsible for color vision, but function only in bright light. In both rods and cones, light absorption occurs in a specialized region of the cell termed the *outer segment*. Because it is attached to the rest of the cell by a narrow stalk containing a typical ciliary axoneme, the outer segment can be considered to be an elaborately modified cilium. The outer segment of each photoreceptor cell contains hundreds of flattened *membrane disks* that store a light-absorbing pigment called **rhodopsin**. Each rhodopsin molecule consists of a protein called *opsin* covalently joined to a light-absorbing derivative of vitamin A known as *11-cis-retinal*. In addition to rhodopsin, cone cells contain blue, green, and red pigments that mediate color perception.

When a photon of light strikes 11-*cis*-retinal, it alters the conformation of the rhodopsin molecule (Figure 17-26). The altered rhodopsin in turn activates a G protein called G_t (or *transducin*), which stimulates the breakdown of cyclic GMP by *cyclic GMP phosphodiesterase*. In photoreceptor cells, cyclic GMP controls the permeability of plasma membrane *cyclic GMP-gated Na^+ channels*; when illumination triggers a decrease in cyclic GMP concentration, the lack of cyclic GMP causes these Na^+ channels to close. The resulting decrease in the flow of Na^+ into the cell leads to an increased negative charge

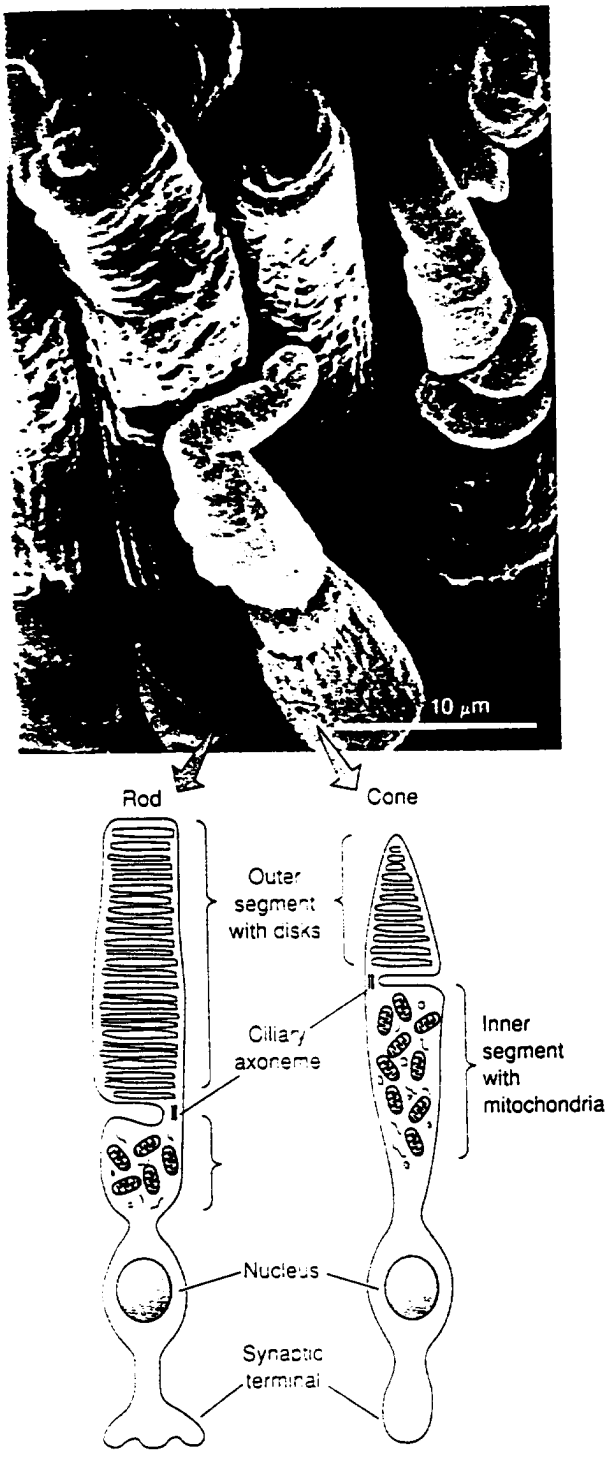


Figure 17-25 *Rod and Cone Cells* (Top) *Scanning electron micrograph of rod and cone cells in the retina of the mud puppy. The outer segments of the rod cells are cylindrical in shape, whereas the outer segments of the cone cells are conical.* (Bottom) *Schematic diagram of typical rod and cone cells. The outer segment contains tightly packed membrane disks that store rhodopsin and other photoreceptor pigments. Micrograph courtesy of E. R. Lewis, Y. Y. Zeevi, and F. S. Werblin.*

inside the cell—that is, a hyperpolarization of the plasma membrane. Hence in vertebrate photoreception, the presence of light is translated into membrane hyperpolarization rather than depolarization. Absorption of a single photon of light by rhodopsin can block the transit of millions of sodium ions across the outer segment plasma membrane. This signal amplification is made possible by the fact that one rhodopsin molecule activates thousands of G_t molecules, and each phosphodiesterase molecule activated by G_t can in turn degrade thousands of cyclic GMP molecules per second.

Sensory Stimuli Alter the Membrane Potential of Sensory Cells by a Variety of Different Mechanisms

Some of the principles illustrated by photoreceptor cells apply to the reception of sensory stimuli by other cells as well. Olfactory cells, for example, perceive odors by a mechanism that also involves G proteins. The binding of an odor molecule to an olfactory cell receptor activates a G protein called G_{olf}, which in turn stimulates adenylyl cyclase to produce cyclic AMP. The cyclic AMP triggers membrane depolarization by opening a plasma membrane cation channel.

G proteins are not always involved in sensory reception, however. Taste bud cells, for example, receive salty sensations through a direct mechanism in which sodium ions in the cell's external environment diffuse through the Na^+ channels in the plasma membrane, depolarizing the taste cell directly. The epithelial *hair cells* found in the inner ear, which are the sensory cells for balance and hearing, act as mechanoreceptors that perceive movements using a mechanism that involves neither G proteins nor plasma membrane receptors. Hair cells exhibit huge microvilli called *stereocilia* (page 591) that are sensitive to tiny movements caused either by sound vibrations or by the movement of fluid in the semicircular canals. These movements trigger membrane depolarization by opening cation channels in the plasma membrane of the stereocilia. The channels open so rapidly that they are thought to be acting as *mechanically gated ion channels*—that is, ion channels that are directly opened by the mechanical movements of the stereocilia.

Responses Triggered by the Nervous System Include Muscular Contraction, Glandular Secretion, and Neurosecretion

Stimuli detected by sensory cells trigger nerve impulses that are transmitted to other neurons by synaptic communication. But in order for these impulses to influence the rest of the organism, some neurons must be capable of triggering responses other than exciting or inhibiting another neuron. Such responses can be grouped into three main categories: muscle contraction, glandular secretion, and neurosecretion.

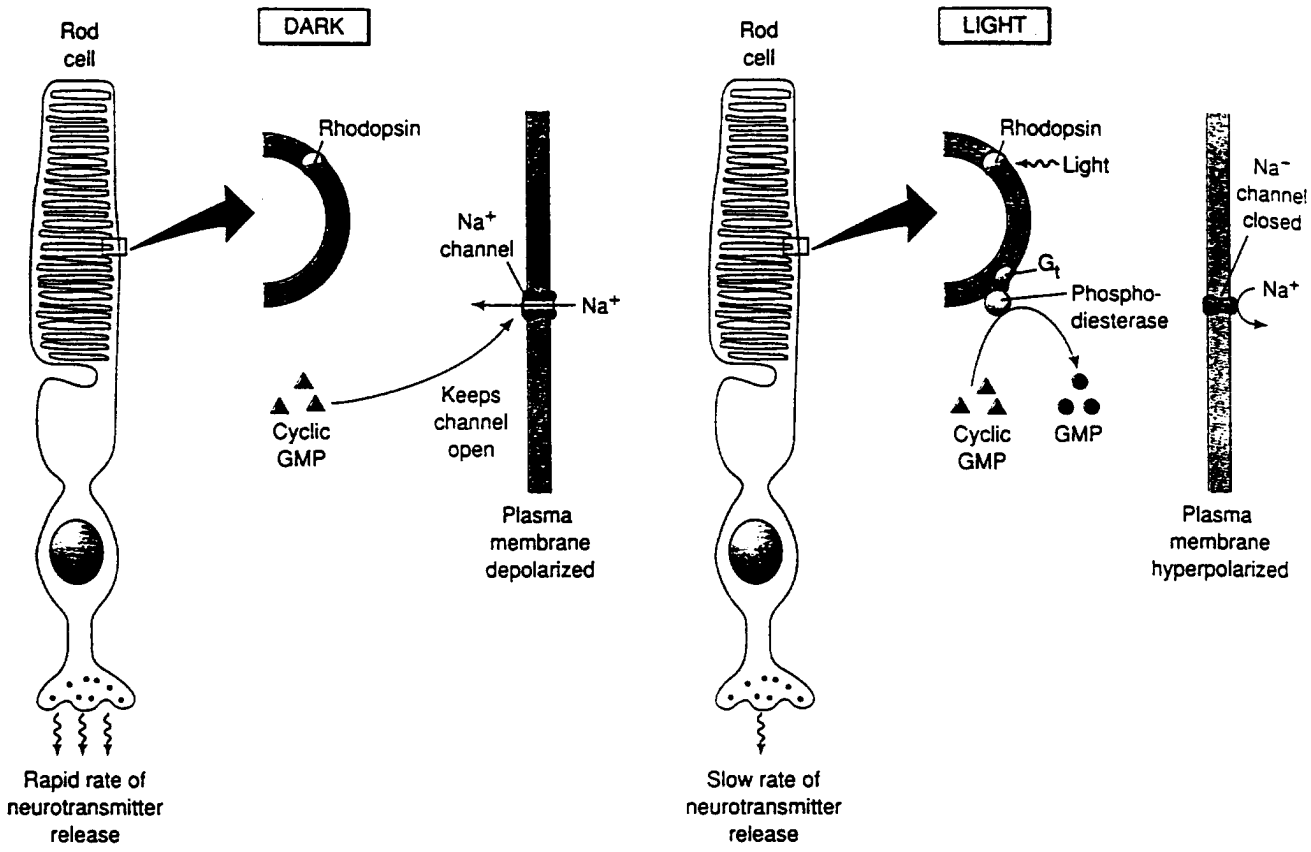


Figure 17-26 *The Effect of Light on Rod Cells* (Left) *In the dark, cyclic GMP keeps plasma membrane Na^+ channels open and the membrane is partially depolarized, thereby leading to neurotransmitter release from the rod cell.* (Right) *Light induces a change in the conformation of rhodopsin, triggering the activation of a G protein (G_t) that stimulates the breakdown of cyclic GMP by phosphodiesterase. The decreased cyclic GMP concentration causes the Na^+ channels to close, leading to hyperpolarization of the plasma membrane and a reduced rate of neurotransmitter release from the rod cell.*

We have already discussed the mechanism by which nerve impulses stimulate *muscle contraction* (pages 564–582). In addition to initiating the contraction process, nerve impulses are also involved in regulating muscle contraction. For example, cardiac muscle and certain smooth muscles exhibit spontaneous rhythmic contractions that do not require nerve impulses for initiation. But the rate of such contractions is under neural control. In the case of the heart, neurons releasing norepinephrine increase membrane excitability and therefore speed the rate of contraction, whereas neurons releasing acetylcholine decrease membrane excitability and therefore slow the rate.

Nerve cells also activate (or inhibit) many types of *glandular secretion*, such as the secretion of saliva from salivary glands or epinephrine from the adrenal medulla. Cells of the adrenal medulla are developmentally related to neurons but fail to sprout axons; instead of utilizing their released epinephrine as a neurotransmitter, they secrete it as a hormone into the bloodstream. Epinephrine circulating throughout the organism exerts effects similar to those produced by neurons that utilize norepinephrine as a neurotransmitter. For example, circulating epinephrine and neurons utilizing norepinephrine as a neurotransmitter can both trigger increases in heart rate, blood pressure, breathing rate, and blood glucose concentration.

The close relationship between neurotransmitters and hormones is underscored by the observation that some neurons secrete hormones directly into the bloodstream by a process called **neurosecretion.** A prominent example occurs in the hypothalamus, where certain neurons secrete peptide *releasing factors* into the circulation that travel to the anterior pituitary gland and stimulate the release of other hormones (Figure 17-27). Among the peptides secreted by hypothalamic neurons are (1) *thyrotropin releasing factor (TRF)*, which promotes the release of thyrotropin from the anterior pituitary, (2) *gonadotropin releasing factor (GnRF)* which promotes the release of the hormones FSH and LH from the anterior pituitary, (3) *corticotropin releasing factor (CRF)*, which promotes the release of the hormone ACTH from the anterior pituitary, (4) *growth hormone releasing factor*, which promotes the release of growth hormone from the anterior pituitary, and (5) *somatostatin*, which inhibits the release of growth hor-

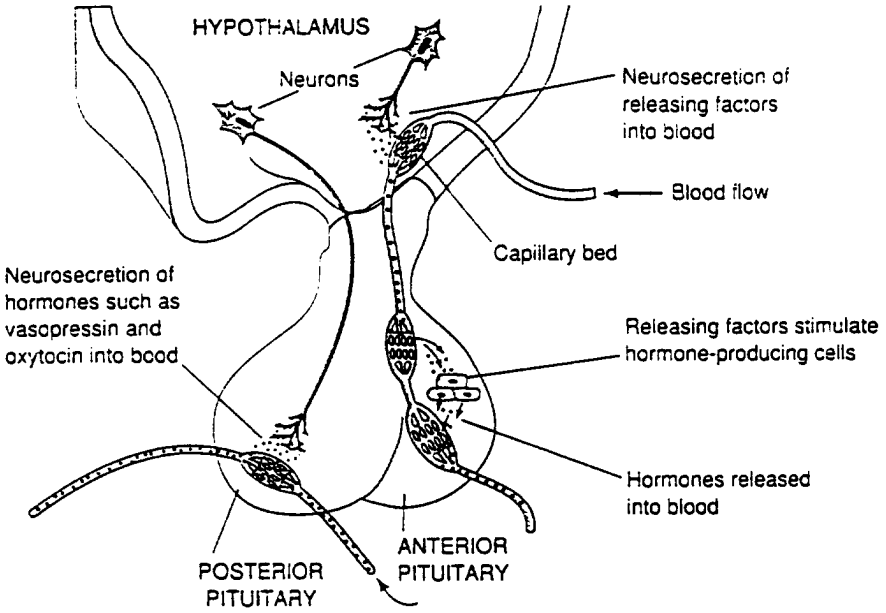


Figure 17-27 *Neurosecretion by Neurons Located in the Hypothalamus* *The neuron on the left has an axon that passes into the posterior pituitary, where it secretes hormones like vasopressin and oxytocin directly into the bloodstream. The neuron on the right secretes peptide releasing factors into capillaries that carry them to the anterior pituitary, where they induce the release of other hormones into the bloodstream.*

mone from the anterior pituitary. Other neurons located in the hypothalamus have axons that pass directly into the posterior pituitary, where they secrete the hormones *vasopressin* and *oxytocin* into the bloodstream. Neurosecretion is accomplished by the same basic mechanism as neurotransmitter release. In both cases the arrival of a wave of membrane depolarization at the nerve ending triggers exocytosis of small vesicles. The only difference is that in typical neurons the vesicles release a neurotransmitter that interacts with the adjacent neuron, whereas in neurosecretory neurons the vesicles release peptide hormones and releasing factors that enter the circulation.

NEURON GROWTH AND DEVELOPMENT

The nervous system of higher vertebrates is an extraordinarily complex network consisting of billions or even trillions of interconnected cells situated in the brain, spinal cord, and peripheral nerves. In order for this system to function properly in analyzing sensory information and triggering appropriate responses, these billions of cells must be properly connected to one another. In the concluding section of the chapter we will briefly consider how neurons establish these connections during embryonic development.

The Growth Cone Directs the Outgrowth of Neurites

In vertebrates, neurons arise during early embryonic development from cells that separate from the primitive ectoderm shortly after gastrulation. These cells, called *neuroblasts*, stop dividing and begin to develop cytoplasmic extensions known as **neurites**, which are destined to become axons and dendrites. Much has been learned about the development of neurites by studying cultures of embryonic neuroblasts induced to form neurites by appropriate incubation conditions. Experiments of this sort were first carried out in the early 1900s by Ross Harrison, one of the pioneers of cell culture techniques. By carefully observing the development of cultured neuroblasts, he was the first to show that the axon is a direct outgrowth of the neuron rather than an independently formed entity.

Shortly before Harrison's pioneering work, Ramón y Cajal had applied the Golgi silver-stain technique to elongating axons and discovered that the tip of each growing axon contains an expanded region, which he named the **growth cone**. Although the cells being examined were fixed and stained, and hence nonliving, Ramón y Cajal suggested that the growth cone represents an area of dynamic change at the growing tip. This hypothesis was soon confirmed by Harrison, who showed that the neurites of cultured nerve cells exhibit growth cones that are in constant motion. In recent years, higher-resolution microscopic techniques have revealed that the growth cone is covered with slender motile filopodia that protrude and wave about (Figure 17-28). These filopodia behave as if they are "sensing" the surrounding environment and determining the direction in which the neurite should grow. In some cases it has been demonstrated that growth cones turn in the direction in which their filopo-

Figure 17-28 ***Scanning Electron Micrograph Showing the Growth Cone of a Neuron in Culture*** *Note the presence of numerous filopodia. Courtesy of S. M. Rothman.*

dia adhere best: as they adhere to new surfaces, the filopodia exert tension on the rest of the axon, pulling it along. If the connection between such a growth cone and its attached axon is experimentally severed, the growth cone continues to move without the axon.

Axon elongation driven by movements of the growth cone requires the cooperation of actin filaments and microtubules. Norman Wessells has shown that treating elongating axons with cytochalasin to disrupt actin filaments causes the filopodia to become immobile and retract, halting axon elongation within a few minutes. In contrast, disrupting microtubules with colchicine does not alter filopodial appearance or motility, nor does it exert an immediate effect on axon elongation. Within a half hour of colchicine treatment, however, the axon begins to shorten and, although filopodial activity remains unaltered, the axon eventually collapses back into the nerve cell body. The contrasting effects of these two drugs on axon elongation suggest that actin filaments are required for growth cone movement and axon elongation, whereas microtubules serve as a skeletal framework whose integrity is essential for maintaining the elongated state (Figure 17-29).

Neurite Growth Is Stimulated by Nerve Growth Factor as Well as a Variety of Other Proteins

The growth and development of nerve cells is controlled by a variety of growth factors. The most thoroughly studied example, called **nerve growth factor** (NGF), was discovered in the early 1950s by Rita Levi-Montalcini and Viktor Hamburger. NGF differs from most growth factors in that it does not actually promote cell proliferation: NGF instead stimulates neurite outgrowth and maintains cell viability. The main targets of NGF are *sensory neurons*, which carry nerve impulses from the periphery to the central nervous system, and *sympathetic neurons*, which are responsible for the regulation of involuntary actions such as breathing and the heartbeat. Sensory neurons are most sensitive to NGF during embryonic development, whereas sympathetic neurons are under the influence of NGF during both embryonic and postnatal development. The selectivity of NGF has been dramatically demonstrated by injecting newborn animals with antibodies directed against NGF: the result is the selective degeneration of the sympathetic nervous system. Both sympathetic and sensory neurons survive poorly when cultured in the absence of NGF, but other cells grow well without it. When NGF is added to a culture of sensory or sympathetic neurons, the cells respond with a striking and massive outgrowth of neurites (Figure 17-30).

Like many other growth factors, NGF binds to a plasma membrane receptor that functions as a protein-tyrosine kinase (page 221). Activation of the tyrosine kinase by NGF is thought to stimulate cell growth through a cascade of events initiated by the phosphorylation of one or more intracellular proteins. Although NGF was the first growth factor shown to promote the growth of nerve cells and foster the development of neurites, a variety of other molecules have subsequently been implicated as well. Included are growth factors such as *insulin-like growth factor II (IGF-II)*, extracellular matrix proteins such as *laminin*, *fibronectin*, and *collagen* (pages 226–232), and cell adhesion molecules such as *N-CAMs* and *cadherins* (page 233).

Axons Are Guided to Their Proper Destination by Cell-Cell Contacts, Matrix Molecules, and Diffusible Substances

Once neurites begin to elongate under the influence of NGF or other appropriate growth factors, they need to follow the proper pathway to link up with the appropriate target cells. The problem is especially pronounced for axons, which must often grow for many centimeters toward a specific region of termination and then make contact with the proper target cell, where they form a synapse at the correct location on the cell body, dendrite, or axon. One of the first attempts to investigate this process was carried out by Roger Sperry using the amphibian visual system as a model. In the visual system, axons emerge from cell bodies located in the retina and grow toward a particular region of the brain called the *optic tectum*. Axons leaving the ventral (lower) portion

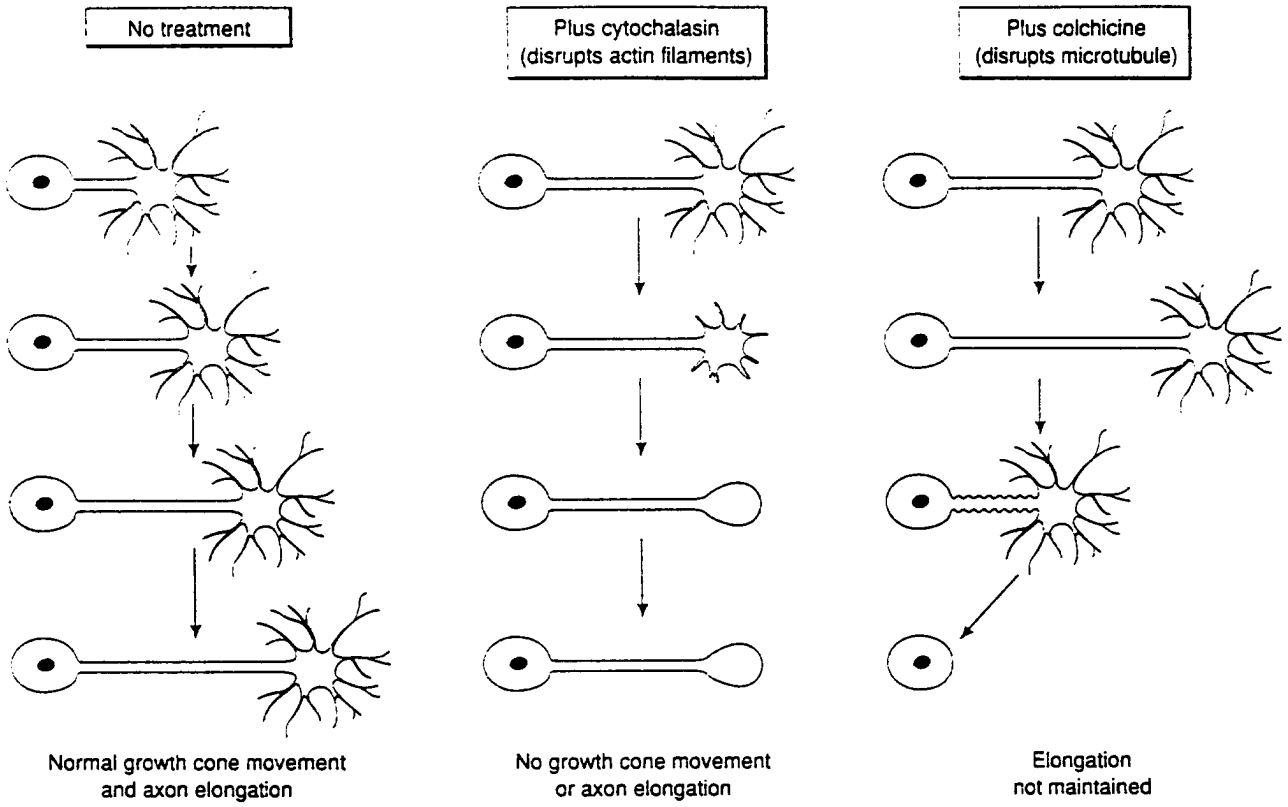


Figure 17-29 ***Effects of Cytochalasin and Colchicine on Axon Elongation in Cultured Neurons*** *The effects of cytochalasin suggest that actin filaments are required for growth cone movement and axon elongation, while the effects of colchicine suggest that microtubules provide a skeletal framework that maintains the elongated state.*

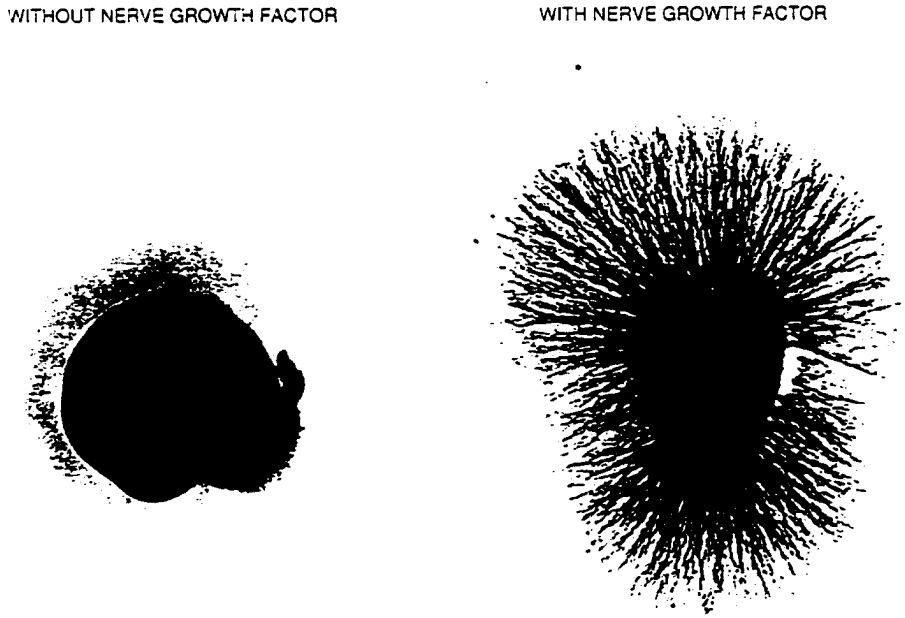


Figure 17-30 ***Effect of Nerve Growth Factor (NGF) on Cultured Nerve Cells*** (Left) *An untreated mass of nerve cells.* (Right) *Nerve cells cultured in the presence of NGF, which induces a massive outgrowth of neurites. Courtesy of R. Levi-Montalcini.*

of the retina migrate into the dorsal (upper) area of the optic tectum, whereas axons originating in the dorsal retina grow toward the ventral tectum. Sperry's approach was to cut the optic nerve, rotate the eye 180°, and then allow the nerves to regenerate. In spite of the disruption, the damaged axons sorted out correctly and migrated to their proper locations.

To explain this remarkable result, Sperry proposed that neurons carry chemical identification tags on their surfaces that permit them to recognize the cells with which they are destined to make synaptic contact. As a test of the hypothesis, Stephen Roth isolated cells from the dorsal and ventral portions of the retina and monitored their ability to adhere to tissue removed from the dorsal or ventral half of the optic tectum. As predicted by Sperry, cells derived from the dorsal retina adhere preferentially to the ventral tectum, and ventral retinal cells bind selectively to the dorsal tectum. The specificity of this cell-cell interaction suggests that neurons carry surface molecules that help guide them to their appropriate targets.

Sperry's pioneering work on the retinotectal system has prompted a vigorous investigation of the mechanisms that guide axons along their proper pathways. In grasshopper embryos, where the developmental trajectories of individual axons have been precisely mapped, several kinds of cell-cell interactions that guide axon extension have been identified. For example, when the axons of developing sensory neurons begin to elongate, their growth cones initially move along epithelial surfaces; but at specific locations, the direction of growth cone extension suddenly changes. These abrupt changes occur at sites where the filopodia of growth cones make contact with specific immature neurons called *guidepost cells*. If the guidepost cells are destroyed with a laser microbeam, the axons wander off in inappropriate directions.

Growth cones also employ the axons of neighboring neurons as guiding scaffolds upon which to extend. In an elegant series of studies investing this phenomenon, Corey Goodman and his associates have found that the growth cone of a particular grasshopper neuron always migrates along the same bundle of axons. If this axon bundle is destroyed before the growth cone of the identified neuron reaches it, the growth cone stops growing or wanders randomly in the vicinity of the missing bundle, even though other axon bundles are within reach of its filopodia. Subsequent investigations have revealed that a family of cell surface glycoproteins called **fasciclins** are involved in the process by which growth cones recognize appropriate axon bundles. Axon bundles differ in the quantities and types of fasciclin they contain: if the ability of an organism to produce a particular type of fasciclin is disrupted by mutation, axon bundles that would have normally contained the missing fasciclin fail to develop.

Fasciclins are but one of several dozen molecules that appear to influence the direction in which axons grow. These guidance factors fall into three main categories: cell surface proteins, extracellular matrix proteins, and diffusible proteins. The long list of molecules implicated in guiding axon outgrowth suggests that the final wiring pattern of the nervous system is achieved not by a single recognition mechanism but by a sequential pathway of recognition steps that gradually guides a growing axon to its proper destination. Some of these guidance interactions involve factors that repel rather than attract growth cones. For example, growth cones of axons derived from the central nervous system have been found to withdraw and move in another direction when they make contact with axons derived from peripheral nerves.

Once axons have entered the appropriate target area, growth cones are able to distinguish among potential postsynaptic partners. The discrimination process involves molecules carried by the target cells as well as chemical gradients that define the positions of given cells within the target tissue. When an axon finally makes contact with the appropriate cell, it induces the formation of a synapse. In the case of skeletal muscle cells, contact with an axon causes acetylcholine receptors that had been diffusely distributed across the muscle cell plasma membrane to form high-density clusters near the site of contact. A protein in the axon terminal called *agrin* has been implicated in triggering this event. If purified agrin is added to cultures of embryonic muscle cells, it causes the formation of multiple clusters of acetylcholine receptors in the absence of nerve cells.

Most Mature Neurons Lose the Capacity to Divide

In higher vertebrates, most neurons lose the capacity to divide after they begin to form axons and dendrites. If neurons are destroyed after this stage, they cannot be replaced. However, injured neurons can sometimes regenerate new axons if their existing axons are cut or damaged. In such an event, the old axon degenerates beyond the point of injury because it has become physically separated from the cell body upon which it depends for nutrition. The cell body then synthesizes new axoplasm and the axon stub begins growing again. The newly forming axon, elongating at a rate of a few millimeters per day, eventually reaches its original destination, where under favorable conditions it may even reestablish proper synaptic connections.

Although most neurons do not divide after a certain stage of development has been reached, tumors of proliferating neuroblasts occasionally arise. These tumors, called **neuroblastomas**, grow and divide readily in culture, and can be induced to differentiate into cells pos-

sessing dendrites, axons, and the ability to synthesize neurotransmitters. Since normal neurons cannot be propagated in culture, the availability of neuroblastoma cells exhibiting many attributes of mature neurons, but capable of growth in culture, has provided biologists with an alternative way of studying nerve cells.

SUMMARY OF PRINCIPAL POINTS

- A neuron consists of a cell body exhibiting long cytoplasmic extensions called axons and dendrites. Dendrites are shorter than axons, exhibit an extensively branched pattern, lack a myelin sheath, and are specialized for receiving signals. Axons are less numerous than dendrites, extend from the cell body for longer distances, often exhibit a myelin sheath, and are specialized for sending signals.
- Slow axonal transport moves proteins and cytoskeletal filaments down the axon at rates of about 1–5 mm per day, whereas fast axonal transport moves vesicles and mitochondria at rates of 10–500 mm per day. Fast transport is mediated by the motor proteins kinesin and dynein, which link organelles to microtubules. Kinesin propels materials down the axon, while dynein moves in the opposite direction.
- Neurons transmit signals by changing the electrical potential across their plasma membranes. Action potentials are caused by stimuli that trigger a transient increase in membrane permeability to Na^+. The resulting diffusion of Na^+ into the cell depolarizes the plasma membrane. Depolarization triggers the opening of K^+ channels, allowing an efflux of K^+ that reestablishes the resting membrane potential.
- Myelination speeds the propagation of action potentials by preventing the leakage of local currents through the plasma membrane and by forcing action potentials to jump from one node of Ranvier to the next.
- Nerve impulses are transmitted from cell to cell at specialized junctions called synapses. At electrical synapses, signals are transmitted by ions that flow directly through gap junctions that link the presynaptic and postsynaptic membranes. At chemical synapses, signals are transmitted by chemical neurotransmitters that are released from the presynaptic cell, diffuse across the synaptic cleft, and bind to receptors on the postsynaptic cell.
- Neurotransmitters are stored in vesicles whose contents are discharged from the axon terminal by exocytosis when the plasma membrane is depolarized. Exocytosis is triggered by calcium ions, which enter the cell when depolarization causes plasma membrane Ca^{2+} channels to open.
- Neurotransmitters involved in fast chemical transmission bind to receptors that function as neurotransmitter-gated ion channels, whereas neurotransmitters involved in slow chemical transmission bind to G protein-linked receptors, activating G proteins that influence ion channels or the production of second messengers such as cyclic AMP or IP_3.
- When acetylcholine binds to nicotinic acetylcholine receptors, fast excitatory transmission ensues because the nicotinic receptor functions as a cation channel that is opened directly by acetylcholine. When acetylcholine binds to muscarinic acetylcholine receptors, slow inhibitory transmission ensues because the muscarinic receptor activates a G protein that causes K^+ channels to close, thereby triggering membrane hyperpolarization.
- GABA and glycine are fast inhibitory transmitters that bind to receptors which function as Cl^- channels. Binding of neurotransmitter opens the channels, causing an influx of Cl^- that hyperpolarizes the plasma membrane.
- Dopamine, serotonin, and norepinephrine bind to receptors that regulate the activity of adenylyl cyclase. In sensory neurons the cyclic AMP produced by adenylyl cyclase acts by closing K^+ channels, which prolongs the action potential by keeping the membrane depolarized for longer periods of time.
- Enkephalins and endorphins are inhibitory neurotransmitters that inhibit pain-signaling pathways by binding to opiate receptors, which are G protein-linked receptors that open K^+ channels, close Ca^{2+} channels, and inhibit adenylyl cyclase.
- The binding of acetylcholine or glutamate to the plasma membrane of certain cells triggers an increase in cytosolic Ca^{2+} concentration, which can stimulate the formation of nitric oxide. The nitric oxide diffuses out of the cell and influences neighboring cells by activating the enzyme guanylyl cyclase.
- Environmental stimuli are detected by sensory cells whose membrane potential is altered by changes in temperature, pressure, chemicals, or light. In vertebrate photoreceptors light is absorbed by rhodopsin, which activates a G protein that stimulates cyclic GMP degradation by phosphodiesterase. The resulting decrease in cyclic GMP concentration causes Na^+ channels to close, thereby hyperpolarizing the plasma membrane.
- During development of the nervous system, axon growth is guided by cell-cell contacts, matrix molecules, and diffusible substances. Once neurons acquire axons and dendrites, they usually lose the capacity to divide.

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