The Nervous System

19

How can we hope to understand the workings of the human brain? This network of some 10^{11} nerve cells, with at least a thousand times that number of interconnections, is more complex, and seems in many ways more powerful, than even the largest of modern computers. Our present understanding of the nervous system is so rudimentary, however, that one can scarcely judge whether the comparison makes sense. We do not know, for example, how many functionally distinct categories of nerve cells the brain contains; nor can we give even an outline of the neural computations involved in hearing a word or reaching for an object, let alone proving a theorem or writing a poem.

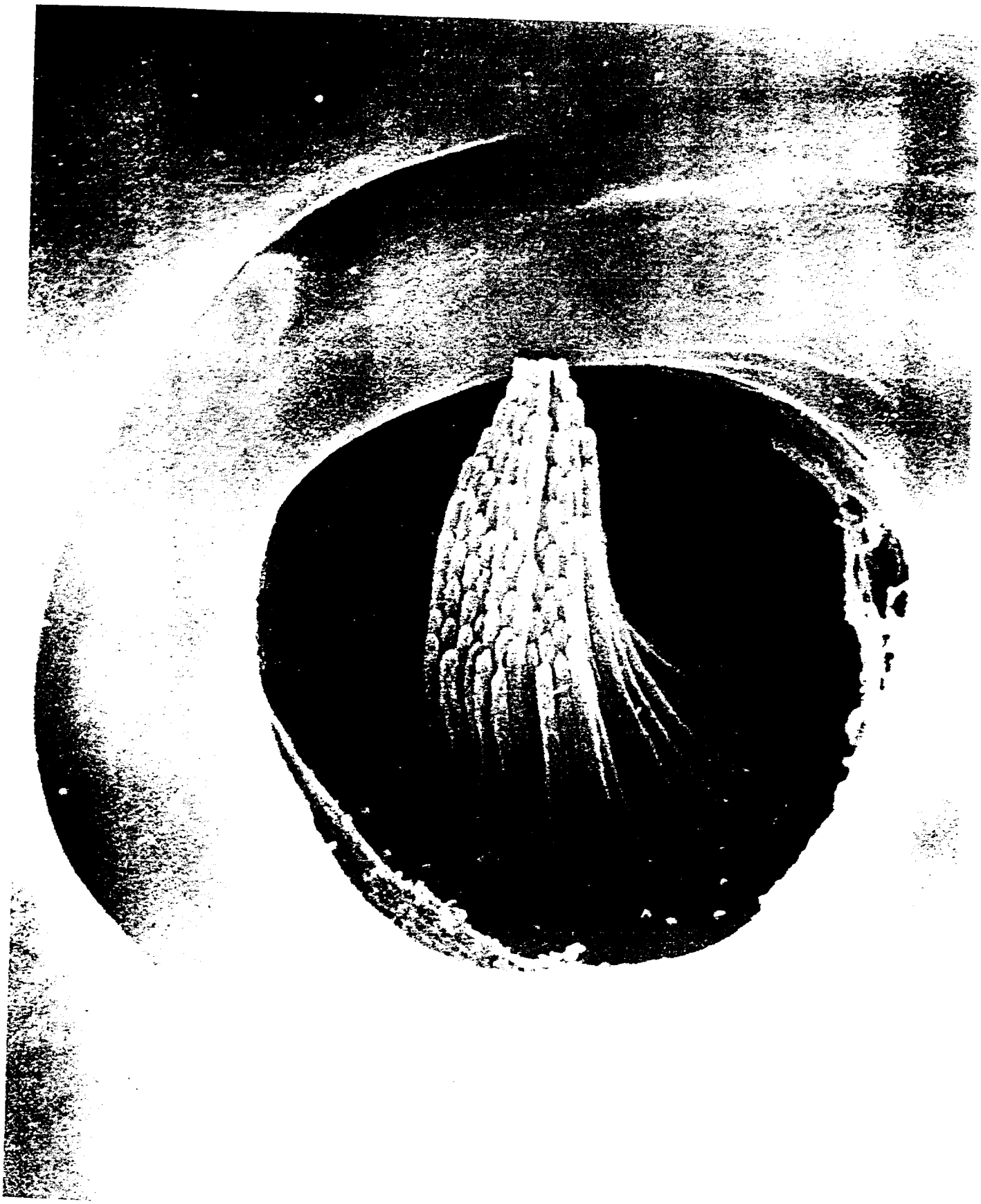
And yet, paradoxically, while the brain as a whole remains the most baffling organ in the body, the properties of the individual nerve cells, or *neurons*, are understood better than those of almost any other cell type. At the cellular level at least, simple and general principles can be discerned. With their help, one can begin to see how small parts of the nervous system work. Important progress has been made, for example, in explaining the cellular machinery of simple reflex behavior, and even of visual perception. From a practical point of view, knowledge of the molecular biology of neurons provides a key to the biochemical control of brain function through drugs, and it holds out the promise of more effective treatment for many forms of mental illness.

In this chapter we shall focus on the nerve cell and try to illustrate how its properties give insight into neural organization at higher levels.

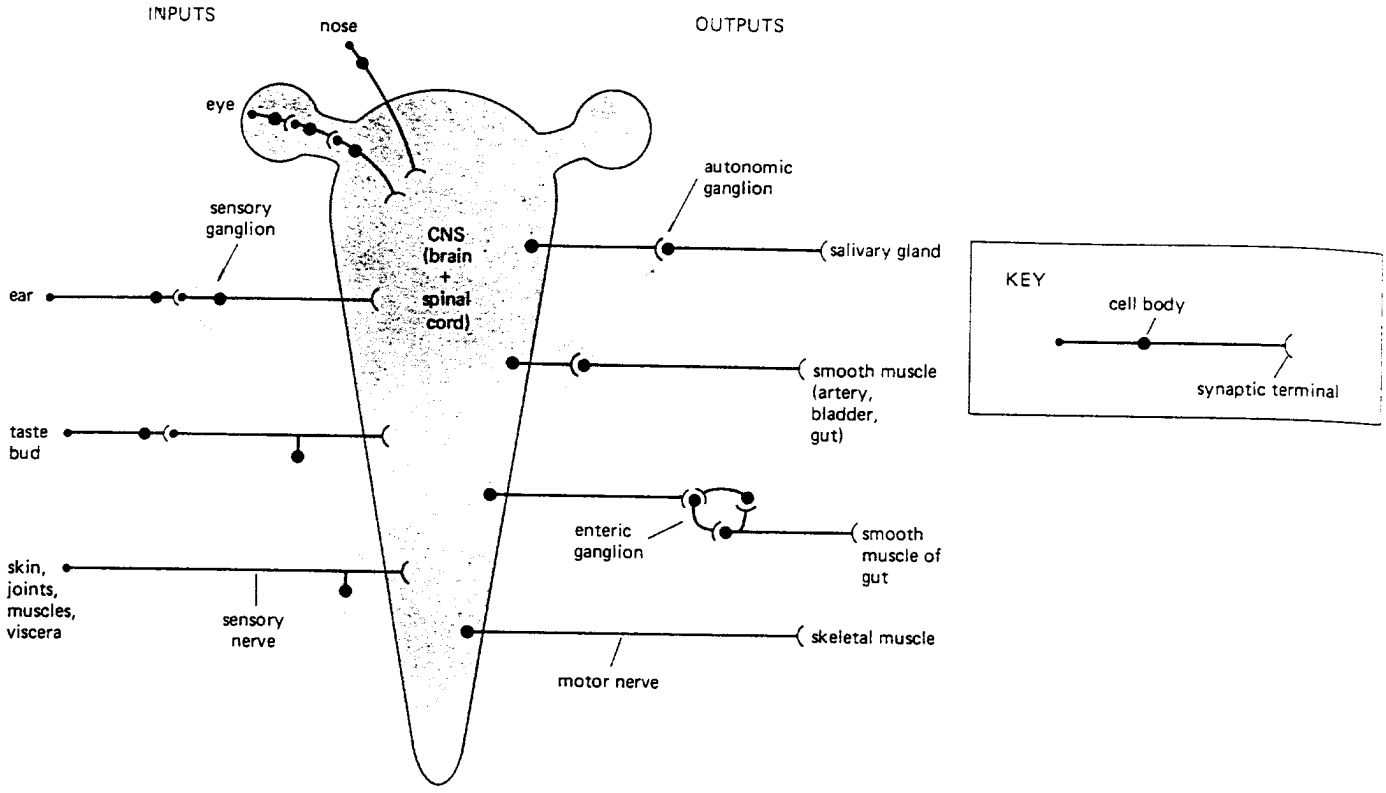
The Cells of the Nervous System: An Overview of Their Structure and Function[1]

The nervous system provides for rapid communication between widely separated parts of the body. Through its role as a communications network, it governs reactions to stimuli, processes information, and generates elaborate patterns of signals to control complex behaviors. The nervous system is also capable of learning: as it processes and records sensory information about the external world, it undergoes adjustments that result in altered future patterns of action.

The major neural pathways of communication were mapped out more than a hundred years ago, before the role of individual nerve cells was understood. Figure 19–1 shows the basic plan. Like a big computing facility, the vertebrate



Scanning electron micrograph of a [illegible]
inner ear of a frog. Courtesy of Ri[illegible]
281-5733), 1979.



nervous system consists of a main processing unit, the **central nervous system,** comprising the brain and spinal cord, which is linked by cables, the *nerves,* to a large number of peripheral structures: sense organs for input, muscles (and to a lesser extent glands) for output. There are also connections to peripheral nerve cell clusters called *ganglia,* which serve in some cases simply for communication between periphery and center and in other cases as accessory minicomputers. The scheme is similar in invertebrates, although they usually do not have such conspicuous central nervous systems, and their ganglia have a larger role and more autonomy.

Within this broad scheme, the detailed patterns of neural connections in different species vary enormously. Yet the properties of the individual neurons are much the same, regardless of whether one looks at a mollusk, an insect, an amphibian, or a mammal.

Figure 19–1 A highly schematized diagram of the nervous system of a vertebrate, showing how sensory inputs are relayed from the periphery to the central nervous system (CNS) and how motor outputs are relayed to the periphery from the CNS. Sensory and motor signals are conveyed from and to the peripheral organs by nerve cells whose cell bodies (*large black dots*) in many cases lie clustered in *ganglia* (*colored circles*) outside the CNS and whose axons are bundled together to form *nerves* (*black lines*). The nerves, ganglia, and sense organs together constitute the *peripheral nervous system.* Some ganglia are simple relay stations; others—notably the subclass of autonomic ganglia that control the peristaltic contractions of the gut (*enteric* ganglia)—are complex systems of interacting neurons capable of functioning even in isolation from the CNS. Within the CNS are masses of interconnected neurons that are not shown.

The Function of a Nerve Cell Depends on Its Elongated Structure[2]

The fundamental task of the **neuron** is to receive, conduct, and transmit signals. To perform these functions, neurons in general are extremely elongated: a single nerve cell in a human being, extending, say, from the spinal cord to a muscle in the foot, may be a meter long. Every neuron consists of a *cell body* (containing the nucleus) with a number of long, thin processes radiating outward from it. Usually there is one long **axon,** to conduct signals away from the cell body toward distant targets, and several shorter branching **dendrites,** which extend from the cell body like antennae and provide an enlarged surface area to receive signals from the axons of other nerve cells (Figure 19–2). Signals are also received on the cell body itself. The axon commonly divides at its far end into many branches and so can pass on its message to many target cells simultaneously. Likewise, the extent of branching of the dendrites can be very great—in some cases sufficient to receive as many as 100,000 inputs on a single neuron. Neurons of different functional classes show an astonishing variety in the pattern of branching of their axons and dendrites (Figure 19–3).

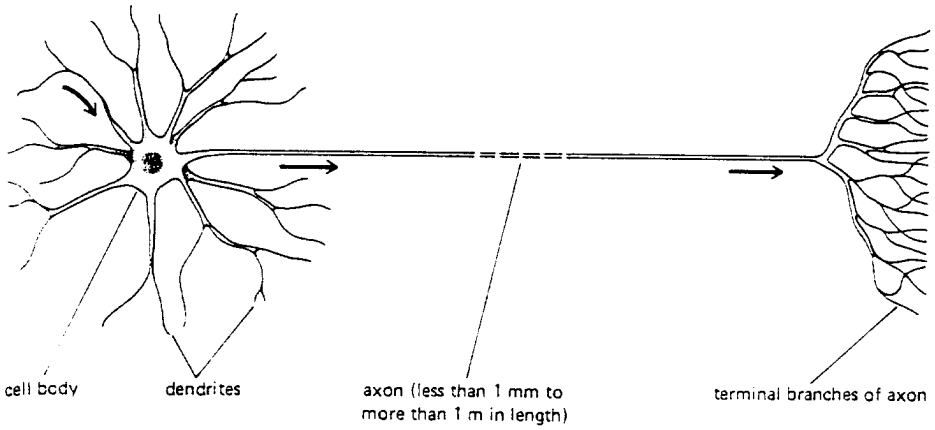


Figure 19–2 Schematic diagram of a typical neuron of a vertebrate. The arrows indicate the direction in which signals are conveyed. The longest and largest neurons in a human extend for about a meter and have an axon diameter of about 15 μm.

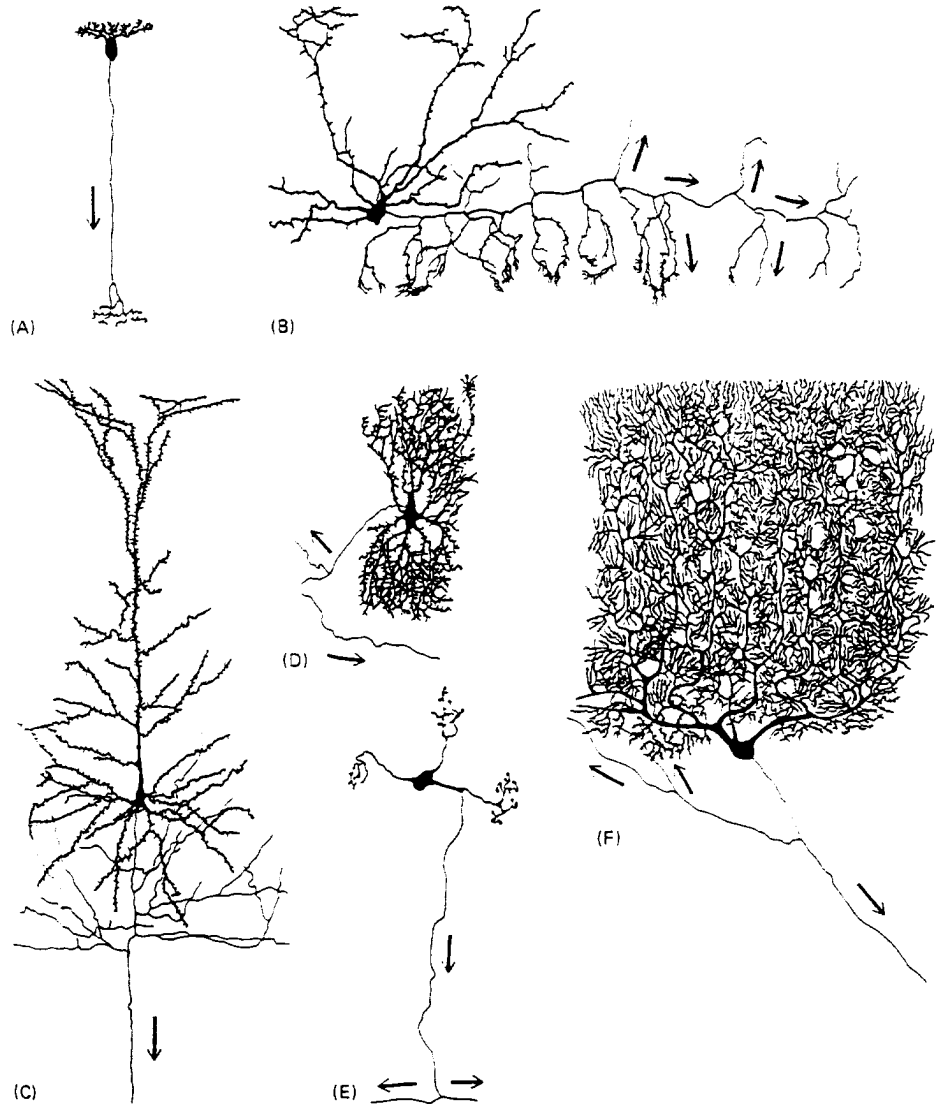


Figure 19–3 A few of the many types of neurons in the vertebrate nervous system as they appear when stained by the Golgi technique. This procedure, which involves immersing the tissue in a solution of metallic salts, picks out at random a small proportion of the cells, staining them an intense black so that all their delicate tracery of branching processes can be seen. Many dendrites, to receive signals, radiate from the cell body of each neuron, and a single thin, branching axon conducts signals away in the direction shown by the arrows. The axon is drawn in color, the cell body and dendrites in black. The cells in (A) and (B) have short axons, which are shown in their entirety. The cells in (C), (D), (E), and (F) have long axons, of which only the initial portion is shown. (A) shows a bipolar cell from the retina of a lizard, (B) is a basket cell from the cerebellum of a mouse, (C) is a pyramidal cell from the cerebral cortex of a rabbit, (D) is a cell from the brainstem of a human being, (E) is a granule cell from the cerebellum of a cat, and (F) is a Purkinje cell from the cerebellum of a human being. This last cell, with its vast array of dendrites, receives inputs from more than 100,000 other neurons. It forms part of the brain's machinery for controlling complex movements. The drawings are not to scale: cell (A) is about 100 μm long, whereas the part of cell (F) shown in the drawing is about 400 μm across (the length of its axon—not shown—is on the order of centimeters). (From S. Ramón y Cajal, Histologie du Système Nerveux de l'Homme et des Vertébrés. Paris: Maloine, 1909–1911; reprinted, Madrid: C.S.I.C., 1972.)

Nerve Cells Convey Electrical Signals[3]

The significance of the signals carried by a neuron depends on the part played by the individual cell in the functioning of the nervous system as a whole. In a *motor neuron* the signals represent commands for the contraction of a particular muscle. In a *sensory neuron* they represent the information that a specific type of stimulus, such as a light, a mechanical force, or a chemical substance, is present at a certain site in the body. In an *interneuron*, forming a connection between one neuron and another, the signals represent parts of elaborate computations that combine information from many different sources and regulate complex behavior.

Despite the varied significance of the signals, their *form* is the same, consisting of changes in the electrical potential across the neuron's plasma membrane. Communication occurs because an electrical disturbance produced in one part of the cell spreads to other parts. Such a disturbance becomes weaker with increasing distance from its source unless energy is expended to amplify it as it travels. Over short distances this attenuation is unimportant, and in fact many small neurons conduct their signals passively, without amplification. For long-distance communication, however, such passive spread is inadequate. Thus the larger neurons employ an active signaling mechanism, which is one of their most striking features: an electrical stimulus that exceeds a certain threshold strength triggers an explosion of electrical activity that is propagated rapidly along the neuron's plasma membrane and is sustained by automatic amplification all along the way. This traveling wave of electrical excitation, known as an *action potential* or *nerve impulse*, can carry a message without attenuation from one end of a neuron to the other at speeds as great as 100 m/sec or more.

Nerve Cells Communicate Chemically at Synapses[4]

Neuronal signals are transmitted from cell to cell at specialized sites of contact known as **synapses**. The usual mechanism of transmission appears surprisingly indirect. The cells are electrically isolated from one another, the *presynaptic cell* being separated from the *postsynaptic cell* by a *synaptic cleft*. A change of electrical potential in the presynaptic cell triggers it to release a chemical known as a *neurotransmitter*, which is stored in membrane-bounded *synaptic vesicles* and released by exocytosis. The neurotransmitter then diffuses across the synaptic cleft and provokes an electrical change in the postsynaptic cell (Figure 19–4). As we shall see, transmission via such *chemical synapses* is far more versatile and adaptable than direct electrical coupling via gap junctions (see p. 799), which is also used, but to a much lesser extent.

The chemical synapse is a site of intense biochemical activity, involving continual degradation, turnover, and secretion of proteins and other molecules. The biosynthetic center of the neuron, however, is in the cell body, where the ultimate instructions for protein synthesis lie. The neuron must therefore have an efficient intracellular transport system to convey molecules from the cell body to the outermost reaches of the axon and dendrites. How is this transport system organized, and what molecules are actually transported?

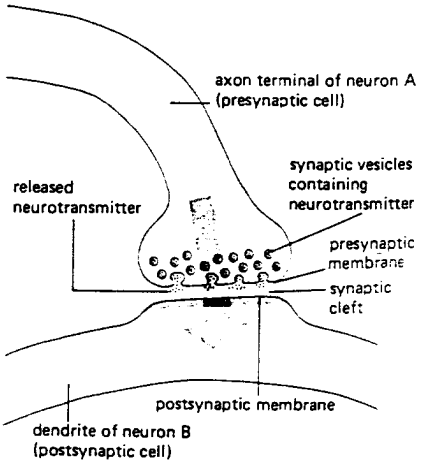
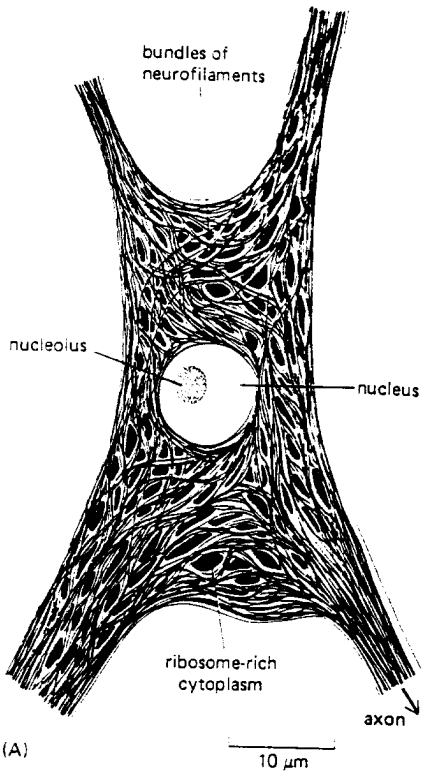


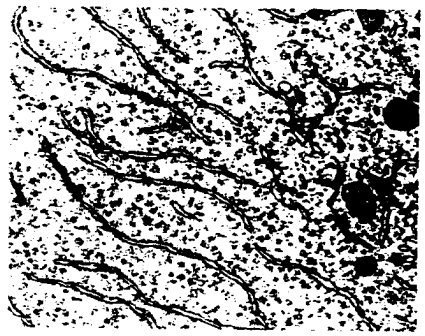
Figure 19–4 Schematic diagram of a typical synapse. An electrical signal arriving at the axon terminal of neuron A triggers the release of a chemical messenger (the neurotransmitter) which crosses the synaptic cleft and causes an electrical change in the membrane of a dendrite of neuron B. A broad arrow indicates the direction of signal transmission.

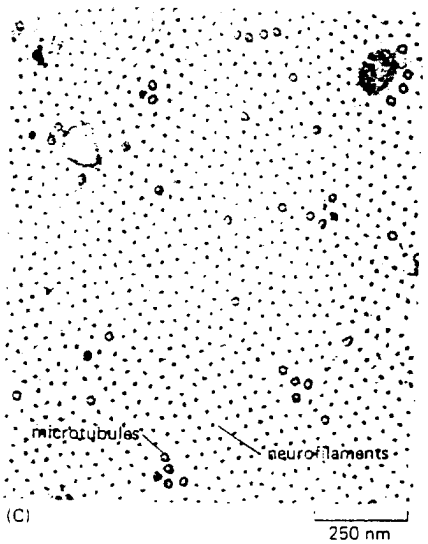
Slow and Fast Transport Mechanisms Carry Newly Synthesized Materials from the Nerve Cell Body into the Axon and Dendrites[5]

Electron microscopy reveals that the cell body of a typical large neuron contains vast numbers of ribosomes, some crowded together in the cytosol, some attached to rough endoplasmic reticulum (ER) (Figure 19–5A). Although dendrites often contain some ribosomes, there are no ribosomes in the axon, and its proteins must therefore be provided by the many ribosomes in the cell body (Figure 19–5B). The needs of the axon are considerable: a large motor neuron in a human being, for example, may have an axon 15 μm in diameter and a meter long, corresponding to a volume of about 0.2 mm^3, which is about 10,000 times the volume of a liver

Figure 19–5 The organization of the cytoplasm in a typical large nerve cell (a motor neuron in the spinal cord). (A) A sketch of the cell body at low magnification, showing how regions of cytoplasm rich in ribosomes are packed in the gaps between bundles of neurofilaments and other cytoskeletal proteins. (B) An electron micrograph of one such ribosome-rich region: some of the ribosomes are free, others are attached to rough ER. (C) An electron micrograph showing part of a cross-section of the axon: large numbers of neurofilaments and microtubules can be seen, but no ribosomes are present. The membranous vesicles in the axon are probably traveling along the adjacent microtubules by fast axonal transport. (B, courtesy of Jennifer La Vail; C, courtesy of John Hopkins.)







cell. Because such a neuron contains only a single nucleus, its ratio of cytoplasm to DNA is far greater than that of any nonneuronal cell type in the human body.

The most plentiful proteins in the axon are those that form microtubules, neurofilaments (a class of intermediate filaments), and actin filaments (Figure 19–5C). These cytoskeletal proteins are exported from the cell body and move along the axon at speeds of 1 to 5 mm per day by the process of **slow axonal transport.** (A similar transport occurs in the dendrites, which contain a slightly different set of microtubule-associated proteins—see p. 659.) Other cytosolic proteins, including many enzymes, are also carried by slow axonal transport, whose mechanism is not understood.

Noncytosolic materials required at the synapse, such as secreted proteins and membrane-bound molecules, move outward from the cell body by a much faster mode of transport. These proteins and lipids pass from their sites of synthesis in the endoplasmic reticulum to the Golgi apparatus, which lies close to the nucleus, often facing the base of the axon. From here, packaged in membrane vesicles, they are carried by **fast axonal transport,** at speeds of up to 400 mm per day, along tracks formed by microtubules in the axon or the dendrites (see p. 660); mitochondria are conveyed by the same means. Since different populations of proteins are sent out in this way along axons and dendrites, the transported molecules are presumed to be sorted in the cell body into separate and distinctive types of transport vesicles (see p. 463).

Among the proteins rapidly transported along the axon are those to be secreted at the synapse, such as the *neuropeptides* that many neurons release as neurotransmitters, often in conjunction with nonprotein transmitters. From the point of view of their internal organization, neurons can thus be thought of as secretory cells in which the site of secretion has been removed to an enormous distance from the site where proteins and membranes originate (Figure 19–6).

Retrograde Transport Allows the Nerve Terminal to Communicate Chemically with the Cell Body[5,6]

Fast axonal transport is required during development for the growth of axons and dendrites, which elongate by adding new membrane to their tips. Fast axonal transport also occurs in a full-grown neuron, in which there is no net accumulation of membrane at the ends of the axon and dendrites. In this case the fast transport of membrane outward from the cell body, called *fast anterograde transport,* must be exactly balanced by *fast retrograde transport* of membrane back from the ends of the cell processes. The mechanisms of fast transport in the two directions are similar but not identical. The fast retrograde transport has a speed about half that of fast anterograde transport, is driven by a different motor protein (see p. 660), and carries somewhat larger vesicles on average. The structures returning to the cell body consist partly of aging cytoplasmic organelles, such as mitochondria, and partly of vesicles formed by the extensive endocytosis required for membrane retrieval at the axon terminal after neurotransmitter release (see

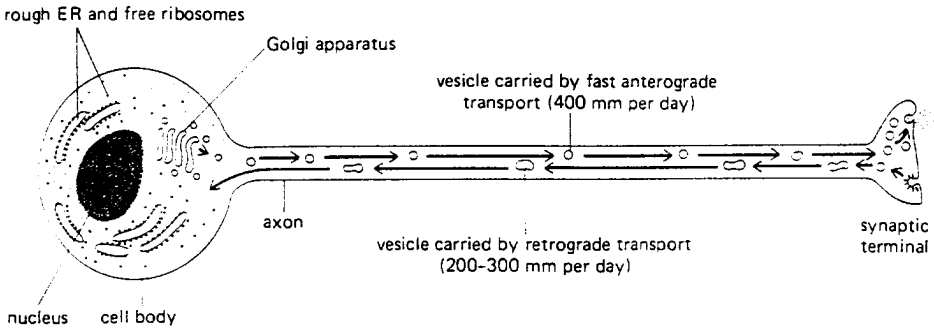


Figure 19–6 A neuron viewed schematically as a secretory cell in which the site of secretion (the axon terminal) lies at a great distance from the site of macromolecular synthesis (the cell body). This mode of organization creates a need for a rapid axonal transport mechanism. The diagram is not meant to imply that all synaptic vesicles have to be transported from the cell body; in most neurons, synaptic vesicles are formed largely by local recycling of membrane in the axon terminal.

Figure 19–20). Molecules present in the extracellular medium surrounding the axon terminal are liable to be captured in these endocytic vesicles and thereby carried back from the axon terminal to the cell body. Thus the biosynthetic machinery in the cell body can sample conditions at the axon terminal and make an appropriate response, as we shall see later (see p. 1119).

Retrograde transport is extremely useful to neuroanatomists, who routinely exploit it to trace neural connections, as explained in Figure 19–7.

Neurons Are Surrounded by Various Types of Glial Cells[7]

All neural tissue, both peripheral and central, consists of two major classes of cells. Neurons play the star role, but they are outnumbered, by about 10 to 1 in the mammalian brain, by a supporting cast of **glial cells.** The glial cells surround neurons (both their cell bodies and their processes) and occupy the spaces between them. The best understood are the *Schwann cells* in vertebrate peripheral nerves and the *oligodendrocytes* in the vertebrate central nervous system, which both wrap themselves around axons to provide electrical insulation in the form of a *myelin sheath* (see p. 1073, below). The three other types of glial cell in the central nervous system are microglia, ependymal cells, and astrocytes (Figure 19–8). The *microglia* belong in a class apart: they are functionally akin to macrophages (see p. 974) and, like them, originate from hemopoietic tissue. With this exception, all the glial cells share a common embryonic origin with the neurons with which they are associated; unlike most neurons, however, they are not as a rule electrically excitable. Moreover, whereas neurons cannot divide after they have differentiated, most glial cells remain capable of dividing throughout life.

Ependymal cells line the internal cavities of the brain and spinal cord (see Figure 19–8), and their epithelial arrangement is a memento of the origin of the central nervous system from an epithelial tube (see p. 1109).

Astrocytes (see Figure 19–8) are the most plentiful and diverse of the glial cells but also the most enigmatic: their functions are still largely uncertain, although it seems clear that they play an important part in guiding the construction of the

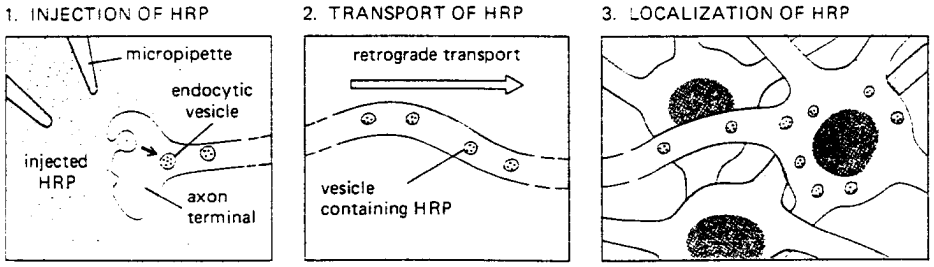


Figure 19–7 How fast retrograde transport is exploited to identify and locate remote nerve cell bodies whose axons project to a given site of interest. The enzyme horseradish peroxidase (HRP) is the most widely used tracer molecule for this purpose, since it can be detected in very small quantities by the colored products of the reaction that it catalyzes.

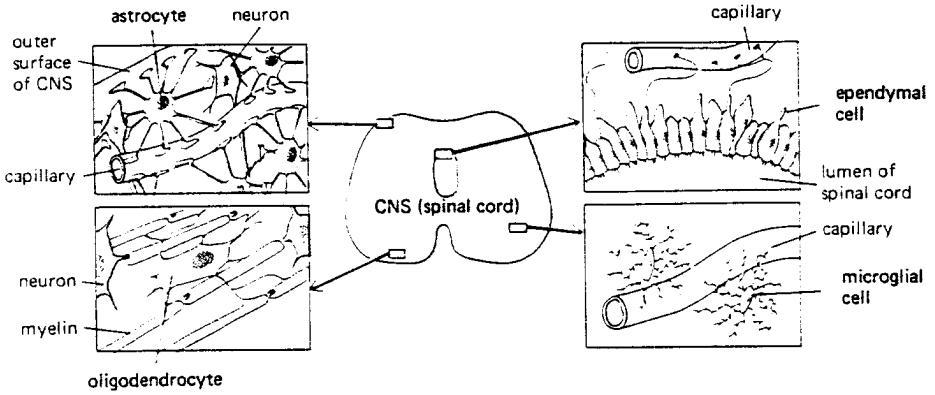


Figure 19–8 The four major classes of glial cells in the vertebrate central nervous system. The glial cells are shown in color. Astrocytes, which are the most plentiful, have many radiating processes. Some of these processes end on the surfaces of neurons; others, with expanded tips, go to form the external surface layer of the CNS and the wrapping that surrounds blood vessels in the CNS, collaborating with endothelial cells of the blood capillaries to create the blood–brain barrier. Ependymal cells form the ciliated epithelial lining of the central cavity of the CNS and, like astrocytes, often have processes ending on blood vessels. Oligodendrocytes form insulating sheaths of myelin around axons in the CNS. Microglial cells are related in function and origin to macrophages and are involved in reactions to tissue damage and infection; they tend to be found in the neighborhood of blood vessels.

nervous system (see p. 1110) and in controlling the chemical and ionic environment of the neurons. Thus one type of astrocyte extends processes that are expanded into "endfeet," which, linked by junctional complexes such as one finds in epithelia (see p. 792), form a sealed barrier at the external surface of the central nervous system. They also extend processes that form similar endfeet on blood vessels, where they induce the endothelial cells (in the case of capillaries and venules) to become sealed together by unusually well-developed tight junctions so as to form the *blood–brain barrier*. This barrier prevents water-soluble molecules from passing into brain tissue from the blood unless they are specifically carried by transport proteins in the plasma membranes of the endothelial cells that line the vessels. The neurons thus occupy a sheltered and controlled environment, on which the molecular machinery for electrical signaling is critically dependent.

Summary

Nerve cells, or neurons, are exceptionally elongated cells that convey electrical signals in the form of action potentials—traveling waves of electrical excitation. Typically, several branching dendrites and a single long axon project from the nerve cell body. Signals are usually received on the dendrites and cell body, sent out along the axon, and passed on to other cells at chemical synapses. Here the electrical signal in the presynaptic axon terminal triggers the secretion of neurotransmitter, which provokes an electrical change in the postsynaptic cell.

The neuron can be viewed as a secretory cell that releases its secretion—the neurotransmitter—at a very large distance from the cell body, where macromolecules are synthesized. Newly synthesized membrane and proteins for secretion are exported along the axon and dendrites by fast axonal transport, in which small membrane vesicles are propelled along tracks formed by microtubules. The microtubules and other non-membrane-bound components of the neuronal cytosol are exported from the cell body by a quite different, slow axonal transport mechanism. Fast axonal transport also operates in a retrograde direction, conveying membrane vesicles back from the axon terminals to the cell body.

Neurons are surrounded by glial cells, which help in various ways to control the chemical and electrical environment of the neurons.

Voltage-gated Ion Channels and the Action Potential[3,4,8]

As discussed in Chapter 6, the voltage difference across a cell's plasma membrane—the **membrane potential**—depends on the distribution of electric charge (see p. 314). Charge is carried back and forth across the nerve cell membrane by small inorganic ions—chiefly Na^+, K^+, Cl^-, and Ca^{2+}—which traverse the lipid bilayer by passing through selective ion channels formed by specific transmem-

brane proteins (see p. 312). When the ion channels open or close, the charge distribution shifts and the membrane potential changes. Neuronal signaling thus depends on channels whose permeability is regulated—so-called **gated ion channels.**

Two classes of gated channels are of crucial importance: (1) *voltage-gated channels*—especially voltage-gated Na^+ *channels*—play the key role in the explosions of electrical activity by which action potentials are propagated along an axon; and (2) *ligand-gated channels,* which convert extracellular chemical signals into electrical signals, play a central part in the operation of synapses. The account in Chapter 6 (pp. 312–319) of ion channels and of their role in electrical signaling forms the basis for the further discussion of neuronal signaling to be given here. Some principles of electrochemistry that are of special relevance to nerve cells are reviewed in Panel 19–1.

Voltage Changes Can Spread Passively Within a Neuron[3,4,8,9]

Action potentials are typically triggered at one end of an axon and propagate along its length. To understand the mechanism it is helpful to consider first how electrical disturbances spread along a nerve cell in the absence of action potentials. As mentioned earlier, such *passive spread* is common, especially in the many neurons that have very short axons or no axon at all; these cells often have few or no voltage-gated Na^+ channels and rely for their signaling entirely on passive spread, manifest as smoothly graded *local potentials.*

In an axon at rest, the membrane potential is uniformly negative, with the interior of the axon everywhere at the same negative potential relative to the external medium. As explained in Chapter 6 (see p. 314), the potential difference depends on the large concentration gradients of Na^+ and K^+, built up by the Na^+-K^+ pump. K^+ *leak channels* make the resting membrane permeable chiefly to K^+, so that the resting potential is close to the K^+ equilibrium potential—typically about -70 mV (see Panel 19–1). An electrical signal may take the form of a *depolarization,* in which the voltage drop across the membrane is reduced, or a *hyperpolarization,* in which it is increased. To illustrate the passive spread of an electrical signal, let us consider what happens when an axon is locally depolarized by injecting current through a microelectrode inserted into it. If the current is small, the depolarization will be *subthreshold:* practically no Na^+ channels open, and no action potentials are triggered. A steady state is quickly reached in which the inflow of current through the microelectrode is exactly balanced by the outflow of current (carried mainly by K^+ ions) across the axonal membrane. Some of this current flows out in the neighborhood of the microelectrode, while some travels down the interior of the axon for some distance in either direction before escaping. The consequence is that the membrane potential is disturbed by an amount that decreases exponentially with the distance from the source of the disturbance (Figure 19–9). This passive spread of an electrical signal along a nerve cell process is analogous to the spread of a signal along an undersea telegraph cable; as the current flows down the central conductor (the cytoplasm), some leaks out through the sheath of insulation (the membrane) into the external medium, so that the signal becomes progressively attenuated. For this reason the electrical characteristics involved in passive spread are often referred to as *cable properties* of the axon.

Axons, though, are much poorer conductors than electric cables, and passive spread is inadequate to transmit a signal over a distance of more than a few millimeters, especially if the signal is brief and transient. This is not only because of current leakage but also because the change in membrane potential that results from current flow is not instantaneous but takes a while to build up. The time required depends on the membrane *capacitance,* that is, on the quantity of charge that has to be accumulated on either side of the membrane to produce a given membrane potential (see Panel 19–1). The membrane capacitance has the effect both of slowing down the passive transmission of signals along the axon and of distorting them, so that a sharp, pulselike stimulus delivered at one point will be detected a few millimeters away as a slow, gradual rise and fall of the potential,

1. Separated layers of charge create a voltage gradient

The voltage gradient across the cell membrane, or *membrane potential*, is created by an excess of positive charge on one side and a matching excess of negative charge on the other. The charge is concentrated in a thin (< 1 nm) layer on each side of the membrane.

2. The membrane capacitance determines the charge required to create a given voltage difference

The amount of charge (in coulombs) required on each side of the membrane to create a voltage difference of 1 V is called the membrane *capacitance* (in farads).

Cell membranes typically have a capacitance of about 1 μF/cm^2, or 0.01 pF/μm^2. Therefore a movement of 0.001 pC of charge across 1 μm^2 of membrane will alter the membrane potential by 100 mV.

UNITS

Charge: couloumb (C) (6.2 X 10^{18} X charge on one electron)
Electric potential: volt (V)
Current: ampere (= coulombs per second) (A)
Capacitance: farad (= coulombs per volt) (F)
Conductance: siemens (= amperes per volt) (S)
mV: millivolt (10^{-3} V)
μF: microfarad (10^{-6} F)
nC: nanocoulomb (10^{-9} C)
pS: picosiemens (10^{-12} S)

3. The number of ions that go to form the layer of charge adjacent to the membrane is minute compared with the total number inside the cell

One coulomb is the charge carried by roughly 6 X 10^{18} univalent ions, so that 0.001 pC is equivalent to 6000 univalent ions. Therefore the movement of 6000 Na^+ ions across 1 μm^2 of membrane will carry sufficient charge to shift the membrane potential by about 100 mV. Because there are about 3 X 10^7 Na^+ ions in 1 μm^3 of bulk cytoplasm, such a movement of charge will generally have a negligible effect on the ion concentration gradients across the membrane.

4. The electrochemical "driving force" is the sum of an effect of the membrane potential and an effect of the concentration gradient

For a univalent positive ion, such as Na^+ or K^+, at room temperature, the net "driving force" across the membrane is proportional to

$$V - 58 \log_{10}\left(\frac{C_o}{C_i}\right),$$

where V is the membrane potential in millivolts, and C_o and C_i are, respectively, the extracellular and intracellular concentrations of the ion. The "driving force" for positive ions is zero when

$$V = 58 \log_{10}\left(\frac{C_o}{C_i}\right) \text{ mV}.$$

This is the *Nernst equation* in its simplest form (see p. 315). It defines the *equilibrium potential* for the given positive ion. For the squid axon, the equilibrium potentials V_{Na}, V_K, and V_{Cl} for Na^+, K^+, and Cl^-, are, respectively, about +55 mV, −75 mV, and −65 mV. The net driving forces for each ion are proportional to $V - V_{Na}$, $V - V_K$, and $V - V_{Cl}$.

5. Ion current is proportional to driving force multiplied by membrane conductance

The current of, say, Na^+ ions passing across the membrane (measured in amperes) is

$$i_{Na} = g_{Na} \times (V - V_{Na}),$$

where g_{Na} is the *Na^+ conductance* of the membrane. The Na^+ conductance is proportional to the number of Na^+ channels that are open at any instant. The conductance of a single open Na^+ channel is about 4 pS in the squid axon, and there are about 75 Na^+ channels/μm^2 of membrane.

6. A flow of current builds up a charge

For a squid axon membrane at the peak of the action potential,

$$g_{Na} = 300 \text{ pS}/\mu\text{m}^2;$$

the corresponding Na^+ current is roughly

$$i_{Na} = 5 \text{ pA}/\mu\text{m}^2.$$

If no other ions crossed the membrane, the charge transferred by the Na^+ current if it were sustained for 0.2 ms at the peak value seen during the action potential would be 0.001 pC/μm^2. This charge would alter the membrane potential—see (1) and (2), above.

 Panel 19–1 Electricity, ions, and membranes: some major principles.

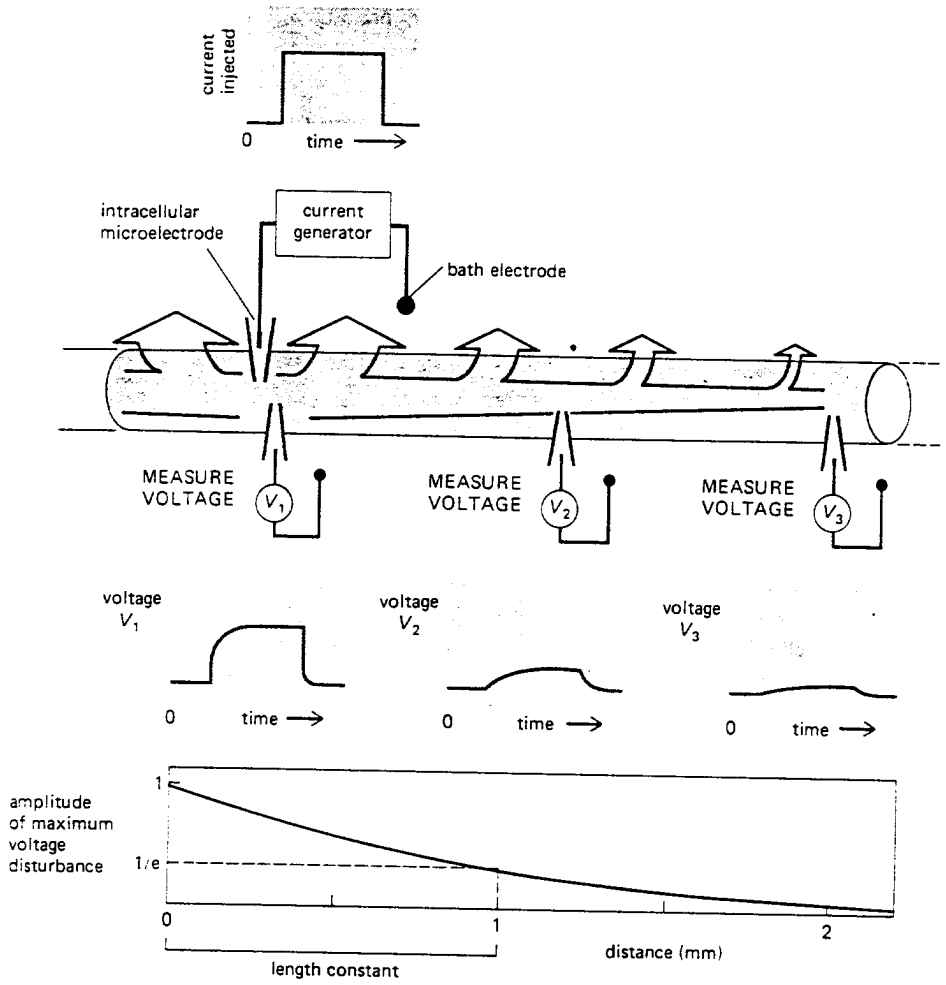


Figure 19–9 Current injected into an axon through a microelectrode flows out again across the plasma membrane; the magnitude of the outflowing current falls off exponentially with distance from the microelectrode. The current flow is assumed to be small causing a subthreshold depolarization of the membrane. The graphs show how the disturbance of membrane potential produced by injection of a pulse of current falls off with distance from the source of the disturbance. The length constant is the distance over which the amplitude of the disturbance of the membrane potential falls off by a factor of 1/*e*. The length constant ranges from about 0.1 mm (for a very small axon with a relatively leaky membrane to about 5 mm (for a very large axon with a relatively nonleaky membrane). Here it is 1 mm.

with a greatly diminished amplitude (see Figure 19–9). To transmit faithfully over more than a few millimeters, therefore, an axon requires, in addition to its passive cable properties, an active mechanism to maintain the strength and waveform of the signal as it travels. This automatically amplified signal is the *action potential.*

Voltage-gated Na^+ Channels Generate the Action Potential; Voltage-gated K^+ Channels Keep It Brief[3,4,8,10]

The electrochemical mechanism of the **action potential** was first established in the 1940s and 1950s. Techniques for studying electrical events in small single cells had not yet been developed, and the experiments were made possible only by the use of a giant cell, or rather a part of a giant cell: a giant axon from a squid (Figure 19–10). Subsequent work has shown that the neurons of most animals conduct their action potentials in a similar way. Panel 19–2 outlines some of the key original experiments. Despite the many technical advances that have been made since then, the logic of the original analysis continues to serve as a model for present-day work. The crucial insight was that the permeability of the membrane to Na^+ and K^+ is changed by changes in the membrane potential: in other words, the membrane contains channels for Na^+ and K^+ that are voltage-gated. The voltage-

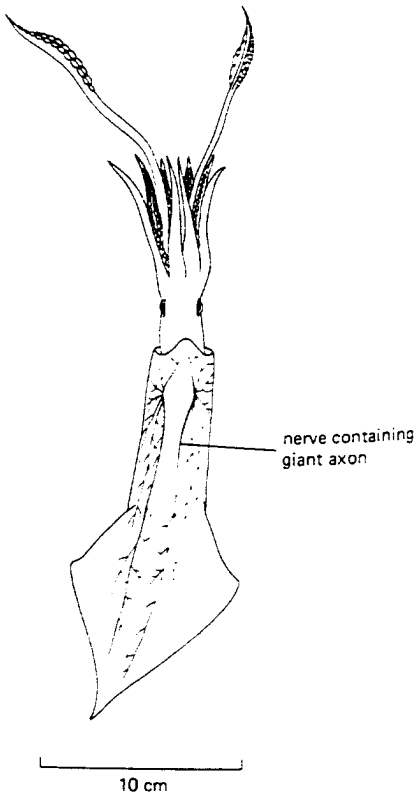
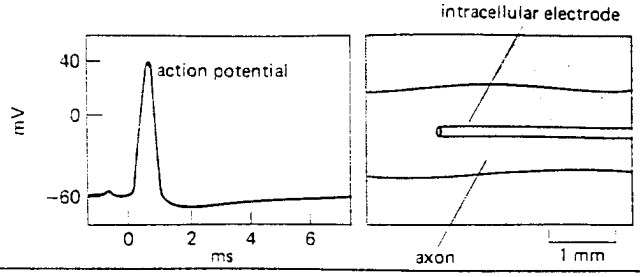


Figure 19–10 A squid, showing the location of the giant axons whose large size made possible the original analysis of the mechanism of the action potential. (From H. Curtis, Biology, 4th ed. New York: Worth, 1983; after Keynes, R.D. The nerve impulse and the squid. *Scientific American*, December 1958. Copyright © 1958 by Scientific American, Inc. All rights reserved.)

1 Action potentials are recorded with an intracellular electrode

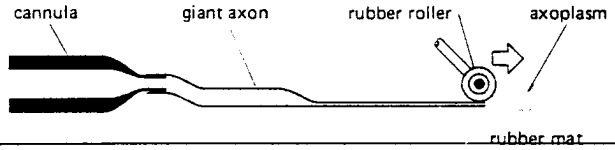
The squid giant axon is about 0.5–1 mm in diameter and several centimeters long (Figure 19–10). An electrode in the form of a glass capillary tube containing a conducting solution can be thrust down the axis of the cell so that its tip lies deep in the cytoplasm. With its help, one can measure the voltage difference between the inside and the outside of the cell—that is, the membrane potential—as an action potential sweeps past the electrode. The action potential is triggered by a brief electrical shock to one end of the axon. It does not matter which end, because the excitation can travel in either direction; and it does not matter how big the shock is, as long as it exceeds a certain threshold: the action potential is *all or none*.

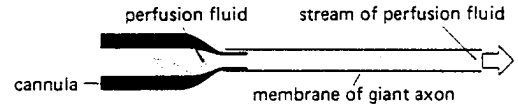


2 Action potentials depend only on the neuronal plasma membrane and on gradients of Na^+ and K^+ across it

The three most plentiful ions, both inside and outside the axon, are Na^+, K^+, and Cl^-. As in other cells, the Na^+-K^+ pump maintains a concentration gradient: the concentration of Na^+ is about 9 times lower inside the axon than outside, while the concentration of K^+ is about 20 times higher inside than outside. Which ions are important for the action potential?

The squid giant axon is so large and robust that it is possible to extrude the cytoplasm from it, like toothpaste from a tube, and then to perfuse it internally with pure artificial solutions of Na^+, K^+, and Cl^- or SO_4^{2-}. Remarkably, if (and only if) the concentrations of Na^+ and K^+ inside and outside approximate those found naturally, the axon will still propagate action potentials of the normal form as shown above. The important part of the cell for electrical signaling, therefore, must be the membrane; the important ions are Na^+ and K^+; and a sufficient source of free energy to power the action potential must be provided by their concentration gradients across the membrane, because all other sources of metabolic energy have presumably been removed by the perfusion.

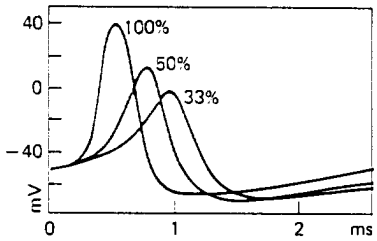




3 At rest, the membrane is chiefly permeable to K^+; during the action potential, it becomes transiently permeable to Na^+

At rest the membrane potential is close to the equilibrium potential for K^+. When the external concentration of K^+ is changed, the resting potential changes roughly in accordance with the Nernst equation for K^+ (see Panel 19–1 and p. 315). At rest, therefore, the membrane is chiefly permeable to K^+: K^+ leak channels provide the main ion pathway through the membrane.

If the external concentration of Na^+ is varied, there is no effect on the resting potential. However, the height of the peak of the action potential varies roughly in accordance with the Nernst equation for Na^+. During the action potential, therefore, the membrane appears to be chiefly permeable to Na^+: Na^+ channels have opened. In the aftermath of the action potential, the membrane potential reverts to a negative value that depends on the external concentration of K^+ and is even closer to the K^+ equilibrium potential than the resting potential is: the membrane has lost its permeability to Na^+ and has become even more permeable to K^+ than before—that is, Na^+ channels have closed, and additional K^+ channels have opened.

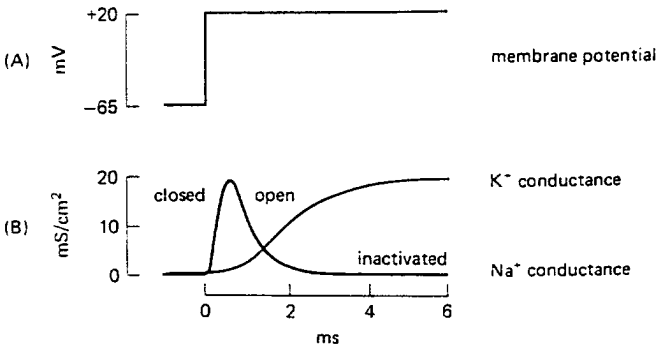


The form of the action potential when the external medium contains 100%, 50%, or 35% of the normal concentration of Na^+

4 Voltage clamping reveals how the membrane potential controls opening and closing of ion channels

The membrane potential can be held constant ("voltage clamped") throughout the axon by passing a suitable current through a bare metal wire inserted along the axis of the axon while monitoring the membrane potential with another intracellular electrode (see Figure 19–11). When the membrane is abruptly shifted from the resting potential and held in a depolarized state (A), Na^+ channels rapidly open until the Na^+ permeability of the membrane is much greater than the K^+ permeability; they then close again spontaneously, even though the membrane potential is clamped and unchanging. K^+ channels also open but with a delay, so that the K^+ permeability becomes large as the Na^+ permeability falls (B). If the experiment is now very promptly repeated, by returning the membrane briefly to the resting potential and then quickly depolarizing it again, the response is different: prolonged depolarization has caused the Na^+ channels to enter an *inactivated* state, so that the second depolarization fails to cause a rise and fall similar to the first. Recovery from this state requires a relatively long time—about 10 milliseconds—spent at the repolarized (resting) membrane potential.

In a normal unclamped axon, an inrush of Na^+ through the opened Na^+ channels produces the spike of the action potential; inactivation of Na^+ channels and opening of K^+ channels bring the membrane back down to the resting potential.



1069 **Panel 19–2** Some classical experiments on the squid giant axon.

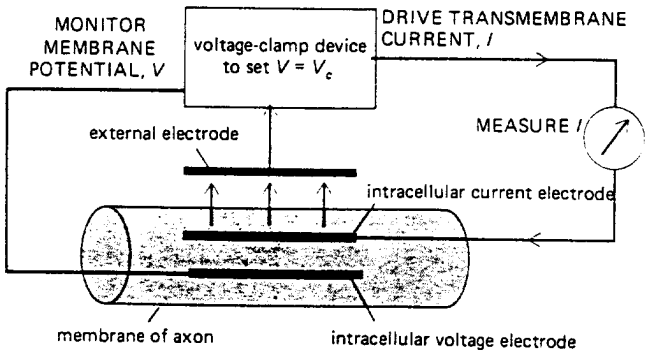


Figure 19–11 The voltage-clamp technique for studying the behavior of ion channels by measuring the current that flows across the plasma membrane when the membrane potential is held fixed at any chosen level. Two intracellular electrodes are used, one to monitor the membrane potential, the other to pass a measured current into the cell. The current passed in via the current electrode flows out again through ion channels in the plasma membrane; the circuit is shown in color. So long as the membrane potential is constant, the current, *I*, passed into the axon via the current electrode is exactly equal to the total current flowing out again across all regions of its membrane (otherwise the net charge inside the cell would change, causing a change in membrane potential). The membrane potential can be altered by forcing more or less current to flow across the membrane. The electronic apparatus marked "voltage-clamp device" monitors the membrane potential, *V*, and controls the current *I* so as to hold *V* constant: any slight departure from a chosen preset "command voltage," V_c, automatically causes a compensating adjustment of the current in such a way that the membrane potential is *clamped* at $V = V_c$. To study the time-dependent behavior of the membrane channels, one can switch abruptly from one fixed value of V_c to another and observe the corresponding current on an oscilloscope. By repeating the current measurements with different concentrations of Na^+ and K^+ in the medium, it is possible to deduce how much of the transmembrane current is carried by each of these ions and so distinguish the contributions due to Na^+-selective and K^+-selective channels. The voltage-clamp technique can be adapted to analyze the behavior of individual ion channel molecules in a tiny patch of membrane covering the mouth of a microelectrode; this is the method of *patch-clamp* recording.

clamp technique (Figure 19–11) made it possible to determine the detailed rules by which a change of membrane potential opens and closes the channels, and the action potential was then shown to be a direct consequence of the operation of these rules.

An action potential is triggered when the membrane is momentarily depolarized beyond a certain *threshold* value. As explained in Chapter 6, such a depolarization, applied to a given area of membrane, causes *voltage-gated Na^+ channels* there to open; this permits an influx of Na^+ down its electrochemical gradient, which causes still further depolarization, causing more Na^+ channels to open, and so on, in an explosive self-amplifying fashion, until the membrane in that region is driven almost all the way to the Na^+ equilibrium potential (see Panel 19–1). At this point, two factors conspire to bring the membrane back down toward its original negative potential: the Na^+ channels convert spontaneously to a closed, *inactivated* state, and *voltage-gated K^+ channels* open. These K^+ channels respond to changes of membrane potential in much the same way as the Na^+ channels do, but with slightly slower kinetics (for this reason they are sometimes called *delayed K^+ channels*). Once the K^+ channels are open, the transient influx of Na^+ is rapidly overwhelmed by an efflux of K^+, and the membrane is driven back toward the K^+ equilibrium potential, even before the inactivation of the Na^+ channels is complete. The repolarization causes the voltage-gated K^+ channels to close again and allows the inactivated Na^+ channels to regain their original closed but activatable state. In this way the patch of membrane can be made ready to fire another action potential in less than a millisecond.

Subsequent experiments have shown that not all neurons depend on voltage-gated K^+ channels to terminate the action potential. In particular, in mammalian myelinated axons (see p. 1073), voltage-gated K^+ channels are present only in very small numbers, and the return to rest is brought about simply by the inactivation of the Na^+ channels. Although voltage-gated K^+ channels are thus not essential for the propagation of action potentials, we shall see (p. 1089) that they play a crucial part in the mechanism for triggering action potentials in response to stimulation of the nerve cell body.

Propagated Action Potentials Provide for Rapid Long-distance Communication[3,4,8,11]

Because of the cable properties of the axon, the large local influx of Na^+ ions during an action potential causes some current to flow along its length, depolarizing neighboring regions of the membrane to threshold levels, so that they in their turn produce action potentials (Figure 19–12). This process continues down the axon, with one region "igniting" the next, at speeds that in a vertebrate range from less than 1 m/sec to more than 100 m/sec, depending on the type of axon.

The speed of propagation depends mainly on the cable properties of the axon: the larger the membrane capacitance, the larger the charge required for depolarization to threshold; and the larger the internal resistance of the axonal cytoplasm, the smaller the currents that will flow along it and the longer it will take for the requisite charge to accumulate. Both the resistance and the capacitance of a unit length of axon depend on the axon's cross-sectional area, and a simple calculation

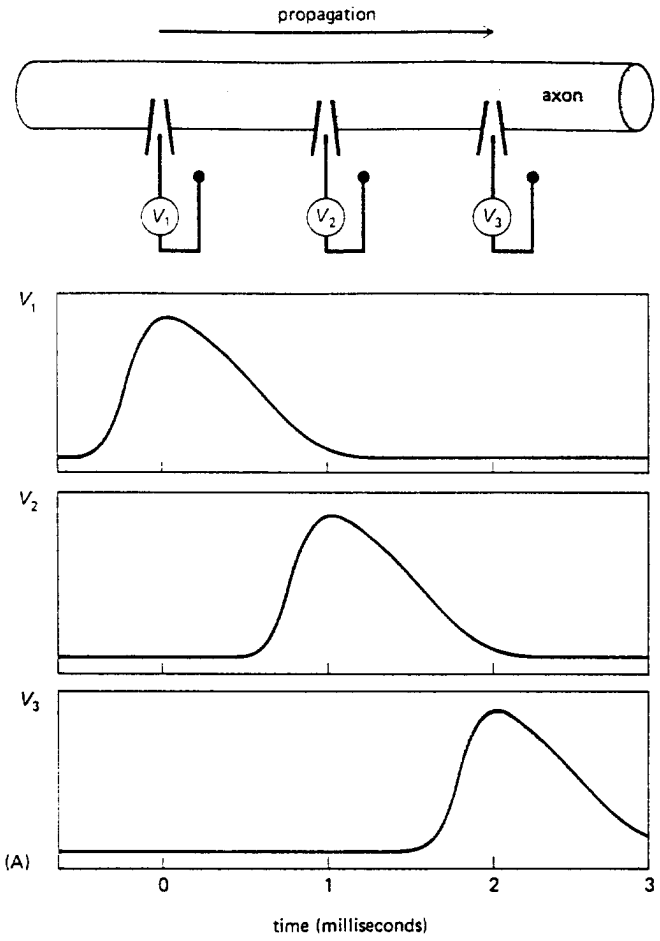
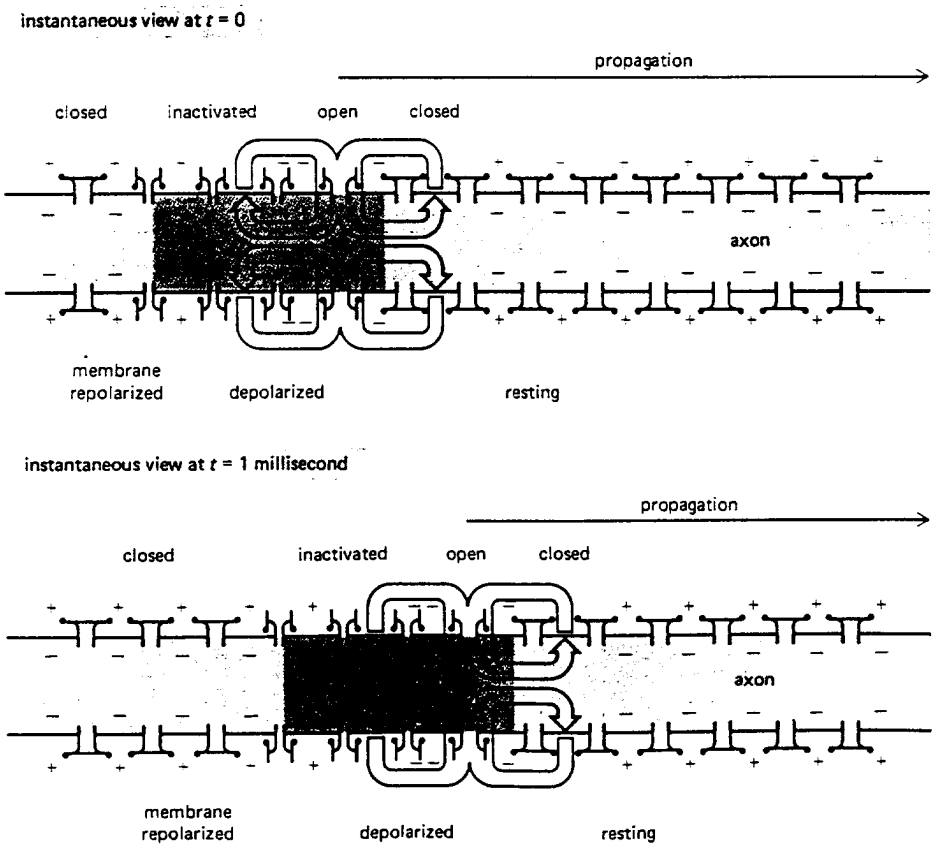
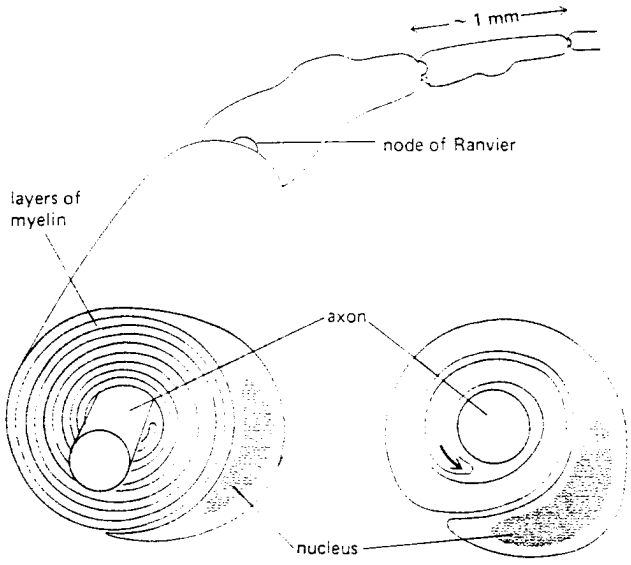
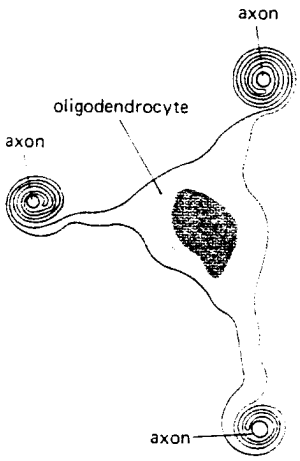


Figure 19–12 The propagation of an action potential. (A) shows the voltages that would be recorded from a set of intracellular electrodes placed at intervals along the axon. (B) shows the changes in the Na^+ channels and the current flows (*colored lines*) that give rise to the traveling disturbance of the membrane potential. The region of the axon with a depolarized membrane is shown in color.



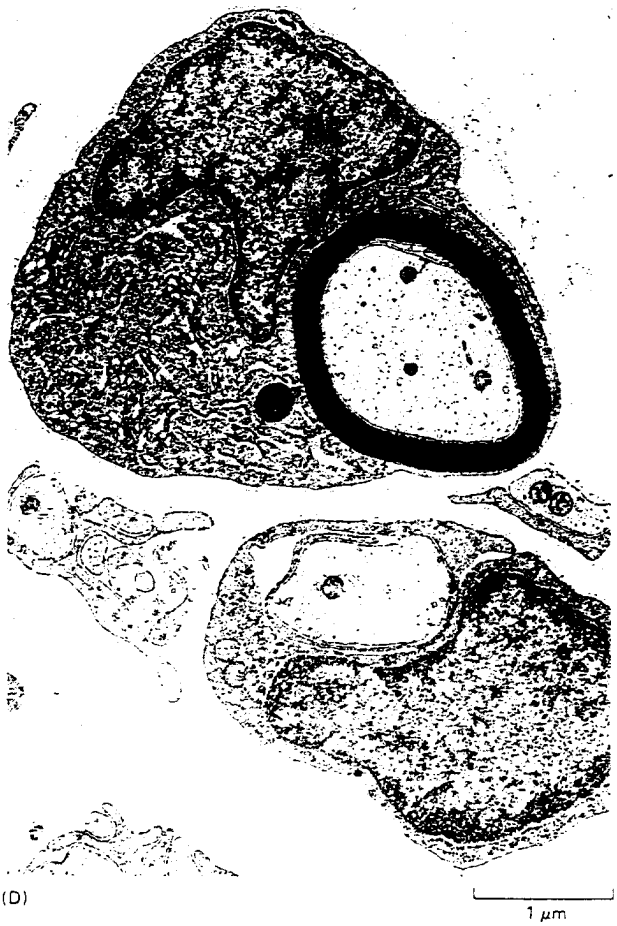


(A) (B)

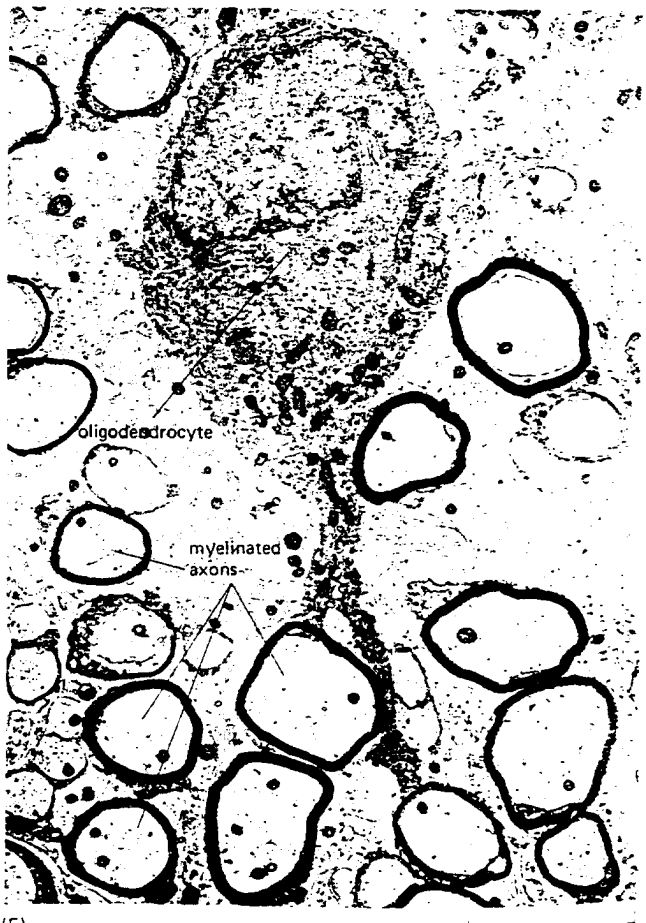


(C)

Figure 19–13 (A) Schematic diagram of a myelinated axon from a peripheral nerve. Each Schwann cell wraps its plasma membrane concentrically around the axon to form a segment of myelin sheath about 1 mm long. For clarity, the layers of myelin are not shown so tightly compacted together as they are in reality (see part D). (B) Schematic diagram of a Schwann cell in the early stages of forming a spiral of myelin around an axon during development. Note that it is the inner tongue of the Schwann cell (marked with an arrow) that continues to extend around the axon, thereby adding turns of membrane to the myelin sheath. (C) Schematic diagram of an oligodendrocyte, which forms myelin sheaths in the central nervous system. A single oligodendrocyte myelinates many separate axons. (D) Electron micrograph of a section from a nerve in the leg of a young rat. Two Schwann cells can be seen: one is just beginning to myelinate its axon, the other has formed an almost mature myelin sheath. (E) Electron micrograph of an oligodendrocyte in the spinal cord of a kitten extending processes to myelinate at least two axons. (D and E, from C. Raine, in Myelin (P. Morell, ed.), New York: Plenum, 1976.)



(D)



(E)

shows that the net effect of enlarging the axon diameter is to increase the speed of propagation of action potentials. The squid and various other invertebrates have thus achieved rapid signaling by evolving axons of giant diameter. Vertebrates, however, have achieved equally high speeds of conduction in a much more compact manner by insulating many of their axons with a *myelin sheath*.

Myelination Increases the Speed and Efficiency of Propagation of Action Potentials in Vertebrates[8,12]

The **myelin sheath** is formed by specialized glial cells—*Schwann cells* in peripheral nerves and *oligodendrocytes* in the central nervous system. These cells wrap layer upon layer of their own plasma membrane in a tight spiral around the axon (Figure 19–13). Each myelinating Schwann cell devotes itself to a single axon, forming a segment of sheath that is about 1 mm long and may consist of up to 300 concentric layers of membrane; oligodendrocytes form similar segments of sheath, but they do so for many separate axons simultaneously.

The insulating layer formed by the myelin sheath drastically reduces the effective capacitance of the axon membrane and at the same time prevents almost all current leakage across it. Between one segment of sheath and the next, small regions of axon membrane remain bare (Figure 19–14). These so-called *nodes of Ranvier*, only about 0.5 μm long, are foci of electrical activity. Practically all the Na^+ channels of the axon are concentrated at the nodes, giving a density of several thousand channels per square micrometer there, with almost none in the membrane covered by myelin sheath. Thus the ensheathed portions of the axon membrane are not excitable but have excellent cable properties—a low capacitance and a high resistance to current leakage. Consequently, when an action potential is triggered at a node, the resulting currents are funneled efficiently by passive spread to the next node, depolarize it rapidly, and trigger it to fire another action potential. Thus conduction is *saltatory:* the signal propagates along the axon by leaping from node to node. Myelination brings two main advantages: action potentials travel faster, and metabolic energy is conserved because the active excitation is confined to the small nodal regions.

Summary

Electrical signaling in nerve cells depends on changes of membrane potential due to movements of small numbers of ions through gated ion channels. The Na^+-K^+ pump builds up a large store of energy to drive these movements by generating

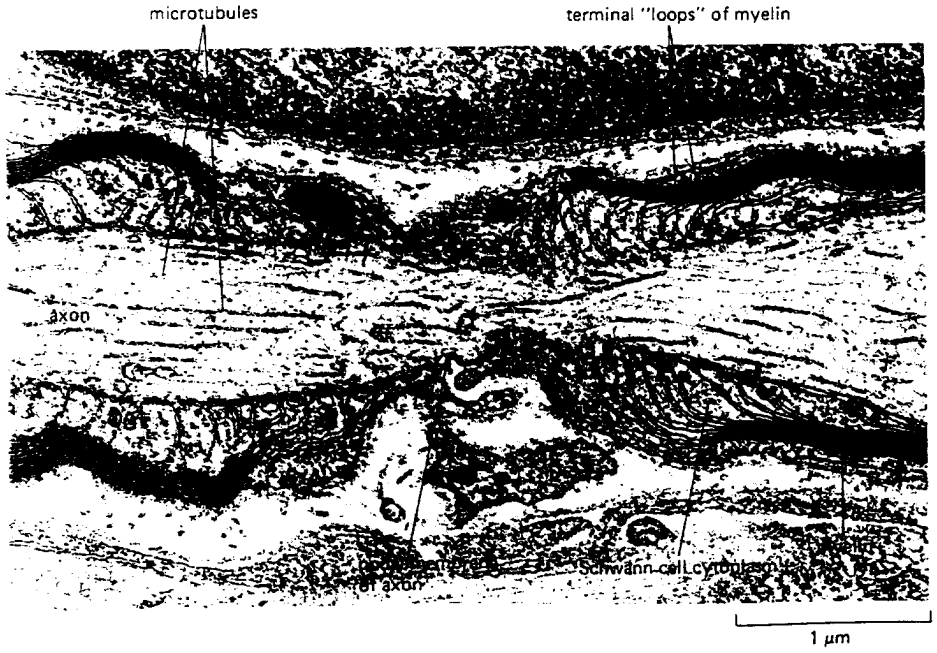


Figure 19–14 Electron micrograph of a longitudinal section of a myelinated axon from a peripheral nerve showing a node of Ranvier, where a small portion of the axon's plasma membrane is left exposed between the ends of two adjacent segments of myelin sheath. (Courtesy of Richard Bunge.)

large concentration gradients of Na^+ and K^+ across the nerve cell membrane. In the resting neuron the K^+-selective leak channels in the membrane make it more permeable to K^+ than to other ions, and the membrane potential is consequently close to the K^+ equilibrium potential of about -70 mV. An action potential is triggered when a brief depolarizing stimulus causes voltage-gated Na^+ channels to open, making the membrane more permeable to Na^+ and further depolarizing the membrane potential toward the Na^+ equilibrium potential. This positive feedback causes still more Na^+ channels to open, resulting in an all-or-none action potential. In each region of membrane, the action potential is rapidly terminated by the inactivation of the Na^+ channels and, in many neurons, by the opening of voltage-gated K^+ channels.

The propagation of an action potential along a nerve fiber depends on the fiber's passive cable properties: when the membrane is locally depolarized and fires an action potential, the current entering through open Na^+ channels at that site spreads passively to depolarize neighboring regions of the membrane, where action potentials are triggered in turn. In many vertebrate axons the speed and efficiency of propagation of action potentials are increased by insulating sheaths of myelin, which change the cable properties of the axon and leave only small regions of excitable membrane exposed.

Ligand-gated Ion Channels and Fast Synaptic Transmission[13]

The simplest way for one neuron to pass its signal to another is by direct electrical coupling through gap junctions. Such **electrical synapses** have the virtue that transmission occurs without delay. But they are far less rich in possibilities for adjustment and control than are the **chemical synapses** that provide the majority of nerve cell connections. Electrical communication through gap junctions was considered in Chapter 14 (pp. 798–801). Here we shall confine our discussion to chemical synapses.

The principles of chemical communication at a synapse are the same as those of chemical communication by water-soluble hormones, as discussed in Chapter 12. In both cases a cell releases a chemical messenger that acts on another cell, or set of cells, by binding to membrane receptor proteins. Unlike a hormone, however, the chemical messenger at a synapse—the **neurotransmitter**—acts at very close quarters.

Electrical stimulation of the presynaptic cell causes the release of a neurotransmitter by exocytosis (see Figure 19–4); once the neurotransmitter has crossed the gap—typically a small fraction of a micrometer—between the pre- and postsynaptic cells, the chemical signal must be converted back into an electrical one. This conversion is mediated by the receptors in the plasma membrane of the postsynaptic cell which fall into two distinct categories: *channel-linked receptors* and *non-channel-linked receptors* (Figure 19–15). Channel-linked receptors can be described equivalently as *ligand-gated channels*. A channel-linked receptor, upon binding neurotransmitter, promptly changes its conformation so as to create an open channel for specific ions to cross the membrane, thereby altering the membrane permeability. This type of receptor underlies the most familiar and the best-understood mode of chemical synaptic signaling, where transmission is very rapid.

Non-channel-linked receptors work by the same mechanisms that mediate responses to water-soluble hormones and local chemical mediators throughout the body (see p. 694). In such receptors the neurotransmitter-binding site is functionally coupled to an enzyme that, in the presence of neurotransmitter, usually catalyzes the production of an intracellular messenger such as cyclic AMP. The intracellular messenger in turn causes changes in the postsynaptic cell, including modifications of the ion channels in its membrane. Compared with channel-linked receptors, these receptors generally provide for neurotransmitter actions that are relatively slow in onset and long in duration. Some of them are believed to mediate the long-lasting neuronal changes that underlie learning and memory (see p. 1094).

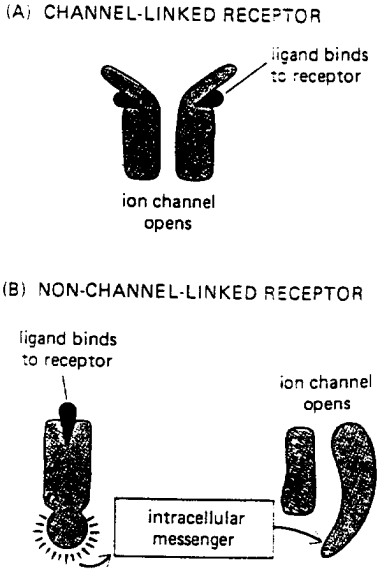


Figure 19–15 A neurotransmitter can exert its effect on a postsynaptic cell by means of two fundamentally different types of receptor proteins: channel-linked receptors and non-channel-linked receptors. Channel-linked receptors are also known as ligand-gated channels.

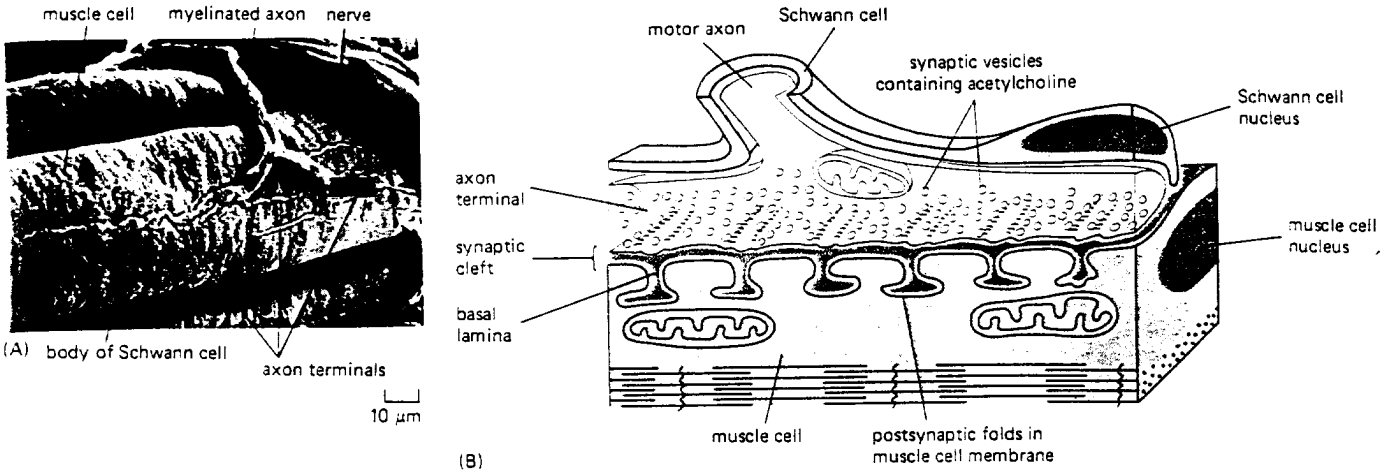


Figure 19–16 A neuromuscular junction in a frog. (A) Low-magnification scanning electron micrograph of the termination of a single axon on a skeletal muscle cell. (B) Schematic drawing of the part of the junction boxed in (A), showing the major features visible by transmission electron microscopy. The pattern of small terminal branches of the axon at the junction varies with the species and with the type of skeletal muscle cell. From its appearance in mammals, the neuromuscular junction is often called the *end plate*. (A, from J. Desaki and Y. Uehara, *J. Neurocytol.* 10:101–110, 1981, by permission of Chapman & Hall.)

In this section we shall discuss rapid synaptic transmission based on ligand-gated ion channels. The special features of synaptic signaling based on non-channel-linked receptors, and their role in long-term synaptic change, will be discussed in a later section (see p. 1091).

The Neuromuscular Junction Is the Best-understood Synapse[14]

The central nervous system is so densely packed with neurons that it is extremely difficult to perform experiments on single synapses within it. Detailed understanding of synaptic function has come instead chiefly from work on the junctions between nerve and skeletal muscle in the frog and, to a lesser extent, on synapses between giant neurons in the squid and other mollusks.

Skeletal muscle cells in vertebrates, like nerve cells, are electrically excitable, and the **neuromuscular junction** (Figure 19–16) has proved to be a valuable model for chemical synapses in general. A motor nerve and its muscle can be dissected free from the surrounding tissue and maintained in a bath of controlled composition. The nerve can be stimulated with extracellular electrodes, and the response of a single muscle cell can be monitored relatively easily with an intracellular microelectrode (Figure 19–17). Figure 19–18 compares the fine structure of a neuromuscular junction with that of a typical synapse between two neurons in the central nervous system.

The neuromuscular junction has been the focus of a long and fruitful series of investigations that began in the 1930s. The background to the early experiments was the discovery, in the early 1920s, that acetylcholine is released upon stimulation of the vagus nerve to the heart and acts on heart muscle to slow its beating. This was the first clear evidence of chemical neurotransmission, and it soon led to the demonstration, in the 1930s, that stimulation of a motor nerve innervating a skeletal muscle also causes the release of acetylcholine and that acetylcholine in turn stimulates skeletal muscle to contract. Acetylcholine was thereby identified as the neurotransmitter at the neuromuscular junction. But how is the release of acetylcholine brought about, and how does it exert its effect on the muscle?

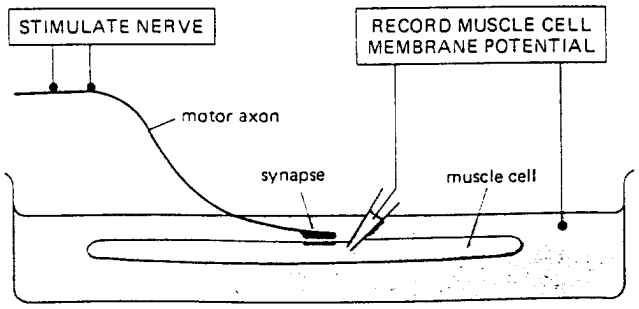
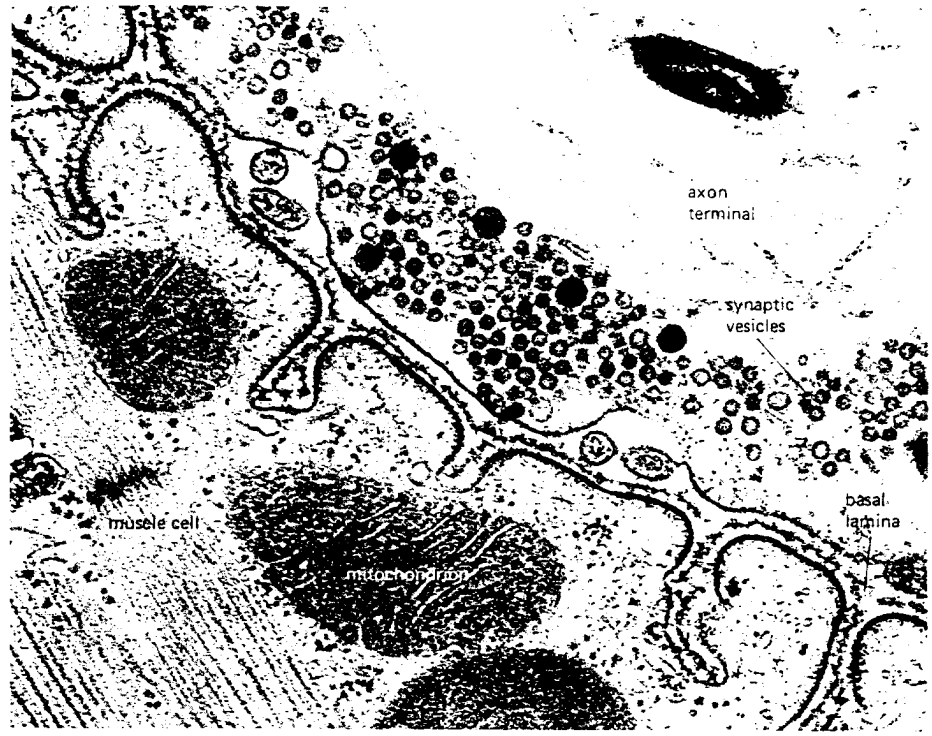
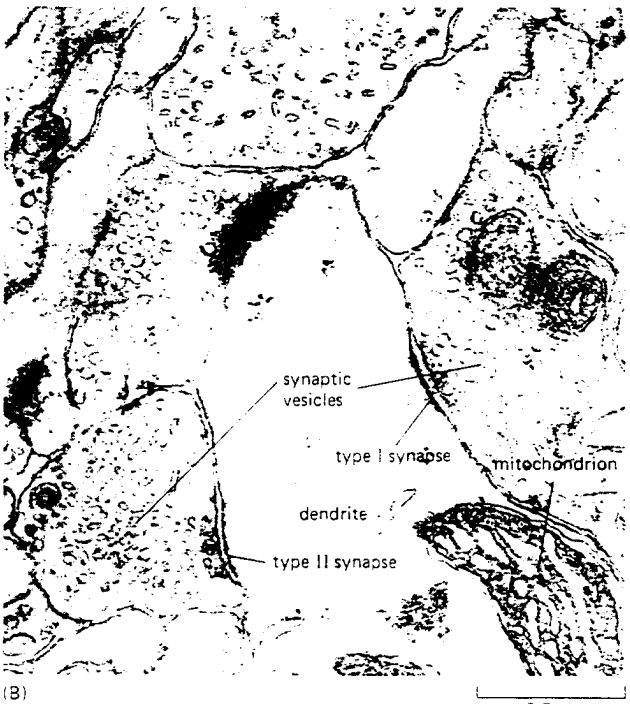


Figure 19–17 An experimental arrangement used to study synaptic transmission at the neuromuscular junction.



(A)



(B)

Figure 19–18 (A) Electron micrograph of part of a neuromuscular junction. (B) Electron micrograph of a small region from the brain of a rat. Two synapses are clearly visible in (B), each showing pre- and postsynaptic membranes, a synaptic cleft between them, and synaptic vesicles on the presynaptic side, as in (A). The two synapses labeled in (B) differ from each other in the size and shape of their vesicles: the vesicles at the *type I* synapse are round, whereas those at the *type II* synapse are flattened and are believed to contain a different neurotransmitter. Note the characteristic "thickened" appearance of the postsynaptic membrane and to a lesser extent, of the presynaptic membrane in both (A) and (B). There is no basal lamina interposed between the pre- and postsynaptic membranes at synapses in the brain, although some extracellular material is faintly apparent in the cleft. The absence of a basal lamina represents the chief structural difference between a synapse in the central nervous system and a neuromuscular junction. (A, courtesy of John Heuser; B, courtesy of G. Campbell and A.R. Lieberman.)

Voltage-gated Ca^{2+} Channels Couple Action Potentials to Neurotransmitter Release[15]

The action potential is propagated along the axon by the opening and closing of Na^+ channels until it reaches the neuromuscular junction. Here the action potential opens *voltage-gated Ca^{2+} channels* in the plasma membrane of the axon terminal, allowing Ca^{2+} to enter and trigger the release of acetylcholine (Figure 19–19).

Three simple observations showed that this influx of Ca^{2+} into the axon terminal is essential for synaptic transmission. First, if there is no Ca^{2+} in the extra-

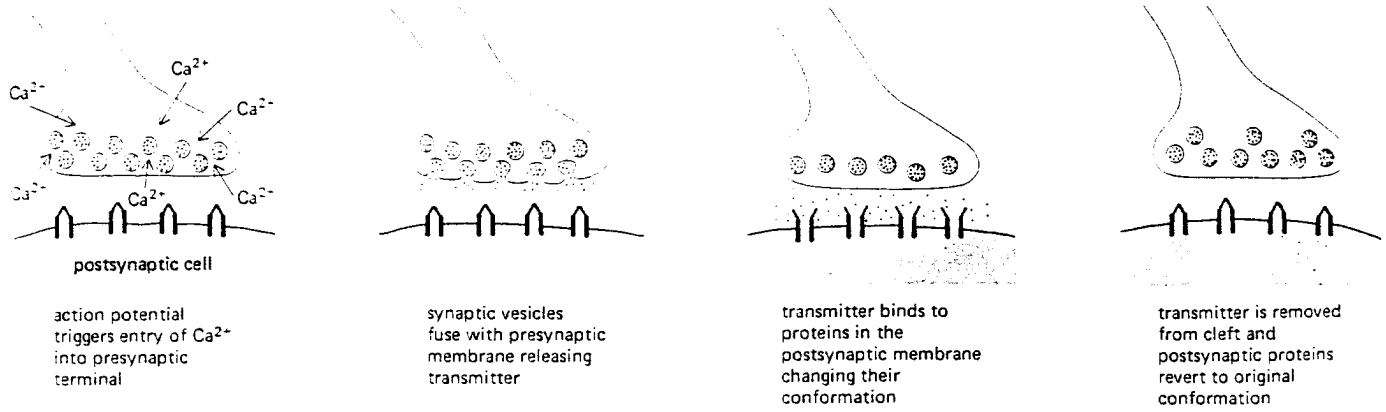


Figure 19–19 Summary of the essential events at a chemical synapse following the arrival of an action potential in the axon terminal.

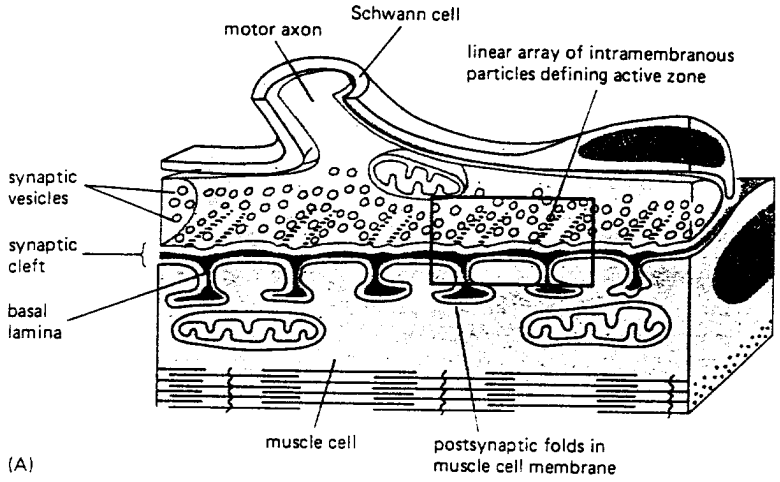
cellular medium bathing the axon terminal when the action potential arrives, no neurotransmitter is released and transmission fails. Second, if Ca^{2+} is injected artificially into the cytoplasm at the axon terminal through a micropipette, transmitter is immediately released even without electrical stimulation of the axon. (This microinjection experiment is difficult to do at the neuromuscular junction because the axon terminal is so small, but it has been done at a synapse between giant neurons in the squid.) Third, artificial depolarization of the axon terminal (again at the squid giant synapse), in the absence of an action potential and with the Na^+ and K^+ channels blocked by specific toxins, leads to Ca^{2+} entry and transmitter release; furthermore, if the depolarization reverses the membrane potential so far as to reduce the electrochemical driving force for Ca^{2+} entry to zero, no transmitter release occurs.

The channel protein that lets the Ca^{2+} into the cell—the **voltage-gated Ca^{2+} channel**—has a uniquely important role. It provides the only known means of converting electrical signals—fleeting depolarizations of the membrane—into chemical changes inside nerve cells. As explained in Panel 19–1, voltage-gated channels for Na^+, K^+, or Cl^- are of no use for this purpose: the ion fluxes driven through them by a single action potential are so small that they do not significantly alter the ion concentrations in the cytosol. The ion flux through voltage-gated Ca^{2+} channels is no larger in absolute terms and generally makes only a small contribution to the electrical current across the membrane; but it is very much larger in relation to the free Ca^{2+} concentration inside the cell, which is normally kept at about 10^{-7} M, corresponding to less than 100 Ca^{2+} ions/μm^3. In 1 millisecond, a single open Ca^{2+} channel would typically pass several hundred Ca^{2+} ions, driven by the membrane potential and the relatively high extracellular concentration of Ca^{2+} (usually ~1–2 mM). Thus a small number of voltage-gated Ca^{2+} channels in the presynaptic terminal, opening in response to an action potential, can easily raise the intracellular concentration of free Ca^{2+} by a factor of 10 to 100. The surge of free Ca^{2+} then acts as an intracellular messenger, triggering the release of neurotransmitter at a rate that increases very steeply with the free Ca^{2+} concentration.

The increase of free Ca^{2+} concentration is short-lived because Ca^{2+}-binding proteins, Ca^{2+}-sequestering vesicles, and mitochondria rapidly take up the Ca^{2+} that has entered the axon terminal, while Ca^{2+} pumps in the plasma membrane, driven either by ATP hydrolysis or by the Na^+ electrochemical gradient, pump it out of the cell (see p. 307 and p. 700). In this way the terminal is ready to transmit another signal as promptly as the axon is ready to deliver one.

Neurotransmitter Is Released Rapidly by Exocytosis[16]

The axon terminal at the neuromuscular junction is crammed with thousands of uniform (~40 nm diameter) secretory vesicles, called *synaptic vesicles*, each containing acetylcholine (see Figure 19–18). The entry of Ca^{2+} induces a synchronized burst of exocytosis in which the vesicles fuse with the presynaptic membrane, discharging their contents into the synaptic cleft to act on the postsynaptic cell.



The exocytosis is restricted to specialized regions known as *active zones*, exactly opposite the receptors on the postsynaptic cell; in this way the delay associated with diffusion of neurotransmitter across the cleft is made negligibly short. The membrane of the discharged synaptic vesicles is subsequently retrieved from the presynaptic plasma membrane by endocytosis.

There is evidence that, as well as triggering exocytosis, the influx of Ca^{2+} into the axon terminal activates a Ca^{2+}/calmodulin-dependent protein kinase (Ca-kinase II—see p. 711), which phosphorylates a number of proteins in the terminal, including *synapsin I*, a protein attached to the surface of synaptic vesicles. Phosphorylation is thought to release the synapsin I and thereby allow the vesicles to dock at the active zone of the presynaptic membrane, where they are needed to replace the vesicles lost from that region by exocytosis. The whole cycle of events that is initiated by a single nerve impulse has been vividly demonstrated by very rapidly freezing the nerve and muscle tissue and then preparing it for electron microscopy. Some of the results are shown in Figure 19–20.

Neurotransmitter Release Is Quantal and Probabilistic[17]

An axon terminal at a neuromuscular junction typically releases a few hundred of its many thousands of synaptic vesicles in response to a single action potential. Each vesicle, by discharging its contents into the synaptic cleft, contributes to the production of a voltage change in the postsynaptic muscle cell, which can be recorded with an intracellular electrode (Figure 19–21). The muscle cell membrane is thus depolarized beyond its threshold and fires an action potential. This excitation sweeps over the cell (Figure 19–22), causing a contraction, as described on page 621.

Even when the axon terminal is electrically quiet, occasional brief depolarizations of the muscle membrane are observed in the neighborhood of the synapse. These **miniature synaptic potentials** typically have an amplitude of only about 1 mV—far below threshold—and they occur at random, with a certain low probability per unit time—typically about once per second (Figure 19–23). Each miniature potential results from a single synaptic vesicle fusing with the presynaptic membrane so as to discharge its contents. The amplitude as recorded in a given muscle cell is roughly uniform because each vesicle contains practically the same number of molecules of acetylcholine, on the order of 5000. This number represents the minimum packet or *quantum* of transmitter release. Larger signals are made up of integral multiples of this basic unit. The Ca^{2+} that enters the axon terminal during an action potential acts for a fraction of a millisecond to increase the rate of occurrence of the exocytic events more than 10,000-fold above the resting spontaneous frequency. Nonetheless, the process remains probabilistic, and identical stimulations of the nerve do not always produce exactly the same postsynaptic effect: if, for example, 300 quanta are released on average, more or less than this number may be released on any particular occasion.

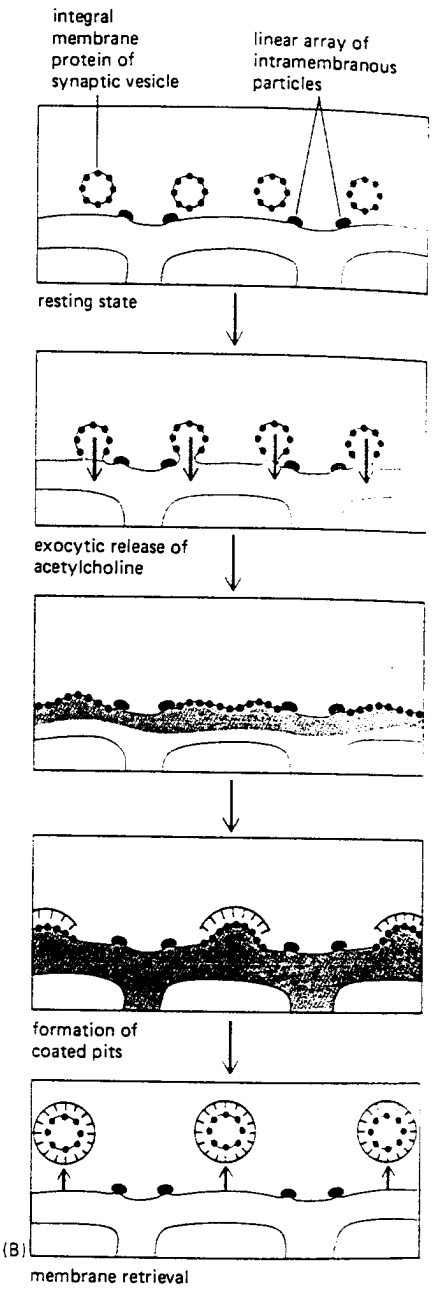


Figure 19–20 The cycle of membrane events in the axon terminal at a neuromuscular synapse following stimulation. To follow the action, samples of tissue are prepared by sudden freezing at measured times after the stimulus. To make the task easier technically, the conditions of excitation are artificially adjusted so as to slow down the normal time course by a factor of 5 or 10 and increase the number of vesicles that undergo exocytosis. (A) Simplified drawing of a neuromuscular junction, showing the active zones where transmitter release occurs. (B) The boxed area in A is enlarged and shown schematically in cross-section at a series of different times after stimulation of the nerve.

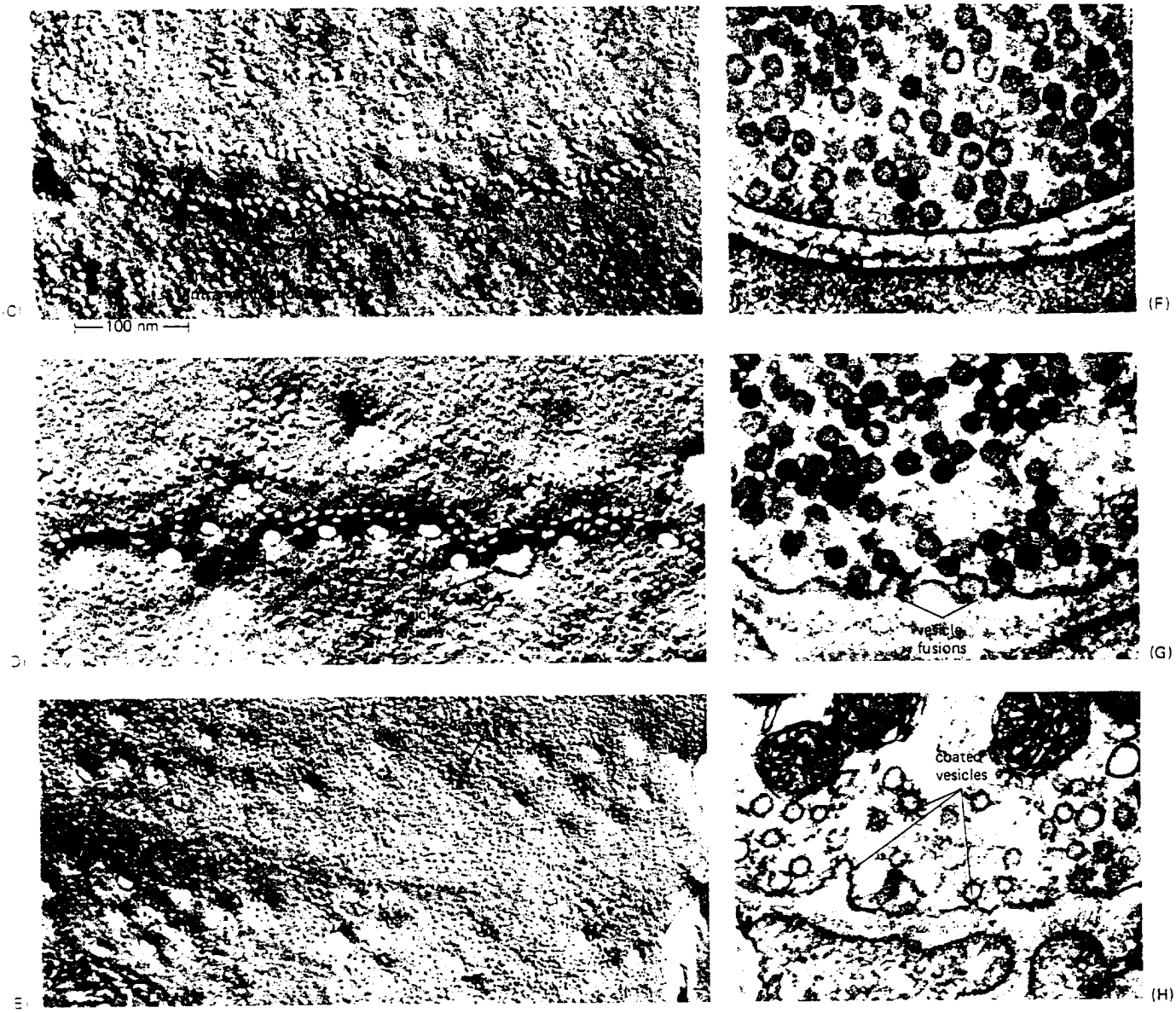


Figure 19–20 *Cont'd.* (C–H) The actual appearance of the membrane as viewed by electron microscopy. Freeze-fracture electron micrographs of the cytoplasmic half of the presynaptic membrane are shown on the left; thin-section micrographs are shown on the right. (C, F) Resting state. (D, G) Fusion of synaptic vesicles with the plasma membrane at an active zone (marked by the linear arrays of intramembranous particles). (E, H) Retrieval of synaptic vesicle membrane via coated pits and coated vesicles.

Synaptic vesicles can be seen to have begun fusing with the plasma membrane within 5 milliseconds after the stimulus (D, G); each of the openings in the plasma membrane apparent in (D) represents the point of fusion of one synaptic vesicle. Fusion is complete within another 2 milliseconds. The first signs of membrane retrieval become apparent within about 10 seconds as coated pits (see p. 326) form and then, after a further 10 seconds, begin to pinch off by endocytosis to form coated vesicles (E, H). These vesicles include the original membrane proteins of the synaptic vesicle and also contain molecules captured from the external medium. The cycle ends when the coat dissociates from the coated vesicle, which refills with acetylcholine to form a smooth-surfaced, regenerated synaptic vesicle. This scheme probably accounts for the strikingly uniform size of the synaptic vesicles, a size defined by the dimensions of the latticelike coat of clathrin (see p. 327).

Further evidence for this retrieval scheme can be obtained by stimulating the nerve in the presence of electron-dense extracellular markers such as ferritin. These markers quickly appear within coated vesicles and eventually show up in synaptic vesicles.

Note, however, that some experts have been skeptical about these experiments, interpreting some of the phenomena as artefacts. (C–H, courtesy of John Heuser.)

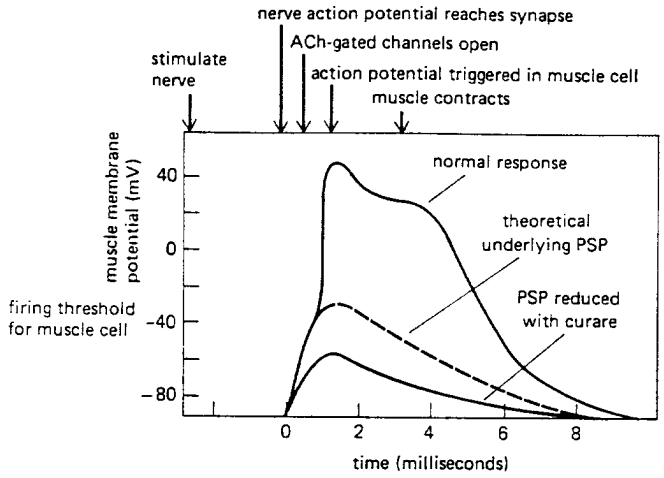


Figure 19–21 The postsynaptic response to a single nerve impulse at the neuromuscular junction: a graph of the voltage change in a frog muscle cell recorded, as in Figure 19–17, with an intracellular electrode close to the synapse. Normally the postsynaptic potential (PSP)—the depolarization directly produced by the neurotransmitter acting on the muscle cell membrane—is large enough to trigger an action potential, which complicates the analysis. A pure PSP, uncomplicated by an action potential, can be obtained by adding a moderate concentration of *curare* to the extracellular medium. This toxin, by binding to some of the receptors and blocking their response to the neurotransmitter, reduces the size of the PSP to the point where no action potential is triggered.

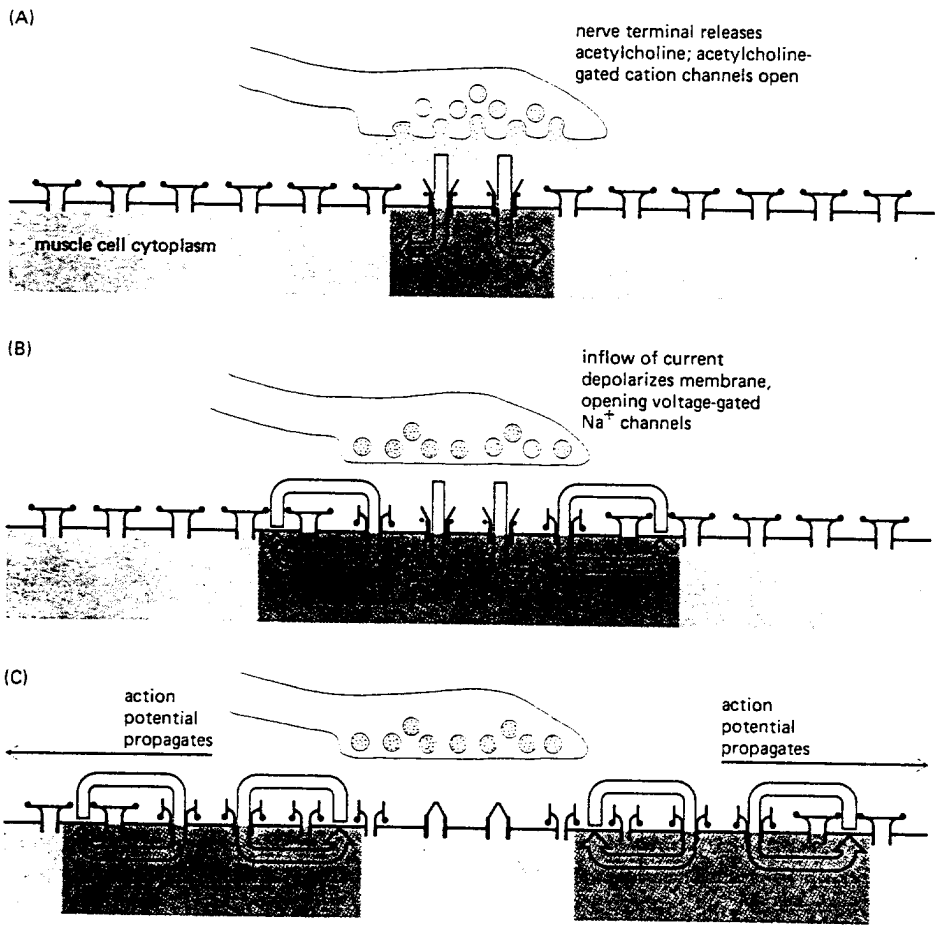


Figure 19–22 Electrical events in the muscle cell membrane at the neuromuscular junction. (A) The opening of ion channels gated by acetylcholine initiates an action potential (B) that propagates along the muscle cell membrane (C), causing contraction of the muscle cell.

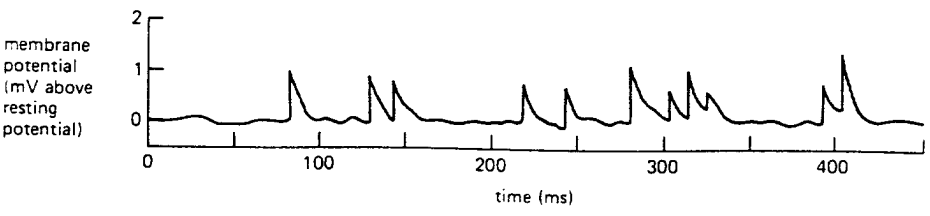


Figure 19–23 Miniature synaptic potentials (often called "miniature end-plate potentials") recorded from a frog muscle cell with an intracellular electrode inserted close to the neuromuscular junction. Each blip in the record is a miniature synaptic potential generated by release of the contents of a single synaptic vesicle from the axon terminal. (Redrawn from P. Fatt and B. Katz, *J. Physiol.* 117:109–128, 1952.)

Ligand-gated Channels Convert the Chemical Signal Back into Electrical Form[18]

The muscle cell membrane at the synapse behaves as a *transducer* that converts a chemical signal in the form of a neurotransmitter into an electrical signal. The conversion is achieved by **ligand-gated ion channels** (that is, **channel-linked receptors**) in the postsynaptic membrane: when the neurotransmitter binds to these proteins, they change their conformation—opening to let ions cross the membrane—and thereby alter the membrane potential. The shift of membrane potential, if it is large enough, will in turn cause voltage-gated channels to open, thereby triggering an action potential (Figure 19–24). Unlike voltage-gated ion channels, the ligand-gated ion channels are relatively insensitive to the membrane potential. They cannot by themselves, therefore, produce an all-or-none, self-amplifying excitation. Instead they produce an electrical change that is graded according to the intensity and duration of the external chemical signal—that is, according to how much transmitter is released into the synaptic cleft and how long it stays there. This feature of ligand-gated ion channels is important in information processing at synapses, as will be discussed later.

Postsynaptic ligand-gated channels have two other important properties. First, in their role as receptors, they have an enzymelike specificity for particular ligands so that they respond only to one neurotransmitter—the one released from the presynaptic terminal; other transmitters are virtually without effect. Second, in their role as channels, they are characterized by different ion selectivities: some may be selectively permeable to K^+, others to Cl^-, and so on, whereas still others may, for example, be relatively nonselective among the cations but exclude anions. We shall see that the ion selectivity of the ligand-gated channels determines the nature of the postsynaptic response.

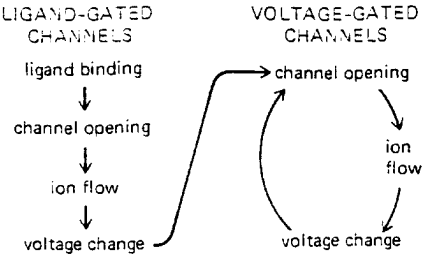


Figure 19–24 Diagram summarizing the function of ligand-gated and voltage-gated channels in the response to a neurotransmitter. The arrows indicate causal connections.

The Acetylcholine Receptor Is a Ligand-gated Cation Channel[19]

The channel in the skeletal muscle cell membrane gated by acetylcholine and known as the **acetylcholine receptor** is the best understood of all ligand-gated ion channels, and its molecular properties have already been discussed (see p. 319).

Like the voltage-gated Na^+ channel, the acetylcholine receptor has a number of discrete alternative conformations (Figure 19–25). Upon binding acetylcholine it jumps abruptly from a closed to an open state and then stays open, with the ligand bound, for a randomly variable length of time, averaging about 1 millisecond or even less, depending on the temperature and the species. In the open conformation the channel is indiscriminately permeable to small cations, including Na^+, K^+, and Ca^{2+}, but it is impermeable to anions (Figure 19–26).

Since there is little selectivity among these cations, their relative contributions to the current through the channel depend chiefly on their concentrations and on the electrochemical driving forces. If the muscle cell membrane is at its resting potential, the net driving force for K^+ is near zero because the voltage gradient nearly balances the K^+ concentration gradient across the membrane. For Na^+, on the other hand, the voltage gradient and the concentration gradient both act in the same direction to drive Na^+ into the cell. (The same is true for Ca^{2+}, but the extracellular concentration of Ca^{2+} is so much lower than that of Na^+ that Ca^{2+} makes only a small contribution to the total inward current.) Opening the acetylcholine receptor channel therefore leads chiefly to a large influx of Na^+, causing membrane depolarization.

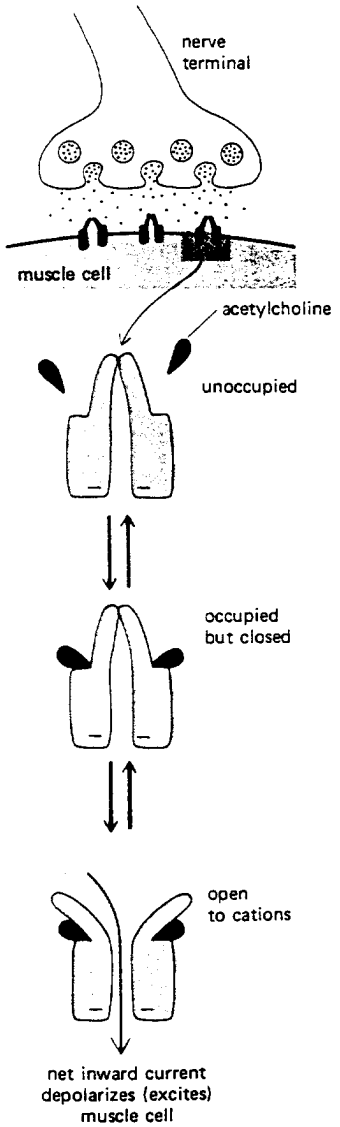
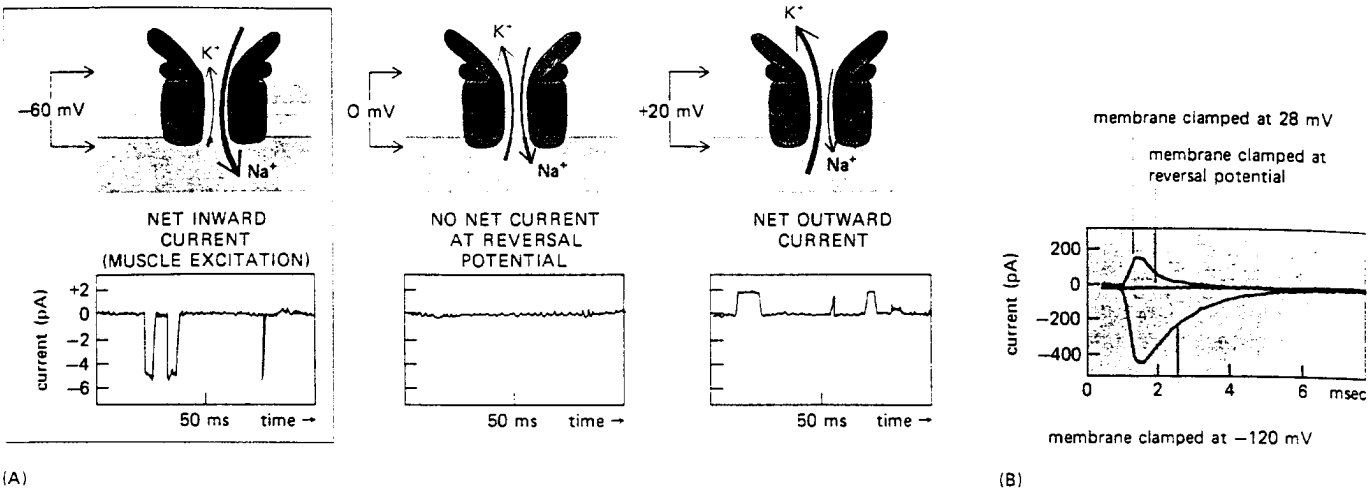


Figure 19–25 The response of the acetylcholine receptor to acetylcholine. Prolonged exposure to high concentrations of acetylcholine causes the receptor to enter yet another state (not shown), in which it is inactivated and will not open even though acetylcholine is present.

Acetylcholine Is Removed from the Synaptic Cleft by Diffusion and by Hydrolysis[20]

If the postsynaptic cell is to be accurately controlled by the pattern of signals sent from the presynaptic cell, the postsynaptic excitation must be switched off promptly when the presynaptic cell falls quiet. At the neuromuscular junction this is achieved by rapidly removing the acetylcholine from the synaptic cleft through two mechanisms. First, the acetylcholine disperses by diffusion, a rapid process because



(A) (B)

Figure 19–26 Measurements of the current through the open acetylcholine-receptor channel at different values of the membrane potential. Such measurements can be used to discover the channel's ion selectivity. A current carried through an open channel by a particular type of ion will vary with the membrane potential in a characteristic way that differs from one type of ion to another according to its concentration gradient across the membrane. Knowing the concentration gradients of the major ions present, one can thus get a useful clue to the channel's ion selectivity simply by measuring its current/voltage relationship; and fuller information can be obtained by repeating the measurements with altered ion concentrations.

(A) Patch-clamp recordings of the current through a single channel kept in a bath containing a fixed concentration of acetylcholine, with the membrane potential clamped at three different voltages. The channel flips randomly between open and closed states in a similar way in each case, but at a certain value of the membrane potential, called the *reversal potential*, the current is zero even when the channel is open. In this particular example the reversal potential happens to be approximately 0 mV. (B) The same phenomenon can be observed by monitoring the total current that flows through the large population of acetylcholine-receptor channels in the postsynaptic membrane at a neuromuscular synapse following a single stimulus to the nerve. The graphs show voltage-clamp recordings of this current made with intracellular electrodes. The channels open during the brief exposure to acetylcholine, but again the current is zero when the membrane potential is clamped at the reversal potential. Because the open channels are permeable to both Na^+ and K^+ and the electrochemical driving forces for these ions are different, zero net current corresponds to balanced nonzero currents of Na^+ and K^+ in opposite directions. (The channels are also permeable to Ca^{2+}, but the Ca^{2+} current is small because Ca^{2+} concentrations are low.) The value of the reversal potential and its sensitivity to ion concentrations in the external medium give a useful indication of the relative permeability of the channel to the different ions. For example, some other ligand-gated channels are selectively permeable to Cl^- (see p. 1083); they can be recognized because they have a reversal potential of about −60 mV, close to the Cl^- equilibrium potential, and the value of this potential depends on the extracellular concentration of Cl^- but not of Na^+ or K^+. (A, data from B. Sakmann, J. Bormann, and O.P. Hamill, *Cold Spring Harbor Symp. Quant. Biol.* 48:247–257, 1983; B, data based on K.L. Magleby and C.F. Stevens, *J. Physiol.* 223:173–197, 1972.)

the dimensions involved are small. Second, the acetylcholine is hydrolyzed to acetate and choline by *acetylcholinesterase*. This enzyme is secreted by the muscle cell and becomes anchored by a short collagenlike "tail" to the basal lamina that lies between the nerve terminal and the muscle cell membrane. Each acetylcholinesterase molecule can hydrolyze up to 10 molecules of acetylcholine per millisecond, so that all of the transmitter is eliminated from the synaptic cleft within a few hundred microseconds after its release from the nerve terminal. Consequently, acetylcholine is available only for a fleeting moment to bind to its receptors and drive them into the open conformation that produces the conductance change in the postsynaptic membrane (Figure 19–27). The sharply defined timing of presynaptic signals is thus preserved in sharply timed postsynaptic responses.

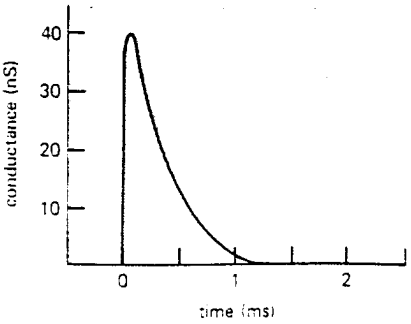


Figure 19–27 The conductance change produced in the postsynaptic membrane by a single quantum (one vesicle) of acetylcholine at the frog neuromuscular junction. About 1600 channels are open at the time of peak conductance, and each channel remains open for an average of 400 microseconds.

Fast Synaptic Transmission Is Mediated by a Small Number of Neurotransmitters[13,21]

Everything about the neuromuscular junction appears to be designed for speed: the large myelinated motor axon; the active zones in the axon terminal, with synaptic vesicles held ready to release their acetylcholine precisely opposite the postsynaptic receptors; the narrow synaptic cleft; the ligand-gated channels in the postsynaptic membrane, ready to open instantly when the transmitter binds; the acetylcholinesterase in the cleft to terminate transmission promptly. The synaptic delay, from the peak of the presynaptic action potential to the peak of the postsynaptic action potential, is on the order of a millisecond or less. Increasing evidence suggests that fast chemical synapses in the central nervous system also employ ligand-gated channels and are constructed on the same principles, with active zones, a narrow cleft, and receptors precisely localized opposite the sites of exocytosis. Moreover, it seems that there are only a handful of neurotransmitters that mediate such rapid signaling. These generalizations must be tentative, however; it is surprisingly difficult to identify conclusively the neurotransmitter acting at a given synapse.

It is probable that the rapid synaptic signaling systems evolved long ago, since the same neurotransmitters are used in the most disparate species of animals, from mollusks to mammals. The rapidly acting neurotransmitters include acetylcholine, γ-aminobutyrate (GABA), glycine, glutamate—and probably aspartate and ATP (Figure 19–28); and in general, a given neuron secretes only one (or occasionally two) of these transmitters—the same at all the synapses it makes. There is direct evidence from patch-clamp studies that the receptors for acetylcholine, GABA, glycine, and glutamate are channel-linked; for the others this is probable but not proved. DNA-sequencing studies indicate that the receptors for acetylcholine, GABA, and glycine are homologous, suggesting that all ligand-gated ion channels share a common evolutionary origin.

Acetylcholine and Glutamate Mediate Fast Excitation; GABA and Glycine Mediate Fast Inhibition[19,22]

Neurotransmitters can be classified according to their actions. We have seen that acetylcholine acting on its receptor in the skeletal muscle cell membrane opens a cation channel and so depolarizes the cell toward the threshold for firing an action potential. This transmitter receptor therefore mediates an *excitatory* effect. **Glutamate** appears to act on a similar type of receptor; it has been shown to be an excitatory transmitter at the neuromuscular junction of an insect and is thought to be the major excitatory transmitter in the central nervous system of vertebrates—a counterpart to acetylcholine, which is the main excitatory transmitter in the vertebrate peripheral nervous system (and also has important central actions). *Aspartate* may act on the same receptors as glutamate, with similar consequences. There is some evidence that *ATP* serves as a fast excitatory transmitter at synapses on certain types of *smooth* muscle.

Counterbalancing these excitatory effects, **GABA** and **glycine** mediate fast *inhibition*. The receptors to which they bind are linked to channels that, when open, admit small negative ions—chiefly Cl^-—but are impermeable to positive ions. The

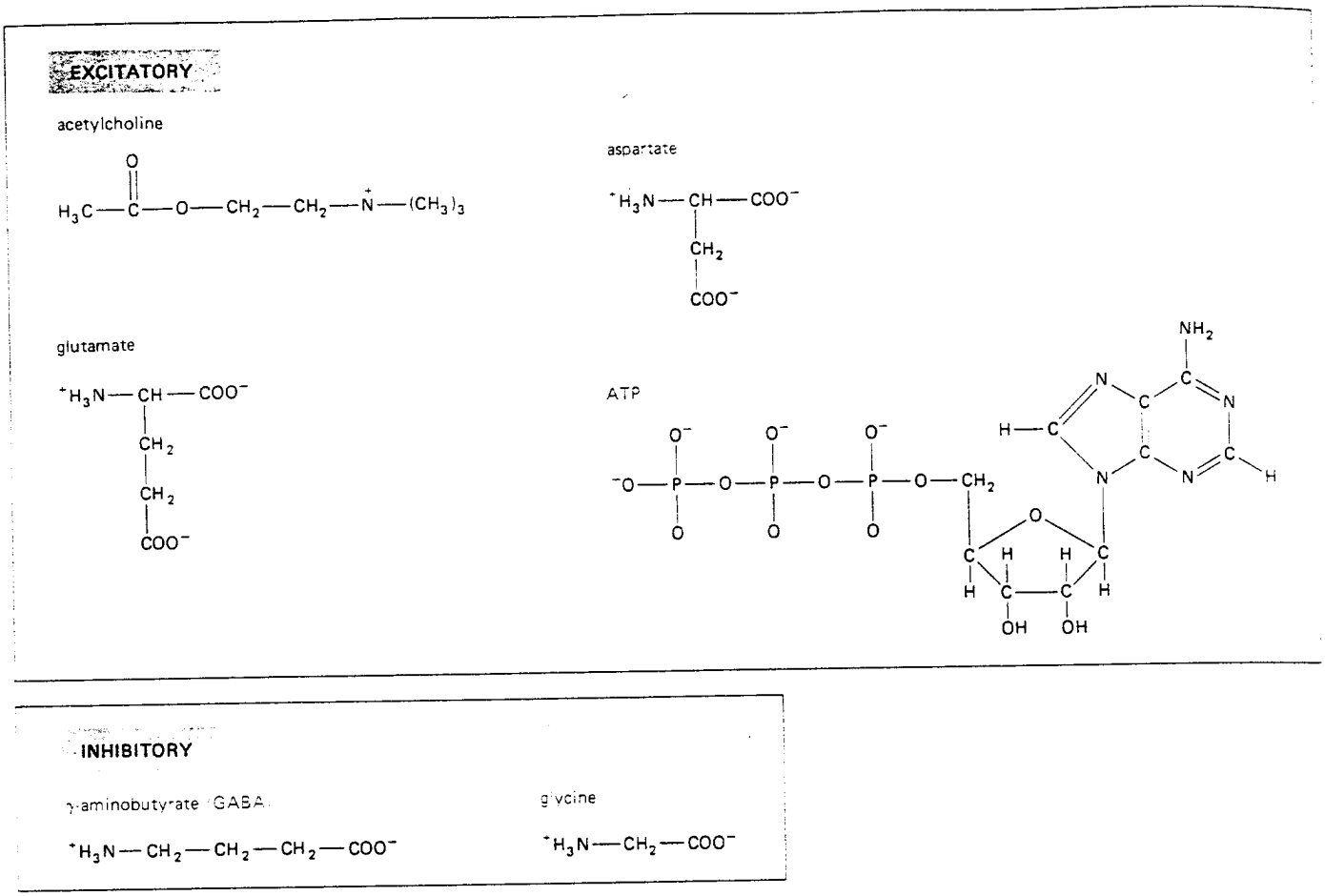


Figure 19–28 The chemical structures of the major neurotransmitters believed to act on channel-linked receptors so as to mediate fast synaptic transmission.

concentration of Cl^- is much higher outside the cell than inside, and the equilibrium potential for Cl^- is close to the normal resting potential or even more negative. The opening of these Cl^- channels, therefore, tends to hold the membrane potential at its resting value or even at a hyperpolarized value, making it more difficult to depolarize the membrane and hence to excite the cell (Figure 19–29). GABA and glycine are thought to be the major transmitters that mediate fast inhibition in the vertebrate central nervous system, and GABA is known also to perform the same function at neuromuscular junctions in insects and crustaceans. The importance of the inhibitory transmitters is demonstrated by the effects of toxins that block their action: strychnine, for example, by binding to glycine receptors and blocking the action of glycine, causes muscle spasms, convulsions, and death.

Several Types of Receptors Often Exist for a Single Neurotransmitter[23]

The action of a neurotransmitter is defined not by its own chemistry but by the receptor to which it binds. In fact there are often several types of receptors for the same neurotransmitter. Acetylcholine in vertebrates, for example, acts in opposite ways on skeletal muscle cells and on heart muscle cells, exciting the former and inhibiting the latter. The acetylcholine receptors are different in the two cases; a non-channel-linked receptor is thought to mediate the inhibitory effect, which is much slower than the excitatory effect on skeletal muscle.

The channel-linked receptors that mediate rapid excitatory actions of acetylcholine are called *nicotinic* because they can be activated by nicotine; the non-channel-linked receptors that mediate the slow actions of acetylcholine, which can be either inhibitory or excitatory, are called *muscarinic* because they can be activated by muscarine (a toxin from a fungus). In addition to such receptor-

specific activators (so-called *agonists*), there are also potent receptor-specific blockers (so-called *antagonists*) that distinguish the two types of acetylcholine receptors. For example, curare and α-bungarotoxin bind specifically to nicotinic acetylcholine receptors, blocking their activity, whereas atropine acts in a similar way on muscarinic receptors. Other agonists and antagonists distinguish among receptors for other neurotransmitters, and it is common to identify, localize, and assay the different receptors according to the agonists and antagonists that bind to them.

Synapses Are Major Targets for Drug Action[23,24]

The receptors for neurotransmitters are important targets for toxins and drugs. A snake paralyzes its prey by injecting α-bungarotoxin to block the nicotinic acetylcholine receptor. A surgeon can make muscles relax for the duration of an operation by blocking the same receptors with curare. The heart, meanwhile, continues to beat normally because the curare does not bind to muscarinic acetylcholine receptors. Thus the distinct ligand-binding properties of the two acetylcholine receptors allow drug action to be precisely targeted.

Most of the psychoactive drugs exert their effects at synapses, and a large proportion of them act by binding to specific receptors. GABA receptors provide an example. The best-studied type, known as $GABA_A$ receptors, are ligand-gated Cl^- channels, mediating rapid inhibition as described above. They are acted upon both by the benzodiazepine "tranquilizers," such as Valium and Librium, and by the barbiturate drugs used in the treatment of insomnia, anxiety, and epilepsy. GABA, benzodiazepines, and barbiturates bind cooperatively to three different sites on the same receptor protein: the drugs apparently alter behavior by allowing lower concentrations of GABA to open the Cl^- channel, thus *potentiating* the inhibitory action of GABA.

Synaptic transmission can also be disturbed in many other ways—for example, by interfering with the degradation or removal of the transmitter from the synaptic cleft. There are drugs that inhibit acetylcholinesterase activity at neuromuscular junctions so that acetylcholine lingers on the muscle cell for a longer time. This helps to relieve the weakness of patients suffering from myasthenia gravis, who have a shortage of functional acetylcholine receptors (see p. 1011). Other neurotransmitters, such as GABA, are not degraded enzymatically in the synaptic cleft but instead are retrieved by the presynaptic terminals that secreted them or by neighboring glial cells. Typically the nerve terminals and glial cells have specific transport proteins in their plasma membranes for active uptake of the neurotransmitter. Some psychoactive drugs block or potentiate the retrieval mechanism at specific classes of synapse, causing clinically useful effects.

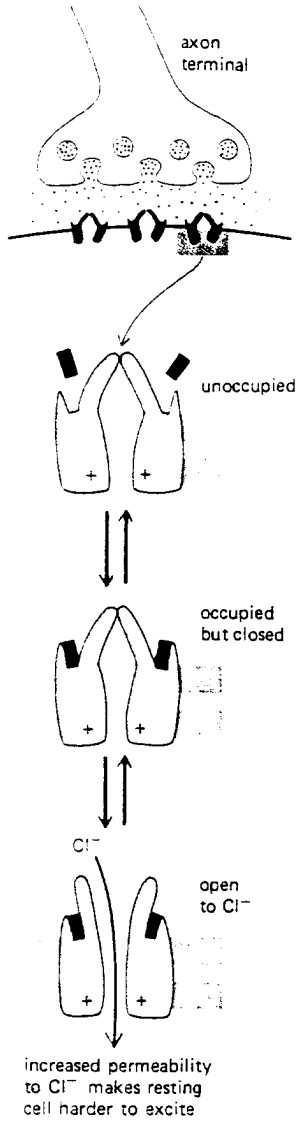


Figure 19–29 The behavior of channel-linked receptors for GABA. In response to binding of GABA, these form an open channel that is selectively permeable to Cl^-. In this way they mediate an inhibitory effect: the open Cl^- channels tend to hold the membrane close to the Cl^- equilibrium potential, which is itself close to the resting potential.

Summary

Neural signals pass from cell to cell at synapses, which can be either electrical (gap junctions) or chemical. At a chemical synapse the depolarization of the presynaptic membrane by an action potential opens voltage-gated Ca^{2+} channels, allowing an influx of Ca^{2+} to trigger exocytic release of neurotransmitter from synaptic vesicles. The neurotransmitter diffuses across the synaptic cleft and binds to receptor proteins in the membrane of the postsynaptic cell; it is rapidly eliminated from the cleft by diffusion, by enzymatic degradation, or by reuptake into nerve terminals or glial cells. The receptors for neurotransmitters can be classified as either channel-linked or non-channel-linked. Channel-linked receptors, also known as ligand-gated ion channels, mediate rapid postsynaptic effects, occurring within a few milliseconds. Only a handful of neurotransmitters are known to act on such receptors. In particular, acetylcholine and glutamate (and probably aspartate and ATP) open ligand-gated channels that are permeable only to cations and thereby produce rapid excitatory postsynaptic potentials, whereas GABA and glycine open homologous channels that are permeable chiefly to Cl^- and thereby produce rapid inhibitory postsynaptic potentials. All these neurotransmitters, as well as many others, may also act on non-channel-linked receptors, with slower and more complex consequences.

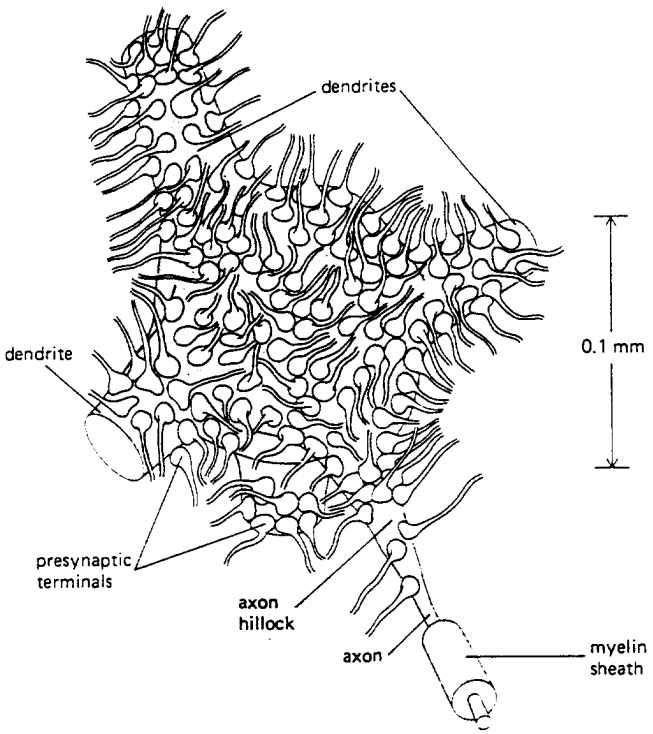


Figure 19–30 A motor neuron cell body in the spinal cord, showing some of the many thousands of nerve terminals that synapse on the cell and deliver signals from other parts of the organism to control its firing. The regions of the motor-neuron plasma membrane that are not covered with synaptic endings are covered by glial cells (not shown).

The Role of Ion Channels in Neuronal Computation[25]

In the central nervous system, neurons typically receive inputs from many presynaptic cells—the number may be anything from one to many thousands. For example, several thousand nerve terminals, from hundreds or perhaps thousands of neurons, make synapses on a typical motor neuron in the spinal cord: its cell body and dendrites are almost completely covered with them (Figure 19–30). Some of these synapses transmit signals from the brain, others bring sensory information from muscles or from the skin, and still others supply the results of computations made by interneurons in the spinal cord. The motor neuron must combine the information received from these many sources and react either by firing signals along its axon or by remaining quiet.

The motor neuron provides a typical example of the way in which neurons play their individual parts in the fundamental task of computing an output from a complex set of inputs. Of the many synapses on the motor neuron, some will tend to excite it, others to inhibit it. Although the motor neuron secretes the same neurotransmitter at all its axon terminals, it makes many different types of receptor proteins, concentrating them at different postsynaptic sites on its surface. At each such site, firing of the presynaptic cell causes a specific set of channels to open or close, leading to a characteristic voltage change or **postsynaptic potential (PSP)** in the motor neuron. An *excitatory PSP* (produced, for example, by the opening of channels permeable to Na^+) is generally a depolarization; an *inhibitory PSP* (produced, for example, by the opening of Cl^- channels) is usually a hyperpolarization. The PSPs generated at the different synapses on a single neuron are highly variable in size and duration. At one synapse on the motor neuron, an incoming nerve impulse might produce a depolarization of less than 0.1 mV, whereas at another there might be a depolarization of 5 mV. However, as we shall now discuss, the properties of the system are such that even small PSPs can combine to produce a large effect.

The Shift of Membrane Potential in the Body of the Postsynaptic Cell Represents a Spatial and Temporal Summation of Many Postsynaptic Potentials[25,26]

The membrane of the dendrites and cell body of most neurons, although rich in receptor proteins, contains few voltage-gated Na^+ channels and so is relatively inexcitable. An individual PSP generally does not trigger the postsynaptic membrane to fire an action potential. Instead, each incoming signal is faithfully reflected in a PSP of graded magnitude, which falls off with distance from the site of the synapse. If signals arrive simultaneously at several synapses in the same region of the dendritic tree, the total PSP in that neighborhood will be roughly the sum of the individual PSPs, with inhibitory PSPs making a negative contribution to the total. At the same time, the net electrical disturbance produced in one postsynaptic region will spread to other regions through the passive cable properties of the dendritic membrane.

The cell body, where the effects of the PSPs converge, is relatively small (generally smaller than 100 μm in diameter) compared with the dendritic tree (whose branches may extend for millimeters). The membrane potential in the cell body and its immediate neighborhood will therefore be roughly uniform and will be a composite of the effects of all the signals impinging on the cell, weighted according to the distances of the synapses from the cell body. The **grand postsynaptic potential** of the cell body is thus said to represent a **spatial summation** of all the stimuli received. If excitatory inputs predominate, it will be a depolarization; if inhibitory inputs predominate, it will usually be a hyperpolarization.

While spatial summation combines the effects of signals received at different sites on the membrane, **temporal summation** combines the effects of signals received at different times. The neurotransmitter released when an action potential arrives at a synapse evokes a PSP in the postsynaptic membrane that rises rapidly to a peak (through the transient opening of ligand-gated ion channels) and then declines to baseline with a roughly exponential time course (which depends on the membrane capacitance). If a second action potential arrives before the first PSP has decayed completely, the second PSP adds to the remaining tail of the first. If, after a period of inactivity, a long train of action potentials is delivered in quick succession, each PSP adds to the tail of the preceding PSP, building up to a large sustained average PSP whose magnitude reflects the rate of firing of the presynaptic neuron (Figure 19–31). This is the essence of temporal summation: it translates the *frequency* of incoming signals into the *magnitude* of a net PSP.

The Grand PSP Is Translated into a Nerve Impulse Frequency for Long-distance Transmission[27]

Temporal and spatial summation together provide the means by which the rates of firing of many presynaptic neurons jointly control the membrane potential in the body of a single postsynaptic cell. The final step in the neuronal computation made by the postsynaptic cell is the generation of an output, usually in the form of action potentials, to relay a signal to other cells that are often far away. The output signal reflects the magnitude of the grand PSP in the cell body. However, while the grand PSP is a continuously graded variable, action potentials are all-or-none and uniform in size. The only variable in signaling by action potentials is the time interval between one action potential and the next. For long-distance transmission, the magnitude of the grand PSP is therefore translated, or *encoded*, into the *frequency* of firing of action potentials (Figure 19–32). This encoding is achieved by a special set of voltage-gated ion channels present at high density at the base of the axon, adjacent to the cell body, in a region known as the **axon hillock** (see Figure 19–30).

Before we discuss how these channels operate, a word of qualification is necessary. The firing of an action potential itself causes drastic changes of the membrane potential of the cell body, which therefore no longer directly reflects the net synaptic stimulation that the cell is receiving. It is therefore a complex

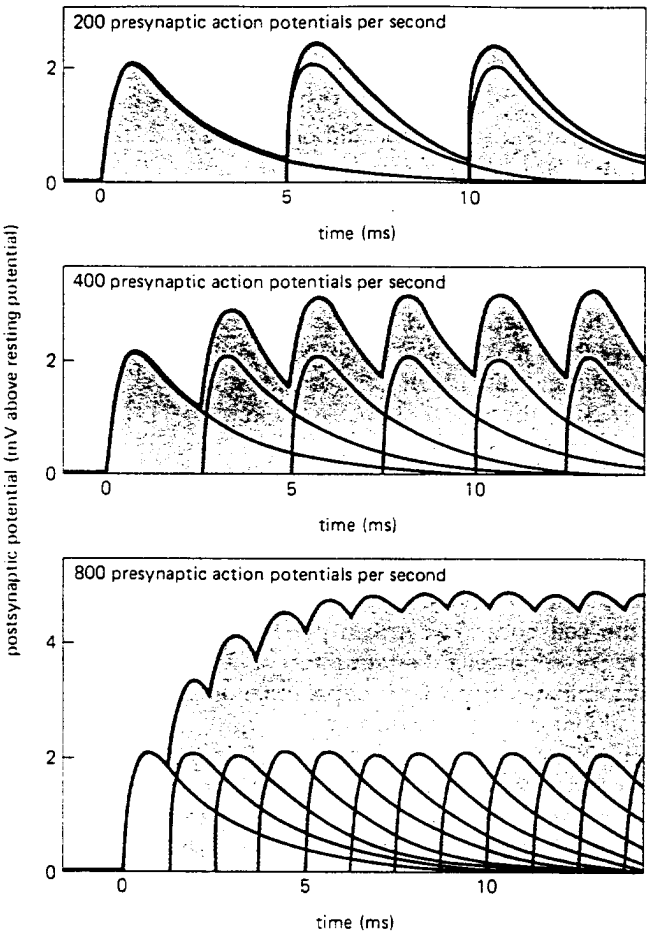


Figure 19–31 Temporal summation. The overlapping curves within the shaded region of each graph represent the individual contributions to the total postsynaptic potential evoked by the arrival of the successive presynaptic action potentials.

problem to give a rigorous analysis of the encoding mechanism. In the nonrigorous, qualitative account that follows, we shall loosely refer to "the strength of synaptic stimulation" or to "the grand PSP," meaning the grand PSP that would be observed if action potentials were somehow prevented from firing; and we shall suppose that this underlying grand PSP is the cause of the firing of action potentials.

Encoding Requires a Combination of Different Ion Channels[28]

The propagation of action potentials depends chiefly, and in many vertebrate axons almost entirely, on voltage-gated Na^+ channels. The membrane of the axon hillock is where action potentials are initiated, and Na^+ channels are plentiful there. But to perform its special function of encoding, the membrane in that neighborhood typically contains in addition at least four other classes of ion channels—three selective for K^+ and one selective for Ca^{2+}. The three varieties of K^+ channels have different properties; we shall refer to them as the *delayed*, the *early*, and the *Ca^{2+}-activated K^+ channels*. The functions of these channels in encoding have been studied most thoroughly in giant neurons of mollusks, but the principles appear to be similar for most other neurons.

To understand the necessity for multiple types of channels, consider first the behavior that would be observed if the only voltage-gated ion channels present in the nerve cell were the Na^+ channels. Below a certain threshold level of synaptic stimulation, the depolarization of the axon hillock membrane would be insufficient to trigger an action potential. With gradually increasing stimulation, the threshold would be crossed: the Na^+ channels would open, and an action potential would fire. The action potential would be terminated in the usual way by inactivation of the Na^+ channels. Before another action potential could fire, these channels would have to recover from their inactivation. But that would require a return of the membrane voltage to a very negative value, which would not occur as long as the

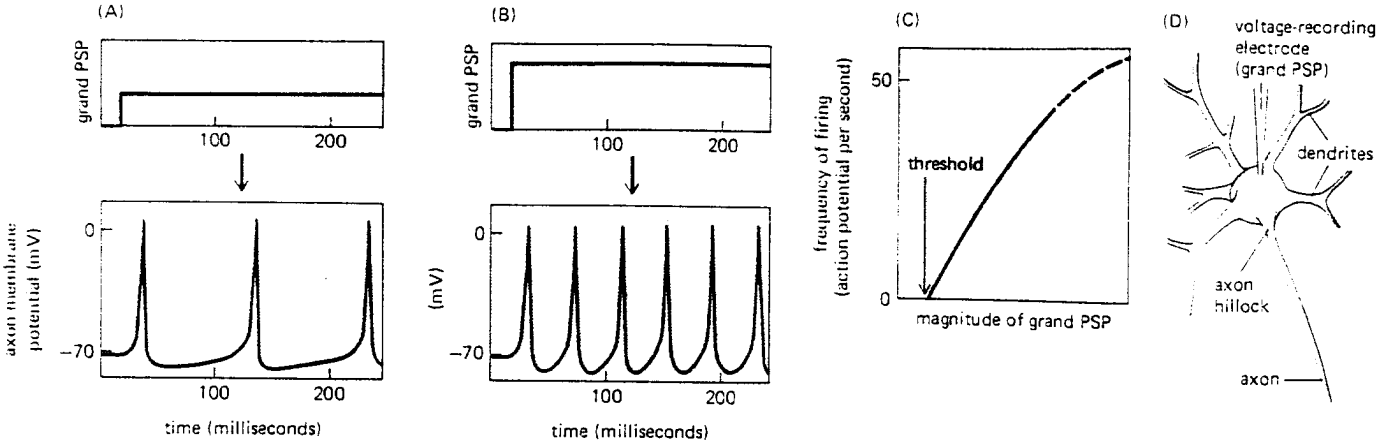


Figure 19–32 The encoding of the grand PSP in the form of the frequency of firing of action potentials by an axon. A comparison of (A) and (B) shows how the firing frequency of an axon increases with an increase in the grand PSP, while (C) summarizes the general relationship. In (D) the experimental setup for measuring the grand PSP is shown. In (A) and (B) the upper graphs (marked "grand PSP") show the net intensity of synaptic stimulation as received by the cell body, while the lower graphs show the resulting trains of action potentials that are transmitted along the axon. The upper graphs can be thought of as representations of the grand PSP that would be observed if the firing of action potentials were somehow blocked.

strong depolarizing stimulus (from PSPs) was maintained. An additional channel type is needed, therefore, to repolarize the membrane after each action potential to prepare the cell to fire another. This task is performed by the **delayed K^+ channels,** which we discussed previously in relation to the propagation of the action potential (see p. 1070). They are voltage-gated and respond to membrane depolarization in much the same way as the Na^+ channels, but with a longer time delay. By opening during the falling phase of the action potential, they permit an efflux of K^+, which short-circuits the effect of even a sustained depolarizing stimulus and drives the membrane back toward the K^+ equilibrium potential. This potential is so far negative that the Na^+ channels recover from their inactivated state. In addition, the K^+ conductance turns itself off: repolarization of the membrane causes the delayed K^+ channels themselves to close again (without ever entering an inactivated state). Once repolarization has occurred, the depolarizing stimulus from synaptic inputs becomes capable of raising the membrane voltage to threshold again so as to cause another action potential to fire. In this way, sustained stimulation of the dendrites and cell body leads to repetitive firing of the axon.

However, repetitive firing in itself is not enough: the frequency of the firing has to reflect the intensity of the stimulation. Detailed calculations show that a simple system of Na^+ channels and delayed K^+ channels is inadequate for this purpose. Below a certain threshold level of steady stimulation, the cell will not fire at all; above that threshold it will abruptly begin to fire at a relatively rapid rate. The **early K^+ channels** (also known as *A channels*) solve the problem. These too are voltage-gated and open when the membrane is depolarized, but their specific voltage sensitivity and kinetics of inactivation are such that they act to reduce the rate of firing at levels of stimulation that are only just above the threshold. Thus they help to remove the discontinuity in the relationship between the firing rate and the intensity of stimulation. The result is a firing rate that is proportional to the strength of the depolarizing stimulus over a very broad range (see Figure 19–32).

Adaptation Lessens the Response to an Unchanging Stimulus[29]

The process of encoding is usually further modulated by the two other types of ion channels in the axon hillock that were mentioned at the outset—*voltage-gated Ca^{2+} channels* and *Ca^{2+}-activated K^+ channels*. The former are similar to the Ca^{2+} channels that mediate release of neurotransmitter at axon terminals: those present in the neighborhood of the axon hillock open when an action potential fires, allowing Ca^{2+} into the axon. The **Ca^{2+}-activated K^+ channel** is different from any of the channel types described earlier. It opens in response to a raised concentration of Ca^{2+} at the cytoplasmic face of the nerve cell membrane.

Suppose that a strong depolarizing stimulus is applied for a long time, triggering a long train of action potentials. Each action potential permits a brief influx of Ca^{2+} through the voltage-gated Ca^{2+} channels, so that the intracellular Ca^{2+}

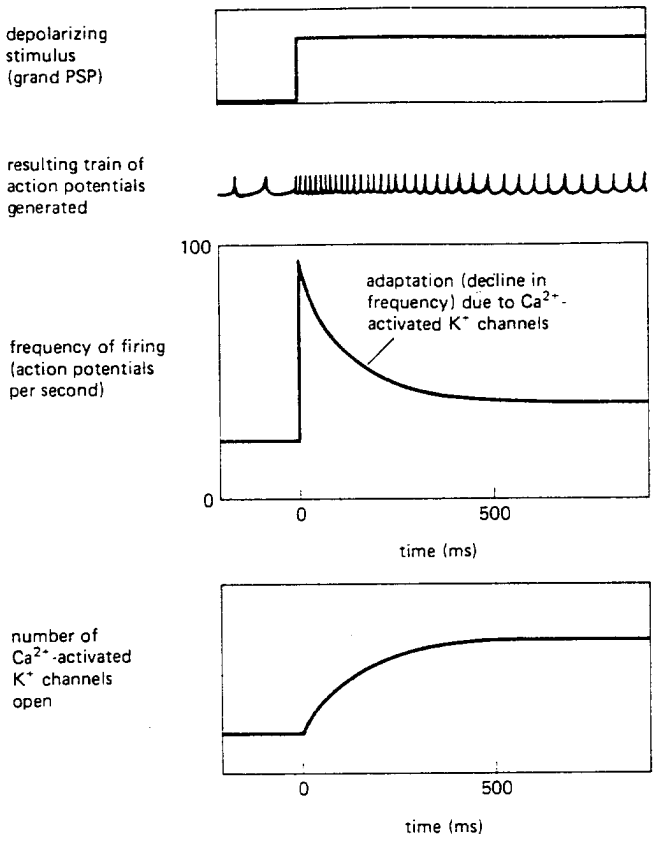


Figure 19–33 Adaptation. When steady stimulation is prolonged, the stimulated cell gradually reduces the strength of its response, as expressed in the rate of firing of action potentials.

concentration gradually builds up to a high level. This opens the Ca^{2+}-activated K^+ channels, and the resulting increased permeability of the membrane to K^+ makes the membrane harder to depolarize and increases the delay between one action potential and the next. In this way a neuron that is stimulated continuously for a prolonged period becomes gradually less responsive to the constant stimulus. The phenomenon, which can also occur by other mechanisms, is known as **adaptation** (Figure 19–33). It allows a neuron, and indeed the nervous system generally, to react sensitively to *change*, even against a high background level of steady stimulation (see p. 1107). It is one of the strategies that help us, for example, to feel a touch on the shoulder and yet ignore the constant pressure of our clothing.

Not All Signals Are Delivered via the Axon[30]

In the typical neuron that we have been describing, there is a clear distinction, in both structure and function, between dendrites and axon. Some neurons, however, do not conform to this model, although the molecular principles of their operation are the same. In most invertebrates, for example, the majority of neurons have a *unipolar* organization: the cell body is connected by a single stalk to a branching system of cell processes, among which it is not always easy to see a structural difference between dendrites and axon (Figure 19–34). The functional distinction can also be blurred, in both vertebrates and invertebrates: processes that are classified structurally as dendrites often form presynaptic as well as postsynaptic specializations and deliver signals to other cells as well as receive them. Conversely, synaptic inputs are sometimes received at strategic sites along the axon—for example, close to the axon terminal, where they can inhibit or facilitate the release of neurotransmitter from that particular terminal without affecting transmission at the terminals of other branches of the same axon (Figure 19–35). We shall discuss later (see p. 1097) an example of this important device of *presynaptic inhibition* or *presynaptic facilitation*.

Synapses at which a dendrite delivers a stimulus to another cell play a large part in communication between neurons that lie close together, within a few millimeters or less. Over such distances, electrical signals can be propagated pas-

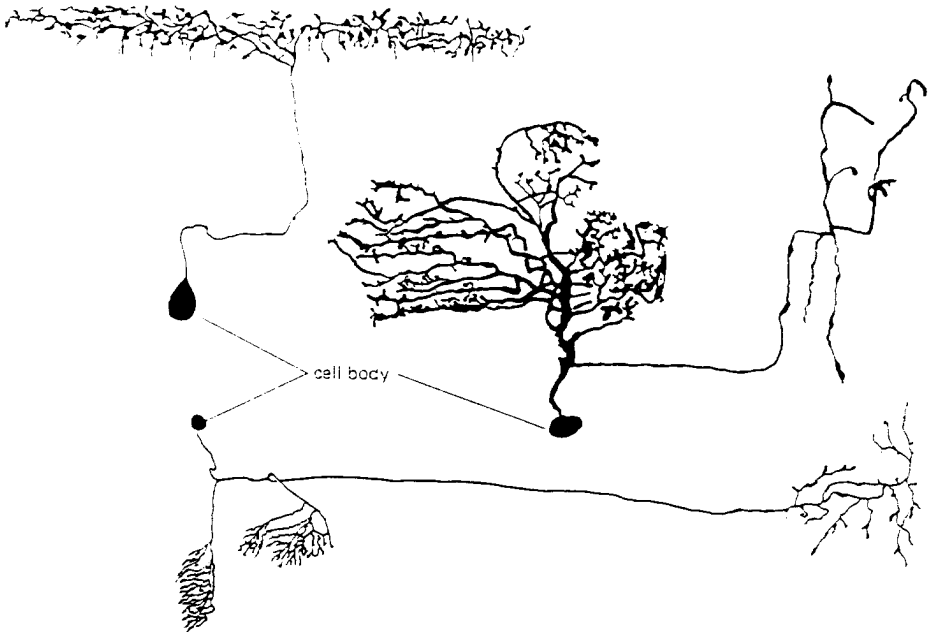


Figure 19–34 Neurons from a fly, showing the structure typical of most neurons in invertebrates, in which the nerve cell body is connected by a stalk to the system of nerve cell processes and does not have dendrites projecting from it directly. The sensory neurons in the spinal ganglia of vertebrates have a similar organization. (From N. Strausfield, Atlas of an Insect Brain. New York: Springer, 1976.)

sively, spreading from postsynaptic sites on the dendritic membrane, where they are received, to presynaptic sites on the same dendritic membrane, where they then control transmitter release. Indeed, there are neurons that possess no axon, do not conduct action potentials, and perform all of their signaling via processes that are conventionally referred to as dendrites. Moreover, if the dendritic tree is large, separate parts of it can behave as more or less independent pathways for communication and for information processing. In some neurons the range of possibilities is still further complicated by the presence of voltage-gated ion channels in the dendritic membrane, which enable the dendrites to conduct action potentials. Thus even a single neuron can behave as a highly complex computational device.

Summary

A typical neuron receives on its dendrites and cell body many different excitatory and inhibitory synaptic inputs, which combine, by spatial and temporal summation, to produce a grand postsynaptic potential in the cell body. The magnitude of the grand postsynaptic potential is translated (encoded) for long-distance transmission into the rate of firing of action potentials, by a system of ion channels in the membrane of the axon hillock. The encoding mechanism often shows adaptation, so that the cell responds weakly to a constant stimulus but strongly to a change of stimulus. There are many variants of this basic scheme: for example, not all neurons produce an output in the form of action potentials, dendrites can be presynaptic as well as postsynaptic, and axons can be postsynaptic as well as presynaptic.

Non-Channel-linked Receptors and Synaptic Modulation[13,31]

At synapses that use channel-linked receptors, the effect of the neurotransmitter is immediate, simple, and brief, and the site of reception of the message is defined with pinpoint accuracy; the transmitter released from one axon terminal acts only on a single postsynaptic cell. By contrast, non-channel-linked receptors allow for effects that are slow, complex, long-lasting, and often spatially diffuse: the transmitter released from one terminal may act on many cells in the neighborhood of that terminal. These slow effects are often described as examples of *neuromodulation* because they modulate the rapid responses mediated by channel-linked

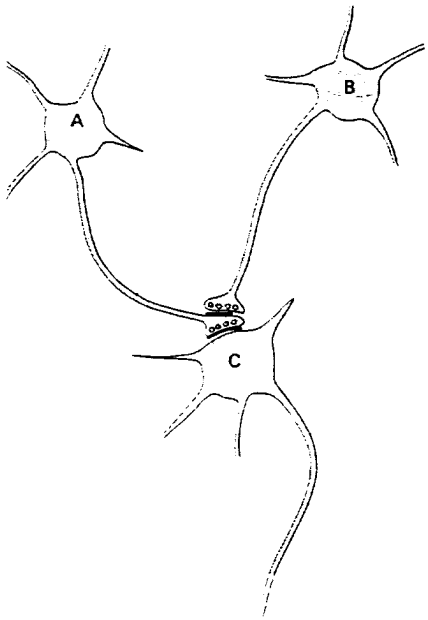


Figure 19–35 An axo-axonic synapse. The neurotransmitter released from the axon terminal of cell B acts on channels in the axon terminal of cell A, thereby altering the number of quanta of neurotransmitter released onto C when A fires. If firing of B causes a reduction in the stimulus delivered by A to C, then B is said to exert a *presynaptic inhibition;* the contrary effect is called *presynaptic facilitation.*

receptors on the same cell. The non-channel-linked receptors act by the same molecular mechanisms as the receptors for hormones and local chemical mediators outside the nervous system—indeed, many of them probably are identical.

As discussed in Chapter 12 (see p. 695), non-channel-linked cell-surface receptors for signaling molecules fall into two large families: (1) **catalytic receptors,** most of which are tyrosine-specific protein kinases, which, when activated by ligand binding, directly phosphorylate tyrosine residues on proteins inside the cell; and (2) **G-protein-linked receptors,** which transmit signals into the cell interior by activating a GTP-binding regulatory protein, or *G protein*, which in turn activates or inactivates a membrane-bound enzyme or ion channel. Most of the non-channel-linked receptors for neurotransmitters studied so far seem to be G-protein-linked, employing their G protein in one of at least three ways:

1. The G proteins may activate or inactivate adenylate cyclase, thereby controlling the cyclic AMP level in the postsynaptic cell. The cyclic AMP then regulates the activity of the cyclic-AMP-dependent protein kinase (*A-kinase*—see p. 709), which, among other target proteins, can phosphorylate ion channels in the plasma membrane, altering their properties. Cyclic AMP may also regulate some ion channels by binding to them directly.
2. The G protein may activate the inositol phospholipid pathway (see p. 702), thereby activating protein kinase C (C-kinase) and releasing Ca^{2+} into the cytosol from a Ca^{2+}-sequestering compartment in the postsynaptic cell. The C-kinase may regulate the behavior of ion channels by phosphorylating them. The Ca^{2+} may alter ion channel behavior directly, or indirectly via a Ca^{2+}-dependent protein kinase that phosphorylates the channel (see p. 711).
3. The G protein may interact directly with ion channels, causing them to open or close.

In each case a set of molecules in the postsynaptic cell act as go-betweens or *intracellular messengers*, diffusing within the cell to relay the signal from the receptor to other cell components. The more steps there are in this cascade of intracellular messengers, the more opportunities there are for amplification and regulation of the signal (see p. 713).

More than 50 neurotransmitters have been identified that act on non-channel-linked receptors to produce these varied and complex effects. Some, such as acetylcholine, also bind to channel-linked receptors, whereas others, such as neuropeptides (see below), apparently do not.

Non-Channel-linked Receptors Mediate Slow and Diffuse Responses[32]

Whereas channel-linked receptors take only a few milliseconds or less to produce electrical changes in the postsynaptic cell, non-channel-linked receptors typically take hundreds of milliseconds or longer. This is to be expected, since a series of enzymatic reactions must intervene between the initial signal and the ultimate response. Moreover, the signal itself is often not only temporally but also spatially diffuse.

A clear example is seen in the innervation of smooth muscle by axons releasing *norepinephrine*, which activates adenylate cyclase via a G-protein-linked receptor. Here, the transmitter is released not from nerve terminals but from swellings or *varicosities* along the length of the axon (Figure 19–36). These varicosities contain synaptic vesicles but no active zones to define the exact sites of release. Moreover, the varicosities are not closely apposed to specialized receptive sites on a postsynaptic cell; instead, the transmitter diffuses widely to act on many smooth muscle cells in the neighborhood, in the manner of a local chemical mediator (see p. 682). It is likely that many of the signaling molecules that operate on catalytic and G-protein-linked receptors in the central nervous system also act in this *paracrine* mode. Indeed, many of these neurotransmitters also serve as hormones or as local chemical mediators outside the nervous system: for example, norepinephrine, together with its close relative *epinephrine*, is also released as a hormone from the adrenal gland.

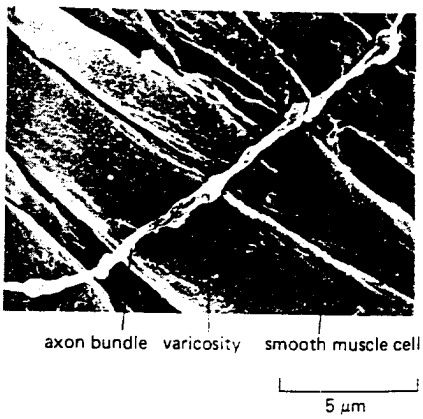


Figure 19–36 Scanning electron micrograph of a small bundle of autonomic motor axons innervating smooth muscle cells in the wall of the ureter. The varicosities (swellings) contain synaptic vesicles loaded with the neurotransmitter norepinephrine. The synapses here are ill-defined structures, with a gap that may be as large as 0.2 μm between the site of release of the neurotransmitter and the nearest muscle cell membrane on which it must act. (From S. Tachibana, M. Takeuchi, and Y. Uehara, *J. Urol.* 134:582–586, 1985. © by Williams & Wilkins, 1985.)

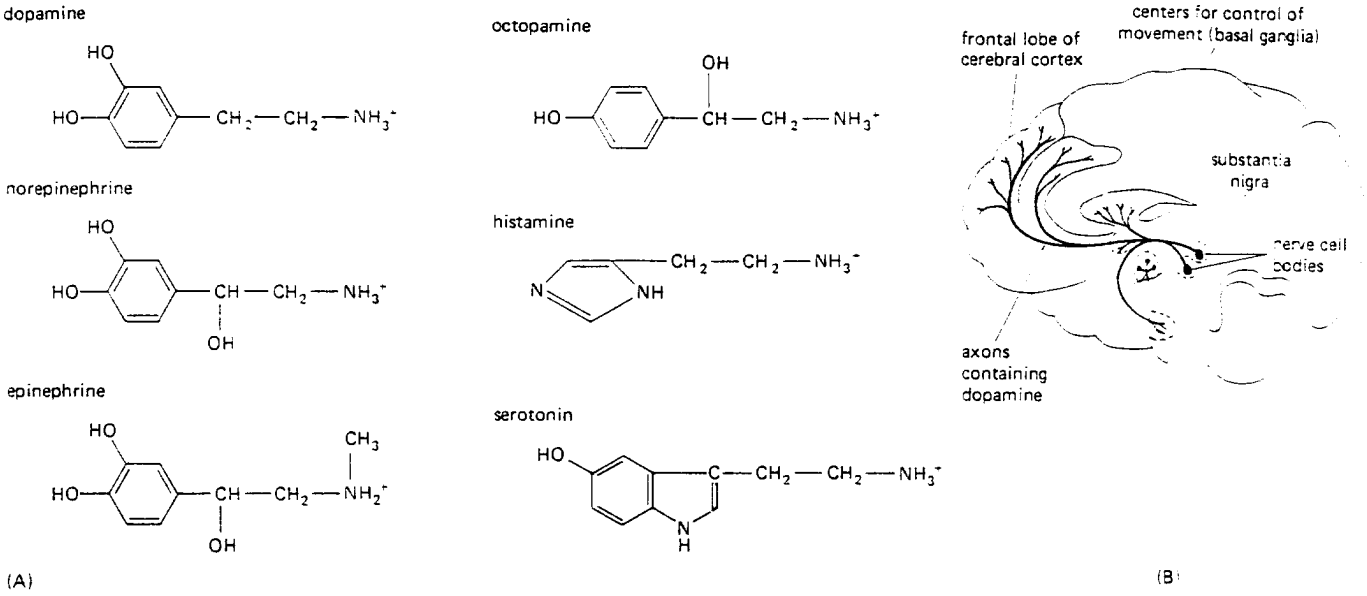


Figure 19–37 (A) The monoamine family of neurotransmitters. (B) Schematized diagram of the distribution of dopamine-containing neurons in the human brain. The movement disorders of Parkinson's disease are due to the death of many of the cells in a particular set of dopamine-containing neurons (those in the substantia nigra); the symptoms can be relieved by drug treatments that boost the synthesis of dopamine and inhibit its breakdown. The distribution of monoamine-containing neurons can be made visible by treating tissue sections with formaldehyde, which reacts with monoamines to give fluorescent products.

Epinephrine and norepinephrine are representatives of the family of **monoamine** neurotransmitters, which have widespread functions both in vertebrates and in invertebrates and are of great medical importance (Figure 19–37A). It is possible to design drugs that interfere with the synthesis, uptake, or breakdown of particular monoamines, or that interact with particular subclasses of monoamine receptors; and some of these drugs have proved to be valuable in the treatment of psychiatric and neurological diseases. Schizophrenia, for instance, can often be treated successfully with drugs that block certain classes of *dopamine* receptors, while drugs that increase the concentrations of dopamine in the brain give dramatic relief from the movement disorders of Parkinson's disease (Figure 19–37B). Drugs that raise synaptic concentrations of noradrenaline and/or serotonin are often effective in the treatment of severe depression.

The Neuropeptides Are by Far the Largest Family of Neurotransmitters[32,33]

Most of the signaling molecules used elsewhere in the body are also employed by neurons. This is true in particular of the array of small protein molecules or peptides that serve as hormones and local chemical mediators to control such bodily functions as the maintenance of blood pressure, the secretion of digestive enzymes, and the proliferation of cells.

Rapid advances in this area over the last ten years or so have largely depended on immunocytochemistry. Once a peptide has been identified in one tissue, it is possible to make antibodies against it and to use these to search elsewhere for that peptide and for others that are structurally related. In this way neurons have been found to contain peptides that were not previously suspected to have a neural function, including many newly discovered varieties. The evidence that these **neuropeptides** (Figure 19–38) serve as neurotransmitters is in most cases persuasive but incomplete. For example, an antipeptide antibody might be shown to label certain neurons and their axon terminals, while the peptide itself, when supplied locally, might mimic the effect of activity of these neurons. Most convincingly, the peptide might be shown to be secreted when the neurons are active, and the effects of activity of the neurons might be shown to be blocked by antibodies against the peptide. Neuropeptides appear to be particularly important in regulating feelings and drives, such as pain, pleasure, hunger, thirst, and sex.

The nonpeptide neurotransmitters are synthesized by enzymes that are usually present both in the cell body and in the axon terminals, so that even if the axon is long, the stores of neurotransmitter at the synapse can be rapidly replen-

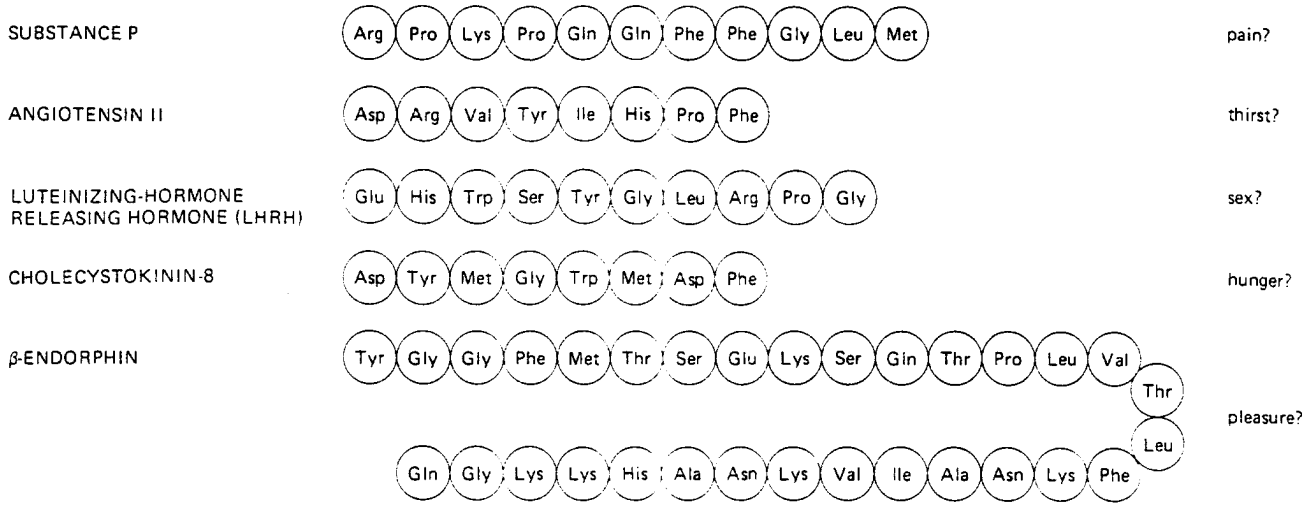


Figure 19–38 A small selection of neuropeptides, with a tentative indication of some of the sensations and drives in which they are thought to be involved.

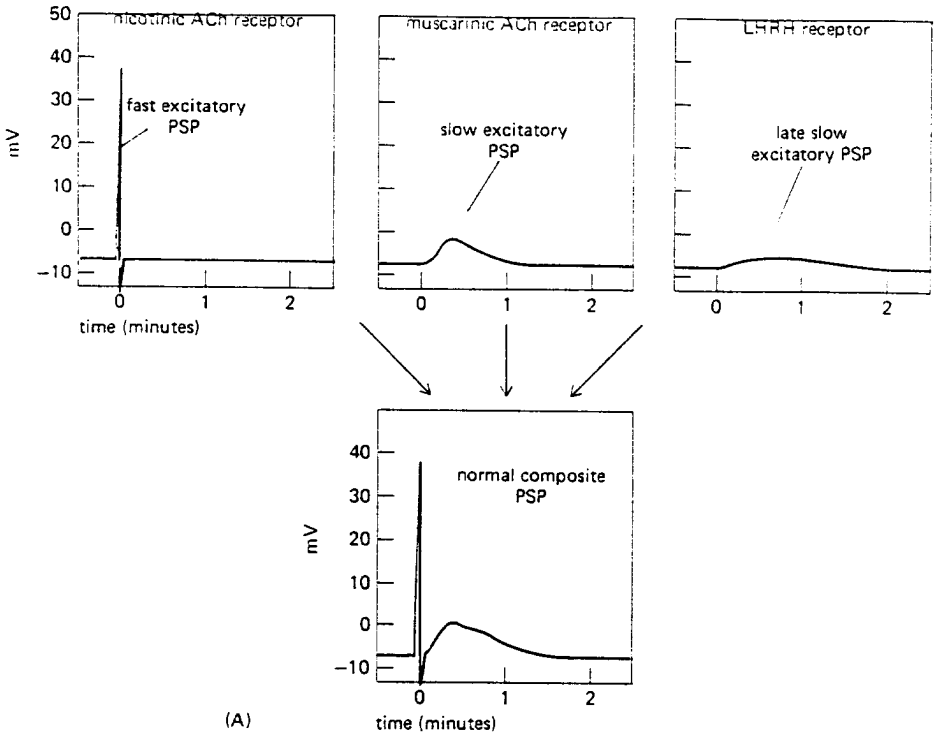
ished. The neuropeptides, by contrast, are made on ribosomes on rough endoplasmic reticulum in the cell body and must be exported to the axon terminals by fast axonal transport—a journey that may take a day or more for a long axon. Neuropeptides are derived from larger precursor proteins, from which they are cleaved enzymatically; in many cases more than one functional peptide is cleaved from a single precursor molecule, which for this reason is called a *polyprotein*. Synaptic vesicles loaded with neuropeptides can usually be recognized by their large size compared with vesicles containing acetylcholine, amino acid transmitters, or monoamines.

At many of the synapses where neuropeptides are secreted, a nonpeptide neurotransmitter is also released, and the two transmitters act side by side but in different ways. The presynaptic axon terminals in certain autonomic ganglia of a bullfrog, for example, contain both acetylcholine and a peptide that closely resembles the reproductive hormone LHRH (luteinizing-hormone releasing hormone). The postsynaptic cell membrane contains at least three types of receptors: a nicotinic (channel-linked) acetylcholine receptor that mediates a fast response, a muscarinic (G-protein-linked) acetylcholine receptor that mediates a much slower response, and a receptor (probably G-protein-linked) for the LHRH-like peptide that mediates the slowest response of all (Figure 19–39A). The action of the LHRH-like peptide is not only slower than that of acetylcholine but also more diffuse, so that the peptide molecules released at a synapse on one postsynaptic cell also evoke a postsynaptic potential in other cells in the neighborhood (Figure 19–39B).

If, as seems likely, other neuropeptide transmitters have similar properties, one can see why the number of neuropeptides needs to be large. Since the peptides diffuse widely, their site of release does not define their site of action; consequently, if peptides released from different presynaptic terminals in the same neighborhood are to act on different postsynaptic targets, the peptides and their receptors must be chemically different.

Long-lasting Alterations of Behavior Reflect Changes in Specific Synapses[34]

The responses mediated by non-channel-linked receptors are long-lasting as well as slow in onset. Therein lies much of their special importance for the control of behavior: they bring about a persistent change in the rules that govern the immediate reaction of the nervous system to the inputs it receives and thus appear to be the basis for at least some forms of memory. This is most strikingly illustrated by studies on the sea snail *Aplysia*, a type of mollusk (Figure 19–40). In this animal, changes in behavior with experience can be traced to identified neural circuits and their molecular mechanisms can be deciphered.



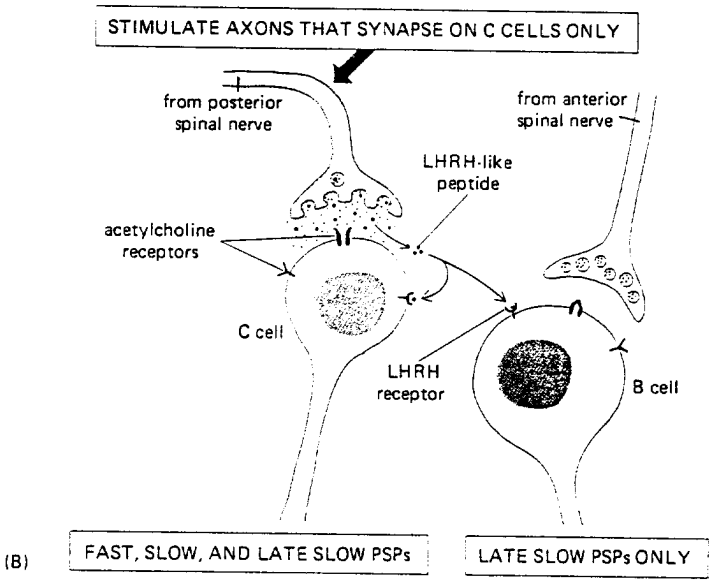


Figure 19–39 The pattern of responses to a peptide neurotransmitter.

(A) The three components of the postsynaptic potential observed in a ganglion cell of a frog following stimulation of the presynaptic nerve. The presynaptic axon terminal releases two neurotransmitters—acetylcholine and a peptide that closely resembles LHRH (luteinizing-hormone releasing hormone). The normal complex PSP is a composite of responses mediated by three kinds of receptors—two for acetylcholine and one for the LHRH-like peptide. Each of the three components can be observed independently by blocking the receptors responsible for the other two components with specific toxins. Only the fast excitatory PSP, mediated by a channel-linked acetylcholine receptor, is large enough to trigger an action potential. The two slow components, mediated presumably by non-channel-linked receptors, serve to modulate the excitability of the cell, making it more responsive to any stimuli that follow soon after the initial stimulus.

(B) Schematic diagram of an experiment on the same ganglion demonstrating the diffuse mode of action of the LHRH-like neuropeptide. This is released together with acetylcholine at synapses made on one population of cells (the C cells), but it diffuses over distances of several tens of micrometers to produce a late slow PSP in other, neighboring cells also (the B cells). (A, after Y.N. Jan *et al., Cold Spring Harbor Symp. Quant. Biol.* 48:363–374, 1983.)

Aplysia withdraws its gill if its siphon is touched (see Figure 19–40). If the siphon is touched repeatedly, the animal becomes **habituated** and ceases to respond. Habituation is similar in function to adaptation, although it operates on a longer time scale and, as we shall see, at a different point in the neural pathway. An unpleasant experience, such as a hard bang or an electrical shock, removes the habituation and leaves the animal **sensitized** so that it responds vigorously again to being touched. The sensitization persists for many minutes or hours, according to the severity of the brief noxious stimulus that caused it, and represents a simple form of *short-term memory*. If the animal is struck or shocked repeatedly on successive days, the sensitization—that is, the memory—becomes

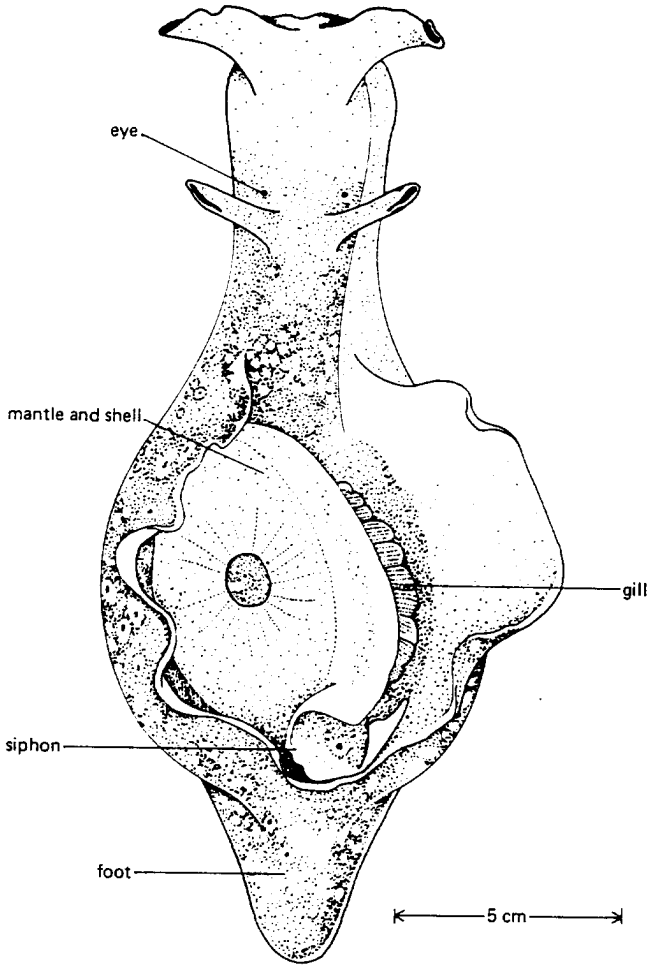


Figure 19–40 The sea snail *Aplysia punctata* viewed from above. An overlying flap of tissue has been drawn aside to reveal the gill under the protective mantle and shell. (After J. Guiart, *Mem. Soc. Zool. France* 14:219, 1901.)

long term, lasting for weeks. These modifications of behavior can be traced to changes occurring in a particular class of synapses in the neural circuit that controls the gill-withdrawal reflex.

The neurons of *Aplysia* are large (~100 μm), relatively few in number (~10^5), and stereotyped in their individual appearance. Touching the siphon stimulates a set of *sensory neurons* to fire. The sensory neurons make excitatory synapses on the *gill-withdrawal motor neurons*, which drive the muscles for gill withdrawal, and changes in these synapses underlie the behavioral phenomena. During habituation, the PSP evoked in the gill-withdrawal neurons is observed to become weaker with repeated firing of the sensory cells. Sensitization has the reverse effect, increasing the PSP. In both cases the changes are due to alterations in the amount of neurotransmitter released from the presynaptic axon terminals of the sensory neurons when they fire. The problem therefore reduces to the question of how transmitter release at these synapses is modulated.

G-Protein-linked Receptors Mediate Sensitization in *Aplysia*[35]

As mentioned on page 1077, the amount of neurotransmitter released at a synapse is controlled by the amount of Ca^{2+} that enters the terminal during the action potential. In habituation, repeated firing of the sensory cells leads to a modification of channel proteins in the terminals such that Ca^{2+} entry is reduced and the amount of neurotransmitter released decreases; in sensitization, by contrast, Ca^{2+} entry is increased, so that more neurotransmitter is released. The detailed molecular changes underlying these simple forms of memory are best understood in the case of sensitization.

In sensitization—provoked, for example, by shocks to the head—the alteration in transmitter release from the sensory neurons is brought about by the firing of

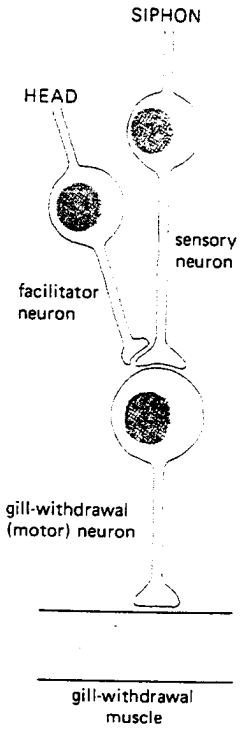


Figure 19–41 Simplified diagram of the neuronal pathways involved in habituation and sensitization of the gill-withdrawal reflex in *Aplysia*. Only one representative neuron of each class is shown.

another set of neurons that are responsive to the noxious stimulus. These *facilitator neurons* synapse on the presynaptic terminals of the sensory neurons (Figure 19–41), where they release serotonin (as well as certain neuropeptides). Their actions can be mimicked by applying serotonin directly to the membrane of the *sensory* neurons, whose presynaptic axon terminals contain serotonin receptors. These receptors operate via a G protein: binding of serotonin activates adenylate cyclase, thereby causing a rise in the intracellular concentration of cyclic AMP, which in turn activates A-kinase (see p. 709). It is this protein kinase that alters the electrical properties of the membrane of the sensory neuron by phosphorylating a special class of K^+ channels (Figure 19–42).

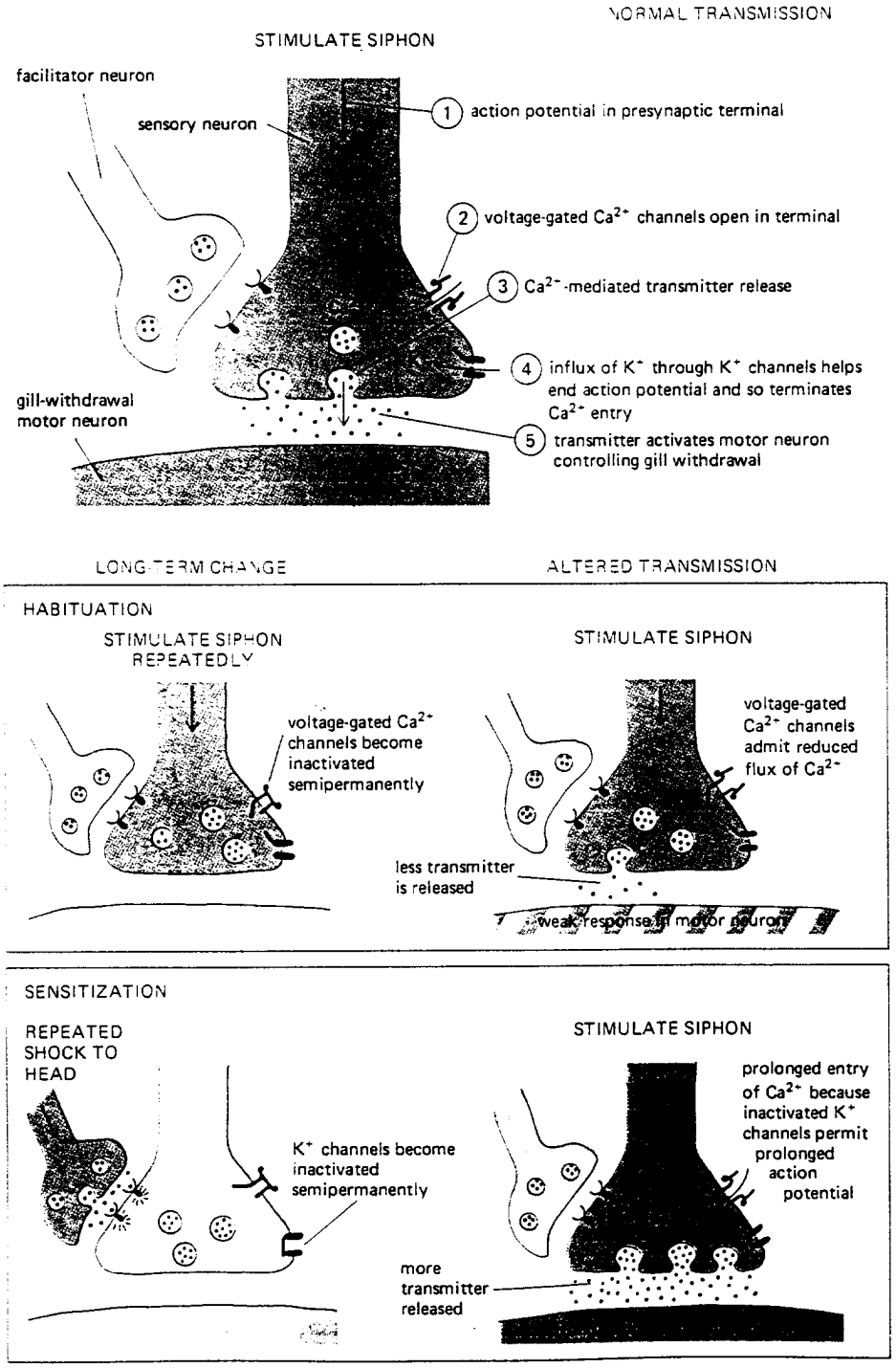


Figure 19–42 The mechanisms that bring about habituation and sensitization of the gill-withdrawal reflex in *Aplysia*. In each diagram the neurons that are electrically active are shown in color. The upper diagram shows the normal mechanism of transmission from the sensory neuron to the gill-withdrawal motor neuron. In each of the lower diagrams, the left-hand drawing shows the nature of the persistent change in the sensory nerve terminal that underlies the memory phenomenon, while the right-hand drawing shows how this change affects synaptic transmission from the sensory neuron to the gill-withdrawal motor neuron. The indicated mechanisms are less certain for habituation than for sensitization.

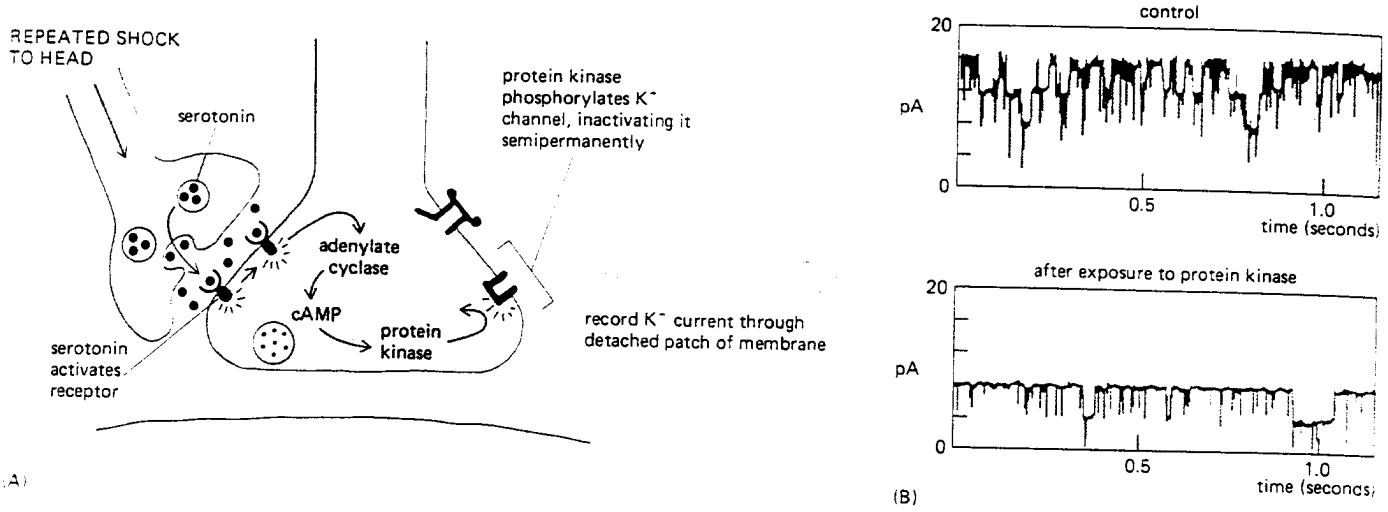


Figure 19–43 (A) The chain of events during sensitization of the gill-withdrawal reflex that leads to inactivation of a special class of K^+ channels (so-called S channels) in the sensory nerve terminal (see Figure 19–42). (B) Patch-clamp recordings of the current through these channels as they flicker between open and closed states. The patch, which has been detached from the cell, contains four channels, and in the control condition these are open most of the time. When the catalytic subunit of the cyclic AMP-dependent protein kinase (A-kinase) is added to the medium bathing the cytosolic side of the patch, two of the four channels become phosphorylated and are thereby locked shut, while the other two continue to spend most of their time in the open state; this reduces the average current through the patch to half its control value. (Patch-clamp data reprinted by permission from M.J. Schuster, J.S. Camardo, S.A. Siegelbaum, and E.R. Kandel, *Nature* 313:392–395, 1985. Copyright © 1985 Macmillan Journals Limited.)

The behavior of these K^+ channels, called *S channels*, can be analyzed by patch-clamp recording (see p. 318). They become locked shut when serotonin is applied to the exterior of the cell (Figure 19–43). Moreover, they close in the same way when the patch of membrane containing them is detached from the cell and transferred to a bath of artificial medium in which the channels are directly subjected to phosphorylation by the catalytic subunit of the A-kinase. This strongly suggests that phosphorylation of the S channels (or of proteins tightly associated with them) is the cause of their long-lasting closure. Because it is the flow of K^+ ions that normally helps to restore the resting potential, closing the S channels prolongs action potentials invading the axon terminal. The prolonged action potentials hold the voltage-gated Ca^{2+} channels open for a longer time, permitting a greater influx of Ca^{2+}, which in turn triggers the release of a larger number of synaptic vesicles; and this produces a larger postsynaptic potential, causing a more vigorous withdrawal of the gill.

These experiments demonstrate how a G-protein-linked receptor can enable a transient signal to cause a persistent change in the electrical properties of a synapse, and hence in the behavior of the animal. The phosphorylation of the S channels represents a form of memory, but it is only a short-term memory, easily erased by the action of phosphoprotein phosphatases (which dephosphorylate the S channels) and limited by the finite lifetime of the S-channel proteins. The mechanism of the *long-term memory* that follows repeated noxious stimulation is not known, but unlike the short-term memory, it requires new RNA and protein synthesis and seems to involve changes in the structure as well as the chemistry of the presynaptic terminals (see p. 1132). Cyclic AMP and A-kinase seem to mediate these changes too, presumably by phosphorylating other proteins in the cell and thereby probably altering the pattern of gene expression. The details are not yet understood, but an intermediate step in the creation of the long-term memory trace appears to be a prolonged activation of the A-kinase itself as a result of a reduction in the concentration of the regulatory subunits that inhibit it (see p. 710). These regulatory subunits are thought to be degraded during the period when cyclic AMP levels are high because, on binding cyclic AMP, they dissociate from the catalytic subunits and thereby become exposed to proteolysis.

Ca^{2+} and Cyclic AMP Are Important Intracellular Messengers in Associative Learning in Invertebrates[36]

Habituation and sensitization as presented above are very simple kinds of learning. An essential feature of the more complex types of learning most widely studied by psychologists is that they are *associative:* thus, in Pavlov's famous experiments,

the dog learned to associate the sound of a bell with food. *Aplysia* is also capable of associative learning. For example, if a sensitizing stimulus (a severe electrical shock, as before) is repeatedly paired in time with a particular mild stimulus that normally excites only a weak withdrawal reflex, the animal behaves as though it has learned that the specific mild stimulus is associated with the shock and becomes strongly and specifically sensitized to the mild stimulus. The same classes of neurons are thought to be involved as in the simple sensitization described earlier. The paired stimuli to different parts of the body cause sensory neurons and facilitator neurons to fire at the same time. Thus, while an action potential is invading the sensory axon terminals, causing their voltage-gated Ca^{2+} channels to open, serotonin (or a neuropeptide) is being released onto their exterior from the facilitator neurons, causing an increase in the intra-axonal cyclic AMP concentration. The cyclic AMP by itself would cause simple sensitization; the simultaneous influx of Ca^{2+} is thought to intensify this effect, producing a much stronger sensitization than would result from firing of facilitator neurons while the sensory neuron was quiet.

It is not clear how far one can extrapolate from these findings in *Aplysia*. Whether memories in other animals are generally recorded in presynaptic changes or in postsynaptic changes, in synaptic chemistry or in synaptic structure, or indeed in synapses at all, are open questions. Experiments on mutants of the fruit fly *Drosophila*, however, suggest that molecular mechanisms like those described above in *Aplysia* may operate in many other forms of learning. In particular, normal *Drosophila* can be trained to avoid a specific odor if they repeatedly receive an electrical shock in association with the odor. Flies that rapidly forget or fail to learn the association can easily be picked out because they will stray into regions where the smell is strong. In this way it has been possible to isolate dim-witted and forgetful mutants. Two of them, *dunce* (*dnc*) and *rutabaga* (*rut*), are capable of learning but have a drastically reduced memory span—on the order of tens of seconds in the case of *dunce*. In *dunce* the mutation turns out to be in a phosphodiesterase that breaks down cyclic AMP; in *rutabaga* it is in a Ca^{2+}-dependent adenylate cyclase, which makes cyclic AMP. It seems that either too much or too little cyclic AMP can interfere with memory formation. Another mutant, called *Ddc*, seems to be unable to learn in the first place; here the deficiency is in a gene for the enzyme dopa decarboxylase, which catalyzes an essential step in the production of serotonin and dopamine. All these mutants with defects in associative learning also show defects in their susceptibility to sensitization. Evidently the two processes share some common mechanisms, and it seems that these mechanisms, like sensitization in *Aplysia*, involve a monoamine neurotransmitter at an initial step and protein phosphorylations—controlled by cyclic AMP and by Ca^{2+}—for the production of a lasting effect.

Learning in the Mammalian Hippocampus Depends on Ca^{2+} Entry Through a Doubly Gated Channel[37]

Practically all animals can learn, but mammals seem to learn exceptionally well (or so we like to think). This may reflect the operation of some unique molecular mechanisms. In a mammal's brain the *hippocampus*, a part of the cerebral cortex, seems to play a special role in learning: when it is destroyed on both sides of the brain, the ability to form new memories is largely lost, although previous long-established memories remain. Correspondingly, some synapses in the hippocampus show dramatic functional alterations with repeated use. Whereas occasional single action potentials in the presynaptic cells leave no lasting trace, a short burst of repetitive firing causes **long-term potentiation,** such that subsequent single action potentials in the presynaptic cells evoke a greatly enhanced response in the postsynaptic cells. The effect lasts hours, days, or weeks, according to the number and intensity of the bursts of repetitive firing. Only the synapses that were activated show the potentiation; synapses that have remained quiet on the same postsynaptic cell are not affected. But if, while the cell is receiving a burst of repetitive stimulation via one set of synapses, a single action potential is delivered

at *another* synapse on its surface, that latter synapse also will undergo long-term potentiation, even though a single action potential delivered there at another time would leave no such lasting trace. Clearly this provides a basis for associative learning.

The underlying rule in the hippocampus seems to be that *long-term potentiation occurs on any occasion where a presynaptic cell fires (once or more) at a time when the postsynaptic membrane is strongly depolarized* (either through recent repetitive firing of the same presynaptic cell or by other means). There is good evidence that this rule reflects the behavior of a particular class of ion channels in the postsynaptic membrane. Most of the depolarizing current responsible for the excitatory PSP is carried in the ordinary way by ligand-gated ion channels that bind glutamate. But the current has in addition a second and more intriguing component, which is mediated by a distinct subclass of channel-linked glutamate receptors, known as **NMDA receptors** because they are selectively activated by the artificial glutamate analog N-methyl-D-aspartate. The NMDA-receptor channels are doubly gated, opening only when two conditions are satisfied simultaneously: the membrane must be strongly depolarized (the channels are subject to a peculiar form of voltage gating that depends on extracellular Mg^{2+} ions), and the neurotransmitter glutamate must be bound to the receptor. The NMDA receptors are critical for long-term potentiation. When they are selectively blocked with a specific inhibitor, long-term potentiation does not occur, even though ordinary synaptic transmission continues. An animal treated with this inhibitor fails in learning tasks of the type thought to depend on the hippocampus but behaves almost normally otherwise.

How do the NMDA receptors mediate such a remarkable effect? The answer seems to be that these channels, when open, are highly permeable to Ca^{2+}, which acts as an intracellular messenger close to its site of entry into the postsynaptic cell, triggering the local changes responsible for long-term potentiation. Long-term potentiation is prevented when Ca^{2+} levels are held artificially low in the postsynaptic cell by injecting the Ca^{2+} chelator EGTA into it and can be induced by transiently raising extracellular Ca^{2+} levels artificially high. The nature of the long-term changes triggered by Ca^{2+} is uncertain, but they are thought to involve structural alterations in the synapse.

Despite the differences between this example of a memory mechanism in mammals and the previous examples in invertebrates, there is a common theme. Neurotransmitters released at synapses, besides relaying transient electrical signals, can also alter concentrations of intracellular messenger molecules, which activate enzymatic cascades that bring about lasting changes in the efficacy of synaptic transmission. But several major mysteries remain, and we do not yet know how these changes endure for weeks, months, or a lifetime in the face of the normal turnover of cell constituents. We shall see later that the development of the nervous system raises some closely related problems.

Summary

Unlike channel-linked receptors, non-channel-linked neurotransmitter receptors respond to their ligand by initiating a cascade of enzymatic reactions in the postsynaptic cell. In most cases studied so far, the first step in this cascade is the activation of a G protein, which may either interact directly with ion channels or control the production of intracellular messengers such as cyclic AMP or Ca^{2+}. These in turn regulate ion channels directly or activate kinases that phosphorylate various proteins, including ion channels. At many synapses both channel-linked and non-channel-linked receptors are present, responding either to the same or to different neurotransmitters. Responses mediated by non-channel-linked receptors have a characteristically slow onset and long duration, and they may modulate the efficacy of subsequent synaptic transmission, thus providing the basis for at least some forms of memory. Channel-linked receptors that allow Ca^{2+} to enter the cell, such as the NMDA receptor, can also mediate long-term memory effects.

Sensory Input[38]

We have seen how nerve cells conduct electrical signals, compute with them, record them, and transmit them to muscles to bring about movement. But how do the signals originate? There are two types of source: spontaneous firing and sensory input. Examples of spontaneously active neurons, such as those in the brain that generate the rhythm of breathing, are common: quite complex patterns of spontaneous firing can be produced in a single cell by appropriate combinations of gated ion channels of the types we have already encountered in discussing neuronal computation. Sensory input likewise involves principles that are already familiar, but they are embodied in cells of the most diverse and remarkable types.

Sense organs have evolved to meet exceptionally stringent engineering specifications: they discriminate precisely between stimuli of different types, operate over phenomenally wide ranges of stimulus intensity, and approach the utmost sensitivity that the laws of physics will allow. An olfactory cell in the male gypsy moth can detect a single molecule of a specific sexual attractant (a so-called *pheromone*) released into the air by a female a mile away. The human eye can see both in bright sunlight and on a starlit night, where the illumination is 10^{12} times fainter; and five photons absorbed in the human retina are perceived as a flash.

We shall concentrate on just two examples where the cellular mechanisms of sensory input are beginning to be understood: the ears and the eyes of vertebrates. At each of these gateways into the nervous system, there stands a highly specialized type of **sensory cell,** very different in the two cases but in both cases remarkable for its selectivity, its operating range, and its sensitivity. Before going into details, however, it will be helpful to discuss some general principles.

Stimulus Magnitude Is Reflected in the Receptor Potential[38,39]

Any signal that is to be fed into the nervous system must first be converted to an electrical form. The conversion of one kind of signal into another is known as transduction, and all sensory cells are therefore **transducers.** Indeed, in a general sense almost every neuron is a transducer, receiving chemical signals at synapses and converting these into electrical signals. Thus, although some sensory cells respond to light, some to temperature, some to a particular chemical, some to a mechanical force or displacement, and so on, transduction in all of them involves many of the same basic principles that were discussed earlier for synaptic activation by neurotransmitters. In some sense organs the transducer is part of a *sensory neuron* that propagates action potentials. In others it is part of a *sensory cell* specialized for transduction but not for long-distance communication; such a cell then passes its signal to an adjacent neuron via a synapse (Figure 19–44).

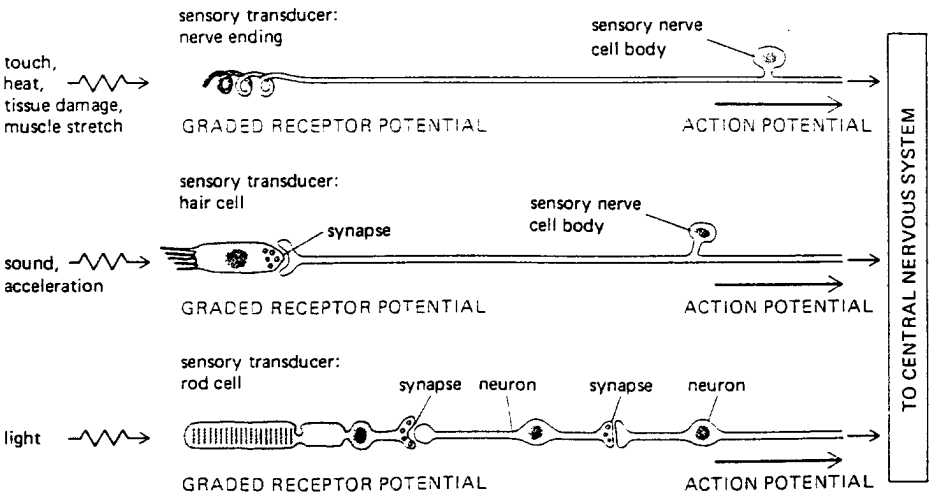


Figure 19–44 Different ways in which sensory stimuli are transmitted to the nervous system. In some cases the sensory transducer is part of a neuron (top drawing); in other cases it is a separate sensory cell (lower two drawings). In all three cases a graded receptor potential (indicated in color) is evoked in the sensory transducer and translated into the frequency of firing of action potentials, which carry the signal rapidly into the CNS.

But in every case the effect of the external stimulus is to cause a voltage change, called the **receptor potential,** in the transducer cell. This is analogous to a postsynaptic potential and likewise serves ultimately to control the release of neurotransmitter from another part of the cell.

Moreover, just as at a synapse, the external stimulus can exert its electrical effect either directly, by acting on an ion channel, or indirectly, by acting on a receptor that generates an intracellular messenger that affects ion channels. Although there are still some uncertainties, it seems that the sensory cells in the ear use the direct mode, based on channel-linked receptors, whereas those in the eye use the indirect mode, based on G-protein-linked receptors.

Hair Cells in the Ear Respond to Tilting of Their Stereocilia[40]

The ear is not only for hearing. It also provides information on acceleration and on the direction of gravity and so is important for balance and coordination of movements. All these sensory functions of the ear depend on *mechanoreception*—that is, the detection of small movements produced by forces acting in the environment of the ear's sensory cells. The movements are rapid oscillations in the case of sound and slower, more sustained displacements in the case of gravity and acceleration. The cells responsible for the various types of mechanoreception in the ear all have a similar and characteristic form: each of them has a tuft of giant microvilli, confusingly called *stereocilia,* projecting from its upper surface (Figure 19–45 and see p. 675). They are consequently known as **hair cells.**

The hair cells in higher vertebrates all lie in the epithelium of the *membranous labyrinth* of the inner ear, where they are grouped in several separate sensory patches. The hair cells in each group are held in place by a framework of interposed *supporting cells,* while above them lies a sheet of gelatinous extracellular matrix attached to the tips of the tufts of stereocilia (Figure 19–46). The movement of this overlying sheet of matrix tilts the stereocilia and produces a mechanical deformation of the hair cells that gives rise to the receptor potential (Figure 19–47). The specific functions of the different groups of hair cells are determined mainly by the nature of the surrounding structures that transmit forces to them. In the case of those hair cells that respond to linear acceleration and to the force of gravity, the overlying matrix is weighted with dense crystals of calcium carbonate: when the head is accelerated or tilted, the weighted matrix shifts relative to the hair cells and the stereocilia are deflected. In contrast, the hair cells that sense rotational acceleration are arranged so that a sideways force is exerted on their overlying matrix by the swirling of fluid in the semicircular canals of the inner ear when the head is turned.

The most elaborate mechanical setting is provided for the hair cells that detect sound in the ears of mammals (see Figure 19–46). These *auditory hair cells* are arrayed on a thin, resilient sheet of tissue—the *basilar membrane*—that forms a long, narrow dividing partition between two fluid-filled spiral channels running in parallel within the portion of the inner ear known as the *cochlea.* Airborne sounds cause vibrations of the eardrum, which are conveyed via the tiny bones in the middle ear to the fluid-filled channels of the inner ear, where they result

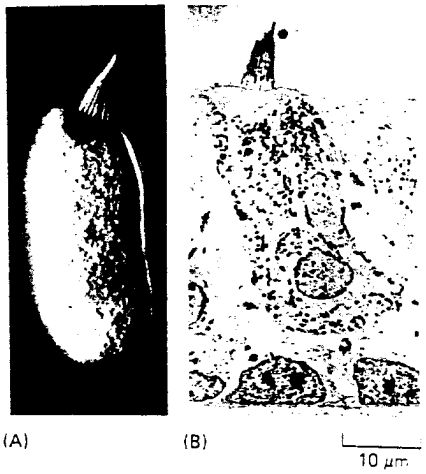


Figure 19–45 (A) Photograph of a sensory hair cell isolated from the inner ear of a bullfrog, showing the bundle of stereocilia on its apical surface. (B) Low-magnification transmission electron micrograph of the hair cell in its normal context, amid supporting cells. (From A.J. Hudspeth, *Science* 230:745–752, 1985. Copyright 1985 by the AAAS.)

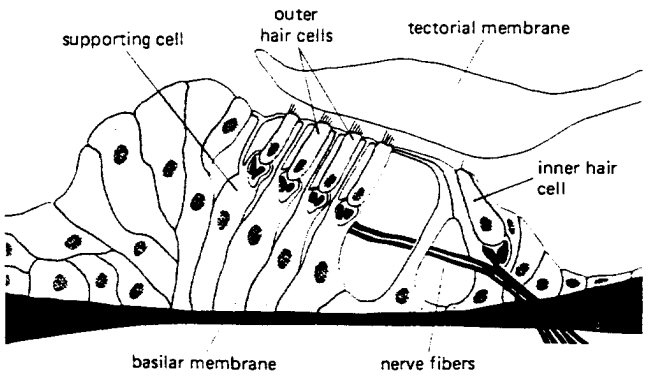


Figure 19–46 Diagrammatic cross-section of the auditory apparatus (the organ of Corti) in the inner ear of a mammal, showing the auditory hair cells held in an elaborate structure of supporting cells and overlaid by the tectorial membrane (a mass of extracellular matrix). The inner hair cells are thought to be the receptors that are primarily responsible for hearing, through the transduction mechanism discussed in the text; they synapse with neurons that convey the auditory signals inward from the ear to the brain. The outer hair cells, by contrast, are richly innervated by an additional set of axons that convey signals outward from the brain, and their function is still a puzzle. There is some evidence to suggest that they are capable of acting (by an unknown mechanism) as transducers in a reverse direction—as loudspeakers rather than microphones—and that they serve as part of a feedback system to modulate the mechanical stimulus delivered to the inner hair cells.

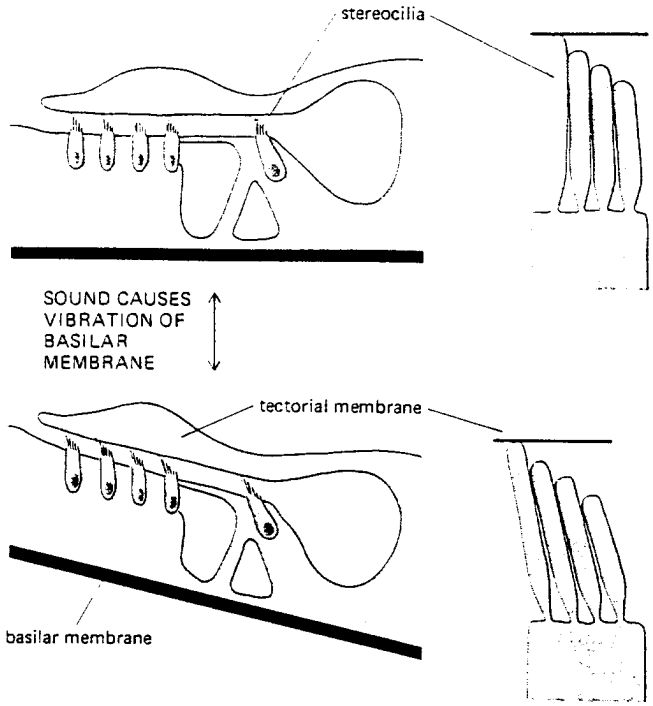


Figure 19–47 How a relative movement of the overlying extracellular matrix (the tectorial membrane) tilts the stereocilia of auditory hair cells in the inner ear of a mammal. The stereocilia behave as rigid rods hinged at the base. The tips of the bundles of stereocilia can be mechanically coupled to the overlying matrix by direct attachment, or indirectly through viscous drag via the intervening fluid.

in vibrations of the basilar membrane and hence of the stereocilia of the auditory hair cells. Hair cells in different positions report on sounds of different pitch because of the mechanics of the cochlea, which resonates most strongly at different positions along its length according to the pitch of the incident sound.

Mechanically Gated Cation Channels at the Tips of Stereocilia Open When the Stereociliary Bundles Tilt[40,41]

When the sheet of matrix overlying a patch of hair cells is abruptly shifted sideways so as to tilt the stereocilia by a few degrees, the hair cells respond by changing their membrane permeability so that a current, called the *receptor current*, flows into them (Figure 19–48). The response reaches a plateau within 100–500 microseconds, which is about the same as the speed of opening of the acetylcholine-activated cation channel at the neuromuscular junction and much faster than the electrical changes produced by any known non-channel-linked receptor. It seems very probable, therefore, that the mechanical stimulus directly opens an ion channel. Studies in which the extracellular ion concentrations are varied have shown that this *mechanically gated ion channel*, like the acetylcholine receptor, is rather

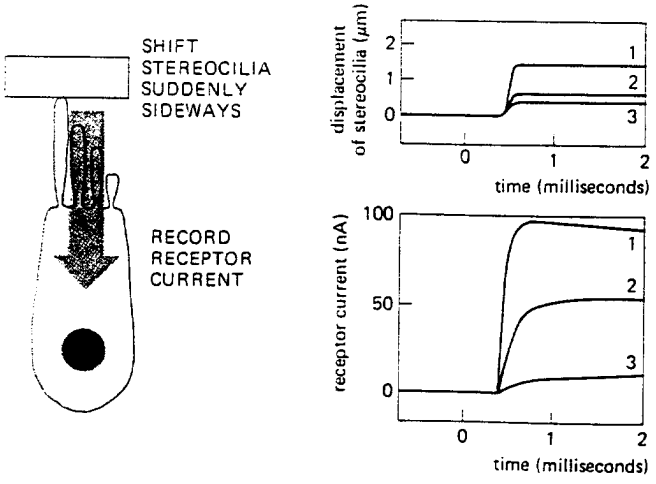


Figure 19–48 Recordings of the receptor current that enters hair cells in the inner ear of a bullfrog in response to a sudden deflection of the bundle of stereocilia. The amount of current is larger for larger deflections. (Data from D.P. Corey and A.J. Hudspeth, *J. Neurosci.* 3:962–976, 1983.)

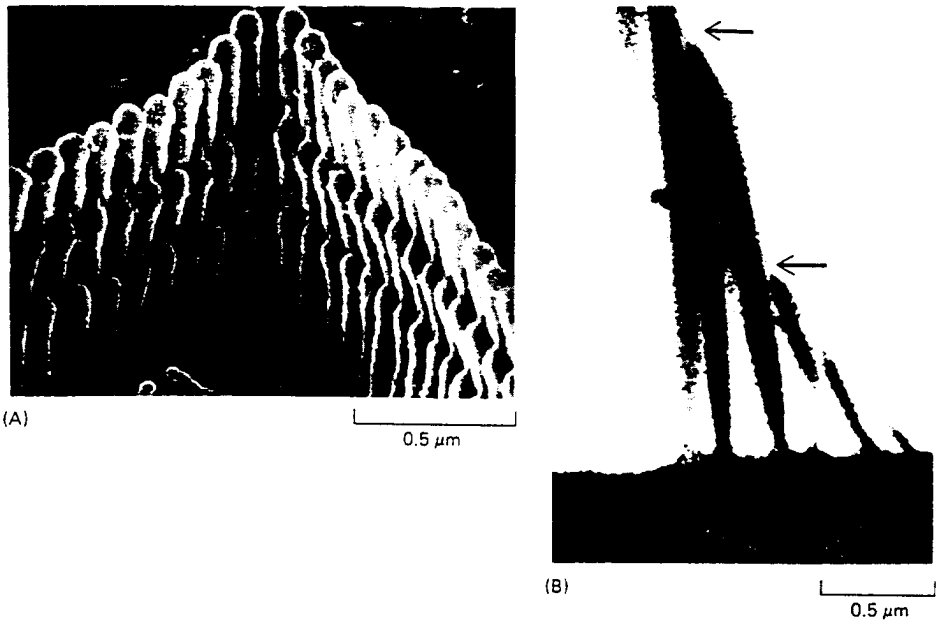


Figure 19–49 (A) Scanning electron micrograph of an auditory hair cell from a mammal, showing the fine filaments that run from the tips of the shorter stereocilia to attach higher up on the sides of stereocilia in the next, taller, row. (B) A cross-section of the same structures as seen by transmission electron microscopy. Arrows point to the filaments. (B, photograph by M.P. Osborne; A and B, reprinted with permission from J.D. Pickles, *Prog. Neurobiol.* 24:1–42, 1985. Copyright 1985, Pergamon Press plc.)

indiscriminately permeable to small cations and that the current through it is largely carried by K^+. (Ionic conditions in the ear are unusual, and there is a large electrochemical gradient for K^+ across the hair-cell membrane.) But where in the hair cell are such channels located, and how is the elaborate structure of the cell related to the process of transduction?

The stereocilia on each cell are arranged like organ pipes in close-packed ranks of graded height (see Figures 19–45 and 19–49). There is often a single true cilium, or *kinocilium*, behind the middle of the tallest row; this is always present during development, but it plays no part in transduction and is sometimes (as in mammalian auditory hair cells) lost during maturation. When deflected with a microprobe, the stereocilia behave as a coherent bundle of rigid rods, individually pivoted about their points of attachment to the apex of the hair cell and therefore sliding parallel to one another so that their tips undergo a relative displacement. Electron microscopy reveals, in addition to lateral attachments that hold the stereocilia together in a bundle, a fine filament running more or less vertically upward from the tip of each shorter stereocilium to attach at a higher point on its adjacent taller neighbor (Figure 19–49). When a microelectrode is used to record from hair cells, the maximal depolarizing response is seen when the stereocilia are deflected in the direction that would be expected to stretch the fine vertical filaments maximally. Moreover, the transmembrane current induced by the deflection (which gives rise to the receptor potential) appears to enter the hair cell near the tips of the stereocilia. Thus the whole structure seems to be designed so that ion channels near the tips of the stereocilia will be opened by a mechanical tug when the bundle of stereocilia is deflected (Figure 19–50).

The hair-cell mechanism is astonishingly sensitive: the faintest sounds that we can hear have been estimated to stretch the vertical filaments attached to the tops of the stereocilia by an average of about 0.04 nm, which is just under half the diameter of a hydrogen atom. Moreover, analyses of the receptor current in a hair cell indicate that there are probably only between one and five mechanically gated ion channels in each stereocilium: the hair cells responsible for human hearing each have about 100 stereocilia; and there are about 3500 such hair cells in each ear. Apparently, we owe our hearing to fewer than 4 million transducer molecules.

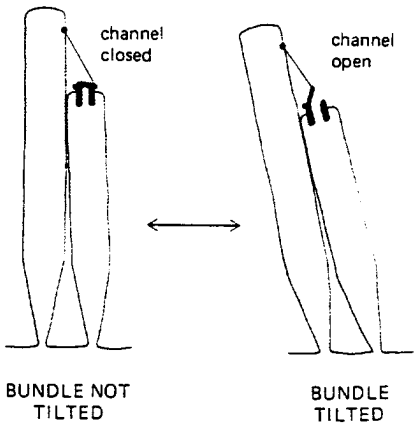


Figure 19–50 Cartoon drawing suggesting how the tilting of stereocilia on a hair cell may be coupled to the opening of ion channels. By extraordinarily delicate mechanical measurements, correlated with electrical recordings from a single hair cell as the bundle of stereocilia is deflected by pushing with a flexible glass probe, it is in fact possible to detect an extra "give" of the bundle as the mechanically gated channels yield to the applied force and are pulled open, just as the model would predict. In this way it can be shown that the force required to open a single one of the hypothesized channels is about 2×10^{-13} newtons and that its gate swings through a distance of about 4 nm as it opens.

Photoreceptors Are Sensitive and Adaptable but Relatively Slow[42]

The sensitivity of the photoreceptors in the vertebrate eye approaches the ultimate limit set by the quantal nature of light. Moreover, the operating range, from the brightest light tolerable to the dimmest light perceptible, is extraordinarily wide.

But the speed of response, by comparison with the auditory transducers, is very slow. Under optimal conditions the fastest of the photoreceptors in the human eye take about 25 milliseconds to reach the peak of their electrical response to a flash of light—more than a hundred times longer than the response time of a typical hair cell. The relative slowness of the visual response probably reflects an essential limitation imposed by the nature of the visual transduction mechanism.

The Receptor Potential in a Rod Cell Is Due to Closure of Na^+ Channels[43]

As described in Chapter 17 (see p. 956), the photoreceptors in the vertebrate eye are of two classes. **Cone cells** (or **cones**) serve for color vision and perception of fine detail, and they require fairly bright light. **Rod cells** (or **rods**) provide for monochromatic vision in dim light, and they can produce a measurable electrical signal in response to a single photon (Figure 19–51). Rods and cones appear to operate on similar principles, but rods have been studied more intensively.

The rod cell (Figure 19–52) consists of an *outer segment*, containing the photoreceptive apparatus; an *inner segment*, containing many mitochondria; a *nuclear region*; and, at the base, a *synaptic region*, which makes synaptic contact with nerve cells of the retina (see Figure 17–6, p. 957). In the dark, paradoxically, the cell is strongly depolarized; the depolarization holds voltage-gated Ca^{2+} channels open in the synaptic region, and the resulting influx of Ca^{2+} produces a steady release of neurotransmitter. The depolarization is due to open Na^+ channels in the plasma membrane of the outer segment. Illumination causes these channels to close, so that the receptor potential takes the form of a *hyperpolarization*, which leads to a decrease of Ca^{2+} influx at the synapse and consequently a decrease in the rate of transmitter release (see Figure 19–52). Because the transmitter acts to inhibit many of the postsynaptic neurons, illumination frees these neurons from inhibition and thus, in effect, excites them. The rate of release of transmitter from the photoreceptors is graded according to the intensity of the light: the brighter the light, the greater the hyperpolarization and the greater the decrease in transmitter release. When background lighting is very dim, so that the cell is in its most

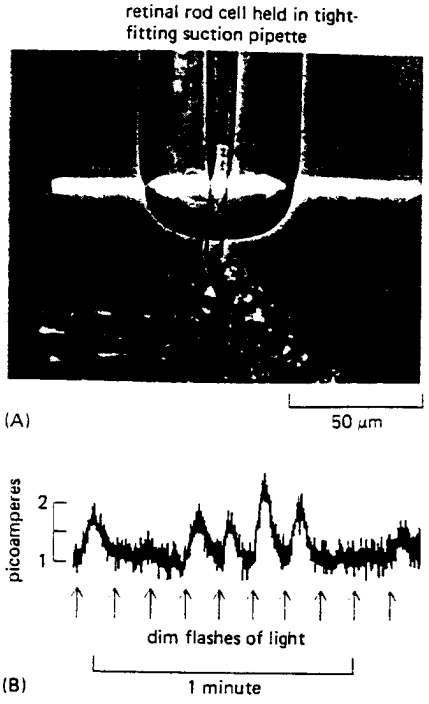


Figure 19–51 The electrical responses of a rod cell to single photons. (A) Photomicrograph to show the recording technique. A fragment of the retina of a toad is dissected out, and the outer segment of a single rod cell is sucked into the mouth of a tight-fitting glass pipette, which then serves as an electrode to collect and record the current passing through the rod cell membrane. (B) Recording of the changes in the rod cell current in response to a series of 10 dim flashes; the number of photons absorbed by the rod from each flash is randomly variable but always a whole number; most of the large peaks in the record correspond to absorption of one or two photons, but many of the flashes fail to evoke a response because no photon is absorbed. (From D.A. Baylor, T.D. Lamb, and K.-W. Yau, *J. Physiol.* 288:589–611, 1979.)

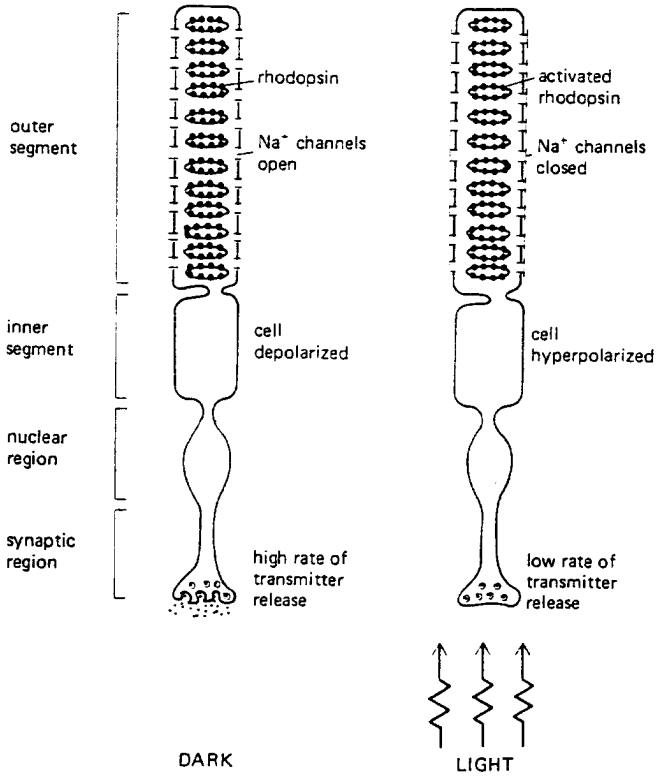


Figure 19–52 The response of a rod photoreceptor cell to illumination. Photons are absorbed, at random, by rhodopsin molecules in the outer segment; this leads to the closure of Na^+ channels in the plasma membrane and reduces the rate of transmitter release from the synaptic region.

sensitive, "dark-adapted" state, the absorption of a single photon blocks the influx of a million or more Na^+ ions that would otherwise have entered the cell and thereby generates a hyperpolarization of about 1 mV.

Photons Change the Conformation of Rhodopsin Molecules[43,44]

How is the light initially detected by the cell, and what chain of events then causes the Na^+ channels to close? These crucial steps in the transduction of the light signal occur in the outer rod segment, which is a cylindrical structure containing a stack of about a thousand *discs* (see Figure 17–7). Each disc is formed by a closed membrane in which photosensitive **rhodopsin** molecules are embedded, packed closely together at a density of about $10^5/\mu m^2$. Each rhodopsin molecule consists of a transmembrane glycoprotein called *opsin* (348 amino acid residues long), with a covalently attached prosthetic group, 11-*cis*-retinal, embedded in its interior. The 11-*cis*-retinal is light-sensitive; absorption of a photon causes it to isomerize almost instantaneously to all-*trans*-retinal, changing its own shape and thereby forcing a slower change in the conformation of the opsin protein, which takes about 1 millisecond. After a further delay of about 1 minute, the all-*trans*-retinal dissociates from the opsin by hydrolysis of the bond between them and is released into the cytosol, where it eventually reverts to the 11-*cis* form, which then reassociates with opsin to regenerate a photosensitive rhodopsin molecule. It is the early conformational change in the rhodopsin in response to light that causes Na^+ channels in the plasma membrane to close. But since the rhodopsin is in the discs, some distance from the Na^+ channels, a messenger system is required to couple the two events.

A Light-induced Fall in Cyclic GMP in the Cytosol of the Photoreceptor Cell Closes Na^+ Channels in the Plasma Membrane[43,44,45]

When light falls on a rod cell, changes occur in the intracellular concentrations of both Ca^{2+} and cyclic GMP, so that either of these molecules could, in principle, be the intracellular messenger. The technique of patch recording (see p. 156) has revealed that a fall in the cytosolic concentration of cyclic GMP is the crucial signal. In the key experiment a microelectrode was pressed against the side of the outer segment and then pulled away, taking with it a patch of membrane whose cytosolic side was thus exposed to the external medium (Figure 19–53). Cyclic GMP was then added to the medium and an electrochemical potential difference was applied across the membrane patch; this caused a Na^+ current to flow. When the cyclic GMP was removed, the current stopped, regardless of the concentration of Ca^{2+}. Thus cyclic GMP opens the Na^+ channels, and light, by causing a fall in cyclic GMP concentration, makes the channels close. Whereas cyclic nucleotides usually work by activating a protein kinase to phosphorylate specific proteins (see p. 708), cyclic GMP in rod cells acts directly on the Na^+ channels to keep them open. But how does the light-triggered change in the conformation of rhodopsin decrease the concentration of cyclic GMP in the rod cell cytosol?

The absorption of a single photon of light by a single molecule of rhodopsin leads to the hydrolysis of many molecules of cyclic GMP. This amplification is

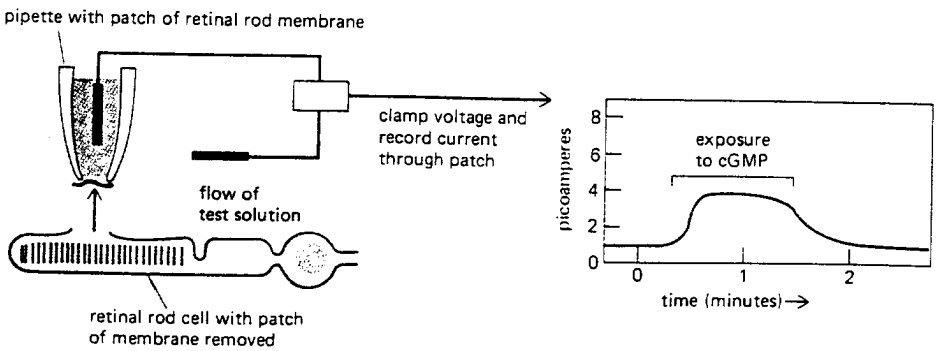


Figure 19–53 An experiment showing that cyclic GMP applied to the cytoplasmic face of the rod cell plasma membrane acts directly to control the opening and closing of ion channels. The relatively slow, smooth rise and fall of the current as cyclic GMP is added and then removed reflect the time taken to change the composition of the test solution; the rate of response of the channels to cyclic GMP is far too rapid to be resolved by this flow technique. (After E.E. Fesenko, S.S. Kolesnikov, and A.L. Lyubarsky, *Nature* 313:310–313, 1985.)

achieved through a catalytic cascade. The single activated rhodopsin molecule catalyzes the activation of a G protein, called *transducin*, at the very rapid rate of about 1000 transducin molecules per second. Transducin is homologous to the G_s protein (see p. 696) that functionally couples various receptors to adenylate cyclase (and rhodopsin itself is homologous to such receptors—see p. 706). Instead of interacting with adenylate cyclase, however, each activated transducin molecule activates a molecule of *cyclic-GMP phosphodiesterase*, which specifically hydrolyzes cyclic GMP at a rate of about 4000 molecules of cyclic GMP per second, causing cyclic GMP levels to fall rapidly. This catalytic cascade lasts for about 1 second and results in the hydrolysis of more than 10^5 cyclic GMP molecules for a single quantum of light absorbed, which transiently closes 250 Na^+ channels in the plasma membrane (Figure 19–34).

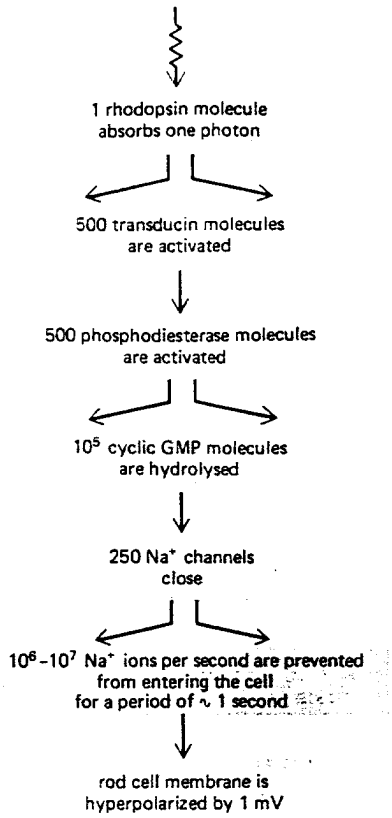


Figure 19–34 The catalytic cascade leading from absorption of a single photon in a dark-adapted rod cell to the production of a receptor potential. Divergent arrows indicate the steps at which amplification occurs.

The Photoreceptor Adapts to the Brightness of the Light[46]

Each reaction in the light-activated catalytic cascade must be counterbalanced by a corresponding inactivation reaction so as to restore the photoreceptor to its resting state after it has been excited by light. It seems that light speeds up the inactivation reactions as well as the activation reactions, but with a slight delay between the two effects, so that when a light is switched on, there is momentarily a strong net response, which is then soon damped down. Besides helping to ensure a brief response to a brief flash of light, this delayed inactivation enables the photoreceptor to *adapt:* steady light, instead of simply driving the cell into a saturated state with a near-zero concentration of cyclic GMP, exerts two contrary effects that nearly cancel, leaving the cell still able to signal subsequent changes of illumination.

A light-induced fall in Ca^{2+} concentration appears to be crucial both in terminating the response to a flash of light and in mediating adaptation. When a Ca^{2+} buffer is artifically introduced into a photoreceptor so that changes in intracellular Ca^{2+} concentration are abnormally delayed, the electrical responses to flashes of light are greatly prolonged, and the cell becomes excessively slow to adapt to steady illumination; and if a photoreceptor is bathed in a solution that completely blocks movement of Ca^{2+} across the plasma membrane, the same effects are seen to an even greater degree, and adaptation is completely abolished. Normally, the channels through which Na^+ enters the outer segment of the photoreceptor are somewhat permeable to other cations, including Ca^{2+}. Light, by closing the channels, blocks the influx of Ca^{2+}, while Ca^{2+} efflux (mediated by a Ca^{2+}-Na^+ antiporter in the rod plasma membrane) continues. Consequently the intracellular concentration of Ca^{2+} falls. This is thought to accelerate those enzymatic reactions (including, in particular, the synthesis of cyclic GMP by guanylate cyclase) that counteract the light-induced fall in cyclic GMP concentration, thereby helping the cell to adapt.

Neuronal Computations Process the Raw Data from the Sensory Receptor Cells[47]

A flood of sensory information enters the nervous system via the sensory receptor cells. The brain must process this information to extract the significant features: it must pick out words from the hubbub of sound, recognize a face in the pattern of light and dark, and so on. This represents a second, neural stage of sensory processing, which is far more subtle and complex than that which occurs in the receptor cells. It involves computations performed by intricate networks of neurons, each one typically receiving a multitude of convergent inputs, some excitatory, some inhibitory. The individual neuron responds by generating an output that represents the presence or absence of some specific pattern in the data supplied by the receptor cells. Certain classes of cells in the visual centers of the brain, for example, fire action potentials when the eye sees a line with a specific orientation. The output signals from one set of neurons are received by yet other neurons, which take the whole process a step further, and so on to increasingly

higher levels of feature detection, culminating in the recognition of such subtle and complex entities as meaningful words and the expressions on faces.

Neuronal computations such as these depend on the bewilderingly complex arrangement of anatomical connections among the nerve cells. The details of the relationship between neuroanatomy and higher neural functions fall outside the scope of a cell biology book. But what are the basic mechanisms by which the complicated yet orderly anatomy develops? This will be the topic of the next section.

Summary

Sensory stimuli are translated into electrical signals by specialized transducers. Sensory hair cells in the vertebrate ear, for example, are mechanoreceptors: each has a bundle of stereocilia (giant microvilli) on its exposed surface, and when this bundle is tilted, the cell responds by opening or closing ion channels so as to change its membrane potential. Photoreceptor cells in the vertebrate eye change their membrane potential when light falls on the rhodopsin molecules that they contain. In both cases the electrical change in the sensory cell—the receptor potential—is signaled to adjacent neurons via chemical synapses. However, the two cases illustrate contrasting strategies for the production of receptor potentials, one based on channel-linked receptors, the other based on non-channel-linked receptors. In the hair cell it appears that physical linkages between the stereocilia bring mechanical forces directly to bear on ion channels in the plasma membrane, which very promptly open or close in response. In the rod cell, light-activated rhodopsin molecules trigger a cascade of enzymatic reactions that lead to the hydrolysis of cyclic GMP in the cytosol, causing Na^+ channels in the plasma membrane to close. The catalytic response mechanism, although intrinsically slow, allows detection of single photons.

Birth, Growth, and Death of Neurons[48]

The nervous system poses a unique developmental problem. How do the axons and dendrites from the billions of neurons find their right partners, so as to create a functional network?

Most of the components of the nervous system—the various classes of neurons, sensory cells, and muscles—originate in widely separate locations in the embryo and are initially unconnected. Thus, in the first phase of neural development, the different parts develop according to their own local programs, following principles of cell diversification common to other tissues of the body, as discussed in Chapter 16. The next phase involves a type of morphogenesis unique to the nervous system, in which a provisional but orderly set of connections is set up between the parts of the nervous system through the outgrowth of axons and dendrites along specific routes. Parts that were originally separate can now begin to interact. In the third and final phase, which continues into adult life, the connections are adjusted and refined through interactions among the far-flung components in a way that depends on the electrical signals that they transmit and receive.

Neurons Are Generated Through Finite Programs of Cell Division[48,49]

With a few exceptions, three principles apply to the production of neurons in almost all species, from nematode worms to vertebrates: (1) mature neurons do not divide; (2) once the adult complement of neurons has been generated, no stem cells persist to generate more; and (3) each small region of the developing nervous system generates neurons through its own program of cell divisions, independently of influences from the distant groups of cells with which neural connections will later be made.

In vertebrates the nervous system develops chiefly from two sets of cells—those of the *neural tube* (see p. 887) and those of the *neural crest* (see p. 944)—both originating from the ectoderm. The neural tube forms the central nervous

system (the brain and spinal cord), while the neural crest gives rise to most of the neurons and supporting cells of the peripheral nervous system. In addition, thickenings or *placodes* in the ectoderm on the head give rise to some of the sensory cells and neurons in that region, including those of the ear and the nose (Figure 19–55).

The **neural tube**, with which we shall be mainly concerned here, consists initially of a single-layered epithelium that gives rise to both the neurons and the glial cells of the central nervous system (Figure 19–56). In the process the simple epithelium is transformed into a thicker and more complex structure, with many layers of cells of various types. The pattern of cell proliferation has been analyzed by labeling the cells that are in S phase of the division cycle with ^{3}H-thymidine and fixing the tissue either immediately, to see which cells are currently dividing (see p. 729), or after a long delay, to see which of the mature cells are derived from ancestors that were dividing at the time the ^{3}H-thymidine was given. Such studies on the developing nervous system are especially useful in revealing the "birthdays" of the various neurons: because differentiated neurons do not divide, each neuronal precursor cell must undergo its final division on a particular day before beginning to mature as a neuron. In both vertebrates and invertebrates, the birthdays of the neurons of a given type are generally all found to lie within a strictly limited period of development, after which no further neurons of that type are produced. Each region of the developing neural tube has its own program of cell divisions, and neurons with different birthdays are generally destined for different functions. Since stem cells usually do not persist once the production of nerve cells is complete and neural connections have begun to form, nerve cell numbers thereafter can only be regulated downward, through cell death (see p. 1120, below).

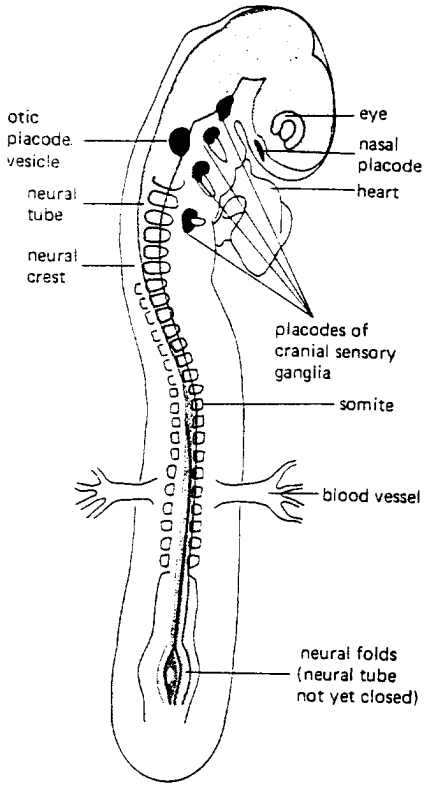


Figure 19–55 Diagram of an early (2½-day) chick embryo, showing the origins of the nervous system. The neural tube (*pale color*) has already closed, except at the tail end, and lies internally, beneath the ectoderm, of which it was originally a part (see Figure 16–13). The neural crest (*gray*) lies dorsally between the roof of the neural tube and the ectoderm. The placodes are thickenings of the surface ectoderm that will give rise to certain sensory cells and neurons. By this stage the otic placode has practically completed its invagination to form the otic vesicle—the rudiment of the inner ear and the source of the neurons in its associated ganglion; the nasal placode will invaginate to form the lining of the nose, including the olfactory neurons responsible for smell; the other cranial placodes will contribute cells to the cranial sensory ganglia, which provide most of the sensory innervation of the head and neck, apart from hearing, smell, and vision. Unlike other sensory cells, those of the eye originate as part of the neural tube.

Radial Glial Cells Form a Temporary Scaffold to Guide the Migrations of Newborn Neurons[50]

Before sending out its axon and dendrites, the immature neuron commonly migrates from its birthplace and settles in some other location. It is possible to trace these migrations with the help of ^{3}H-thymidine: precursor cells going through their final division in a certain location become labeled, and the labeled neurons

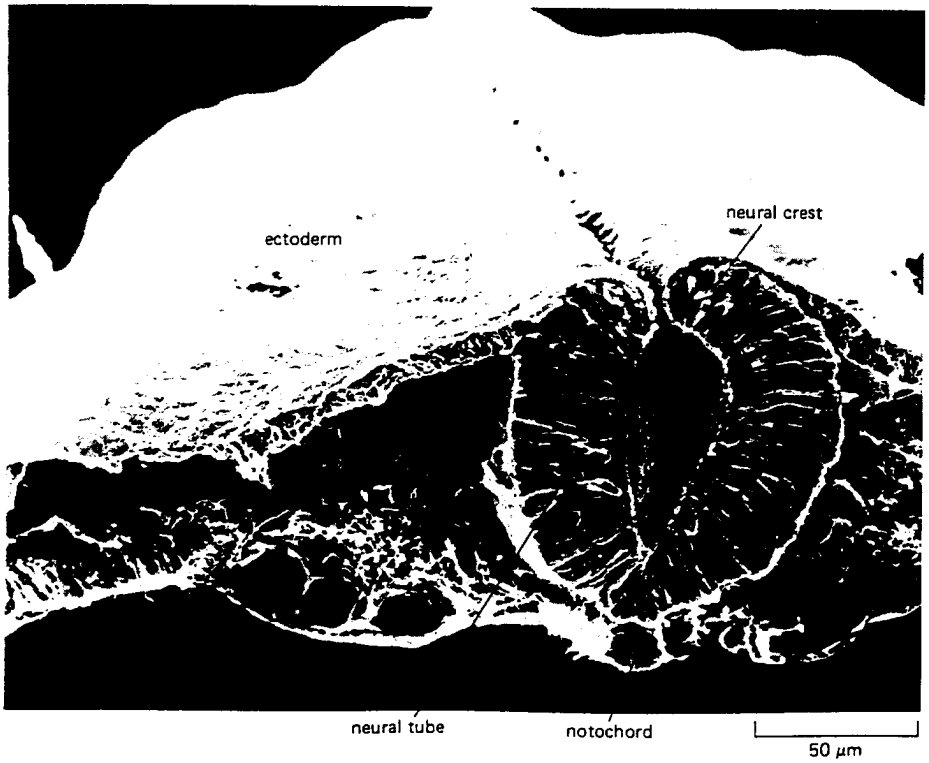
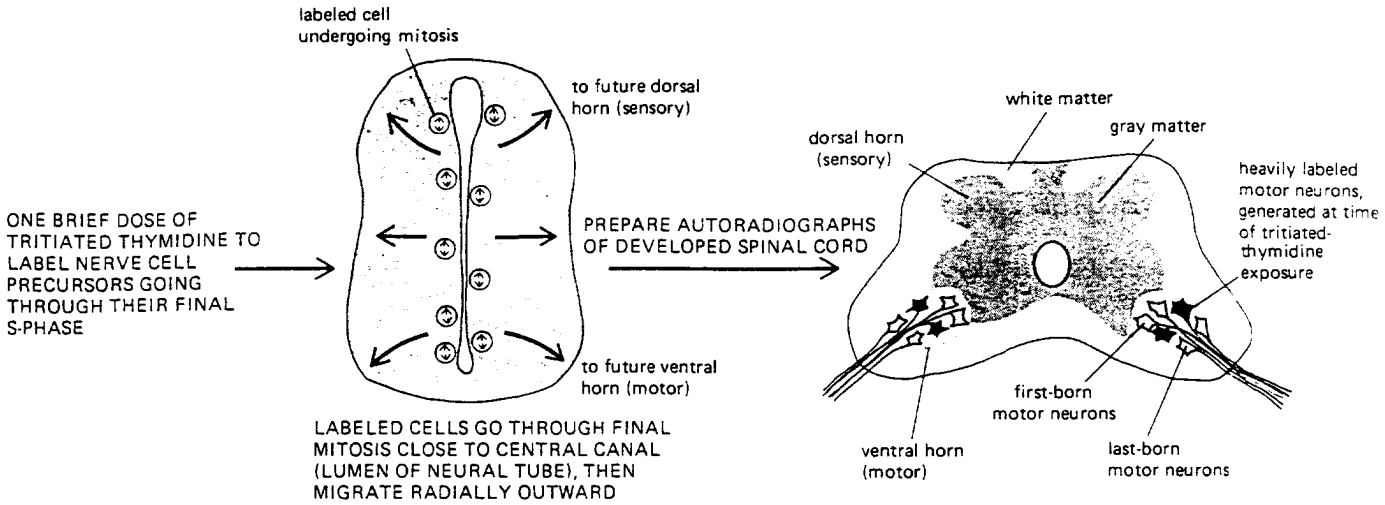


Figure 19–56 Scanning electron micrograph of a cross-section through the trunk of a 2-day-old chick embryo. The neural tube is about to close and pinch off from the ectoderm; at this stage it consists of an epithelium that is only one cell thick. (Courtesy of Jean-Paul Revel.)



descended from them can later be seen elsewhere. The motor neurons that will innervate the limbs, for example, undergo their final division close to the lumen of the neural tube and then move outward to settle in the *ventral horn* of the future spinal cord (Figure 19–57).

Nerve cell bodies are guided in their migrations by a specialized class of cells in the neural tube—the *radial glial cells* (Figure 19–58A). These can be considered as persisting cells of the original columnar epithelium of the neural tube that become extraordinarily stretched as the wall of the tube thickens: each cell extends from the inner to the outer surface of the tube, a distance that may be as much as 2 cm in the cerebral cortex of the developing brain of a primate. Three-dimensional reconstructions from serial electron microscope sections reveal that the immature migrating neurons cling closely to the radial glial cells and evidently crawl along them (Figure 19–58B and C).

The radial glial cells remain for many days—in some species for months—as a nondividing population, clearly distinct from the neurons and their precursors. Eventually, toward the end of development, they disappear from most regions of the brain and spinal cord; it has been suggested that many of them transform into astrocytes, but this has yet to be directly demonstrated. Thus the radial glial cells can be viewed as a developmental apparatus, necessary—like scaffolding—for the complex process of construction but not retained in most parts of the completed structure.

The Character and Future Connections of a Neuron Depend on Its Birthday[50,51]

There is a regular relationship between the birthday of a neuron in the vertebrate central nervous system and the site where it comes to rest (an echo, perhaps, of the rigid relationship between cell lineage and cell location that one sees in invertebrates such as nematodes—see p. 902). In the cerebral cortex, for example, the neurons are arranged in layers according to their birthdays through a migration in which the cells that are born later migrate outward past those born earlier. The cells in the successive layers of the cortex, as they mature, will come to differ in their shape, size, and patterns of connections with other cells. Thus small pyramidal cells, born late, lie in an outer layer and send their axons to other regions of the cerebral cortex, whereas large pyramidal and irregularly shaped cells, born earlier, lie in inner layers and send their axons to regions outside the cerebral cortex.

Is it the birthday or the final location that governs these differences? The *reeler* mouse provides an answer. In this mutant, named for its uncoordinated gait, there is a defect in the mechanism of nerve cell migration, so that the cells born late settle in an inner layer and the cells born early settle in an outer one.

Figure 19–57 The origins of motor neurons in the spinal cord, as revealed by autoradiography following a brief dose of tritiated thymidine given at an early stage. The diagrams represent cross-sections of the early neural tube (*on the left*) and of the relatively mature spinal cord that develops from it (*on the right*); radioactively labeled cells are shown in color. Cells that are heavily labeled at the late stage are those that were going through their final round of DNA synthesis in the early embryo when tritiated thymidine was given. For simplicity, only the motor neurons are indicated in the mature spinal cord, whose *gray matter* (*shaded*) also contains many other nerve cell bodies. The *white matter* (*unshaded*) consists chiefly of bundles of axons traveling along the length of the spinal cord and connecting one region of gray matter to another. (These regions appear white in the adult because they contain large amounts of myelin.) For an account of the production of glial cells during development, see Chapter 16, p. 909.

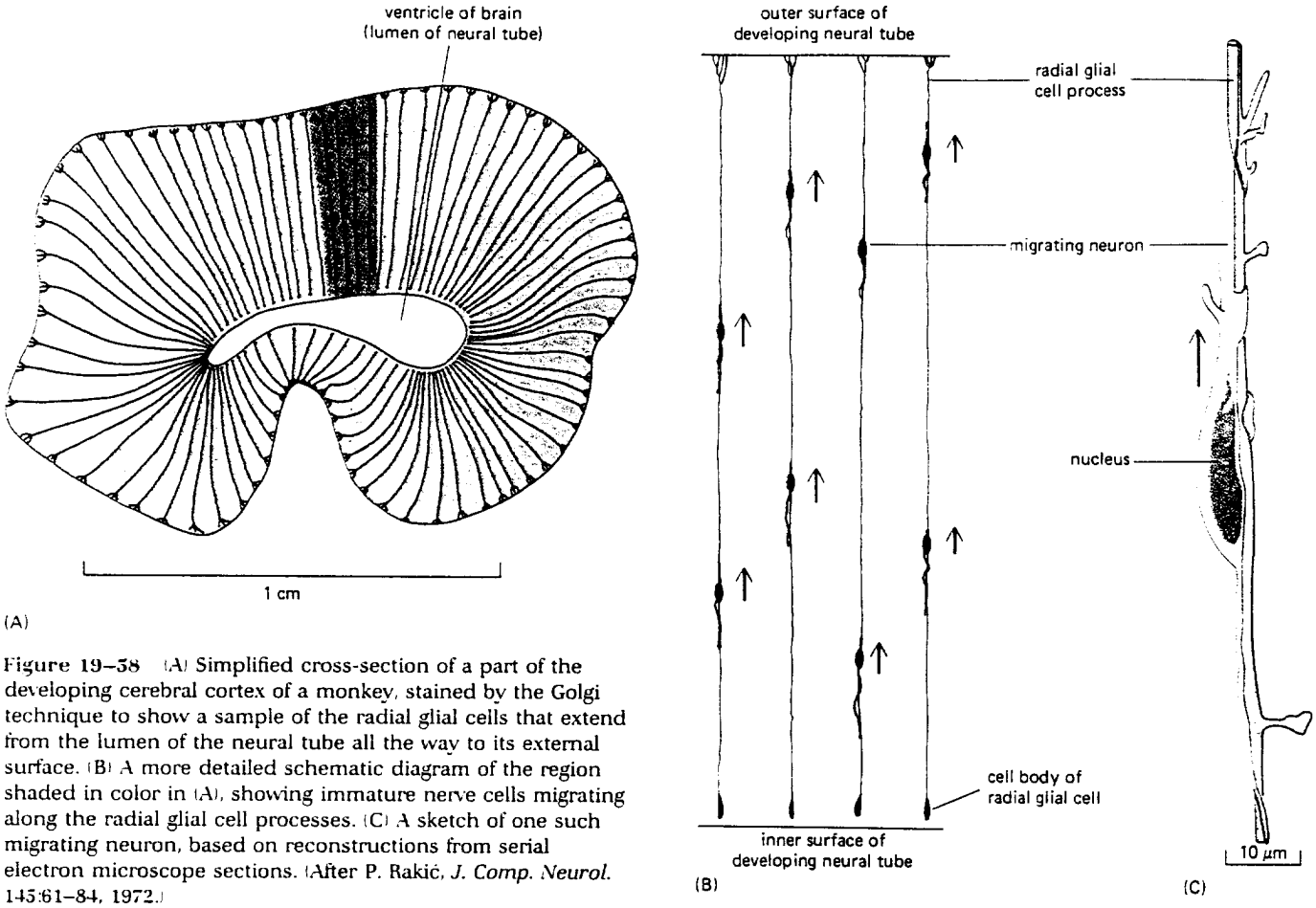


Figure 19–38 (A) Simplified cross-section of a part of the developing cerebral cortex of a monkey, stained by the Golgi technique to show a sample of the radial glial cells that extend from the lumen of the neural tube all the way to its external surface. (B) A more detailed schematic diagram of the region shaded in color in (A), showing immature nerve cells migrating along the radial glial cell processes. (C) A sketch of one such migrating neuron, based on reconstructions from serial electron microscope sections. (After P. Rakić, *J. Comp. Neurol.* 145:61–84, 1972.)

Despite this inversion of their normal positions, the cortical cells differentiate according to their birthdays: late-born cells become small pyramidal neurons, whereas early-born cells become large pyramidal or irregularly shaped neurons. In this system, therefore, it is the birth date rather than the final location that determines cell character (Figure 19–39). Indeed, it seems that in general the character of a neuron is dictated largely by its ancestry and the place and time of its birth.

The intrinsic character of the cell in turn governs the connections it will form—an important general principle about which more will be said later (see p. 1118). Thus the misplaced neurons in the *reeler* mouse, with relatively few errors, make the connections appropriate to their birthdays rather than to their

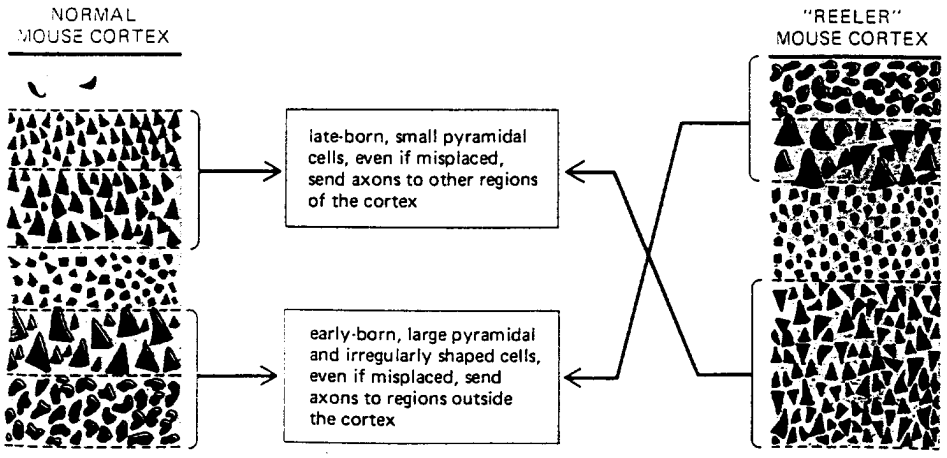


Figure 19–39 Comparison of the layering of neurons in the cortex of normal and *reeler* mice. In the *reeler* mutant an abnormality of cell migration causes an approximate inversion of the normal relationship between neuronal birthday and position. The misplaced neurons nevertheless differentiate according to their birthdays and make the connections appropriate to their birthdays.

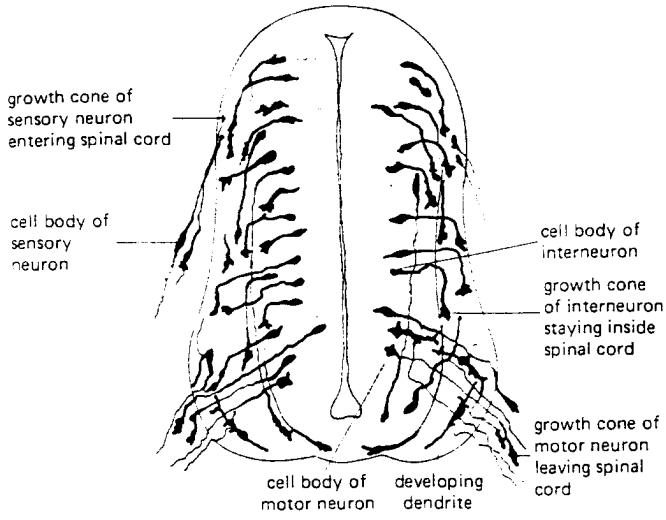


Figure 19–60 Growth cones in the developing spinal cord of a 3-day chick embryo as seen in a cross-section stained by the Golgi technique. Most of the neurons, apparently, have as yet only one elongated process—the future axon. The growth cones of the interneurons remain inside the spinal cord, those of the motor neurons emerge from it (to make their way toward muscles), and those of the sensory neurons grow into it from outside (where their cell bodies lie). Many of the cells in the more central regions of the embryonic spinal cord are still proliferating and have not yet begun to differentiate as neurons or glial cells. (From S. Ramón y Cajal, Histologie du Système Nerveux de l'Homme et des Vertébrés. Paris: Maloine, 1909–1911; reprinted. Madrid: C.S.I.C., 1972.)

positions: the small pyramidal cells send axons to other regions of cortex, while the large pyramidal and irregularly shaped cells send their axons to regions outside the cortex. To understand how such selective connections are made, we must first examine the machinery by which axons and dendrites are produced.

Each Axon or Dendrite Extends by Means of a Growth Cone at Its Tip[52]

As a rule the axon and then the dendrites begin to grow out from the nerve cell body soon after it has reached its final location. The sequence of events was originally observed in intact embryonic tissue by the method of Golgi staining (Figure 19–60). This technique and other methods developed subsequently reveal an irregular, spiky enlargement at the tip of each developing nerve cell process. This structure, which is called the **growth cone**, appears to be crawling through the surrounding tissue. It comprises both the engine that produces the movement and the steering apparatus that directs the tip of each process along the proper path.

Most of what we know about the properties of growth cones has come from studies in tissue or cell culture. Embryonic nerve cells in culture send out processes that are often hard to identify as axon or dendrite and are therefore given the noncommittal name of *neurite*. The growth cone at the end of each neurite moves forward at a speed of about 1 mm per day. It consists of a broad, flat expansion of the neurite, like the palm of a hand, with many long *microspikes* or *filopodia* extending from it like fingers (Figure 19–61). These are continually active: some

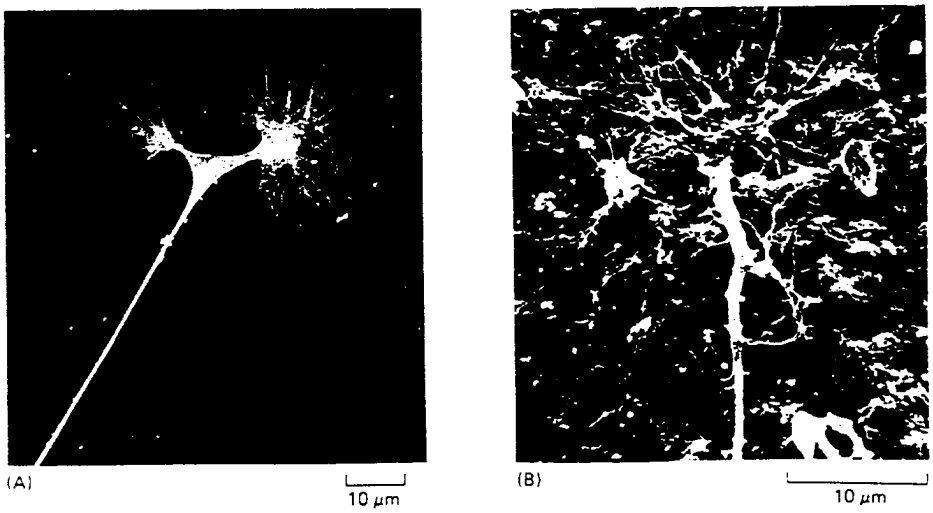


Figure 19–61 (A) Scanning electron micrograph of growth cones at the end of a neurite put out by a chick sympathetic neuron in culture. Here a previously single growth cone has recently divided in two. Note the many filopodia and the taut appearance of the neurite, due to tension generated by the forward movement of the growth cones, which are often the only firm points of attachment to the substratum. (B) Scanning electron micrograph of the growth cone of a sensory neuron *in vivo*, crawling over the inner surface of the epidermis of a *Xenopus* tadpole. (A, from D. Bray, in Cell Behaviour [R. Bellairs, A. Curtis, and G. Dunn, eds.]. Cambridge, U.K.: Cambridge University Press, 1982; B, from A. Roberts, *Brain Res.* 118:526–530, 1976.)

Figure 19–62 Electron micrograph of a section through a growth cone, showing the many membrane-bounded vesicles of varied shapes that it contains. These probably reflect a high rate of exocytosis and endocytosis at the growth cone. (Courtesy of Gerald Shaw.)

are retracting back into the growth cone while others are elongating, waving about, and touching down and adhering to the substratum. The "webs" or "veils" between the filopodia have a ruffling membrane (see p. 638). Electron microscopy shows that the microtubules and neurofilaments present in the neurite terminate in the growth cone and that the broad "palm" is full of flattened membranous sacs and vesicles, together with some mitochondria (Figure 19–62). Immediately beneath the ruffling margins of the growth cone, and filling the filopodia, is a dense meshwork of actin filaments. All these microscopic observations suggest that the growth cone is crawling forward in much the same way as the leading edge of a cell such as a neutrophil or fibroblast (see p. 670).

The Growth Cone Is a Site of Assembly of Materials for Growth[53]

Besides acting as the locomotive for neurite elongation, the growth cone serves as a moving construction site where new components of the growing cell are inserted (Figure 19–63). Because the ribosomes of a developing neuron are largely confined to the cell body, the cell body must be the site of synthesis of the proteins used to extend the neurite. New membrane also is synthesized in the cell body and carried outward in the form of small vesicles by fast axonal transport (see p. 1063) toward the growth cone. Upon arriving there the vesicles are inserted into the plasma membrane by exocytosis. Although some of the membrane is retrieved by endocytosis and recycled, there is a net addition of new membrane at the growth cone. Evidence for this mode of growth has come from watching the movement of small particles of dust clinging to the external surface of the developing neuron: on the growth cone itself these move rapidly, but when bound to the more proximal part of the neurite, they remain stationary relative to the cell body even as the neurite elongates. Microtubules are the "railroad tracks" for fast axonal transport (see p. 660), and membrane vesicles traveling along them are evidently delivered to the sites where the tracks end. Various experiments suggest that microtubules determine where growth cones can occur, and they may do so by controlling the delivery of membrane in this way.

At the same time the microtubules themselves must grow, as must the rest of the cytoskeleton. Tubulin is shipped out from the cell body by slow axonal

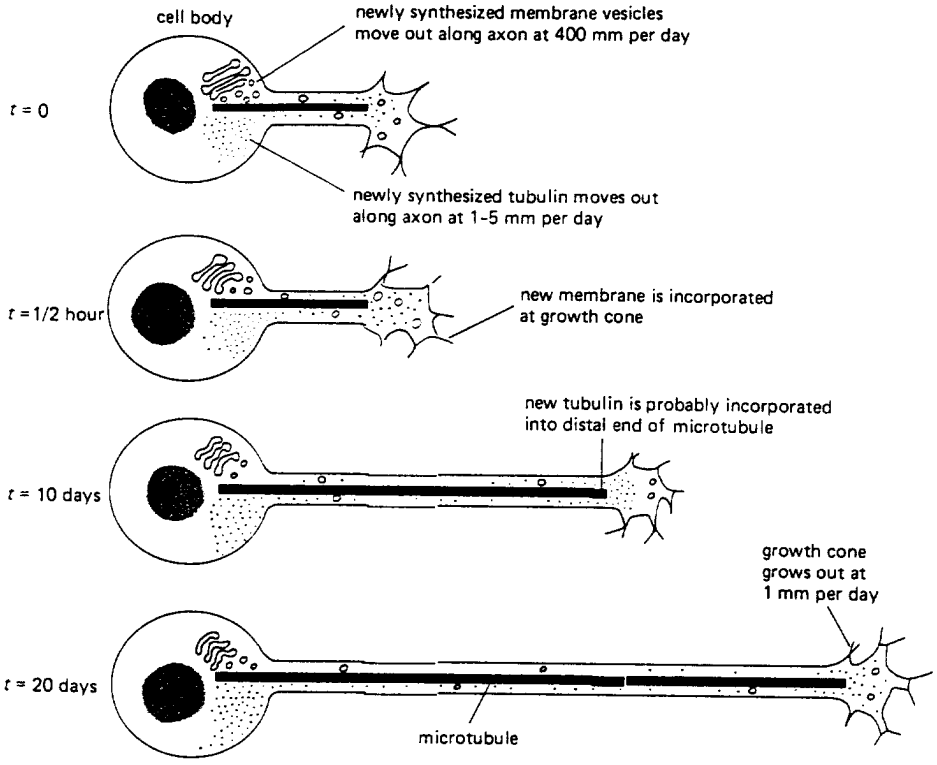


Figure 19–63 How materials for neurite growth are delivered from the nerve cell body to the growth cone and become incorporated there. For simplicity, only one microtubule is shown. Microtubules serve as the tracks for the fast axonal transport of membrane. Tubulin itself is carried outward from the cell body by slow axonal transport; the site of addition of the subunits for microtubule extension is still controversial.

transport (see p. 1063), but it is not certain where the subunits assemble into microtubules. It is known, however, that the microtubules are generally oriented with their "plus" or potentially fast-growing ends (see p. 653) at the growth cone and that the growth cone is uniquely sensitive to local application of drugs that interfere with microtubule assembly. This suggests that microtubules are extended by addition of subunits in the growth cone.

The Growth Cone Can Be Guided *in Vitro* by Selective Adhesion, Chemotaxis, and Electric Fields[32-34]

In the simplified conditions of cell culture, one can explore the mechanisms that might guide the movements of growth cones in the intact animal. Like a neutrophil or a fibroblast, a growth cone confronted with a choice of substrata shows a preference for the surface to which it can adhere most strongly (Figure 19–64). As it moves forward, it continually extends microspikes into the regions that lie ahead and on either side. Some of these projections may contact a less adhesive sub-

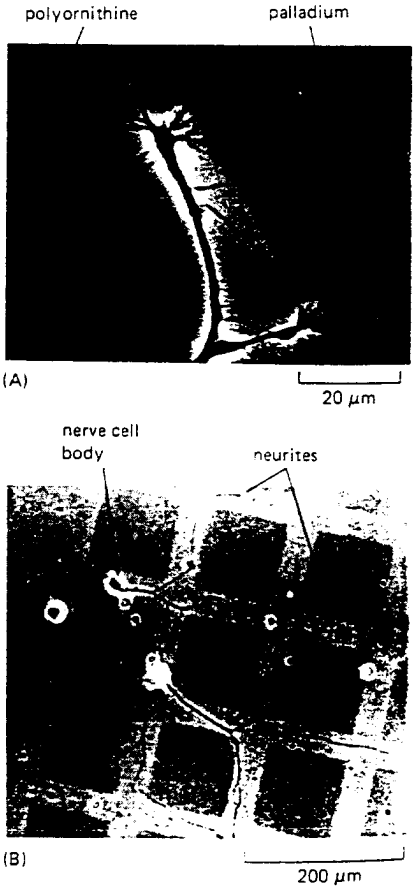


Figure 19–64 Growth cones can be offered a choice of substrata by culturing the cells in a dish whose surface has first been coated with polyornithine and then has had patches of palladium deposited on top of that. Because cell surfaces are largely negatively charged, they adhere strongly to polyornithine, which is positively charged. The growth cones advance along the lanes of polyornithine and stay off the palladium; given a choice between palladium and an even less adhesive substratum, they would travel on the palladium. (A) is a phase-contrast photograph at high magnification showing growth cones at the boundary between the two substrata. (B) is a phase-contrast photograph at lower magnification; the routes taken by the growth cones are recorded in the disposition of the neurites laid out behind them, which have remained adherent to the polyornithine. (From P. Letourneau, *Dev. Biol.* 44:92–101, 1975.)

stratum, in which case they are withdrawn relatively promptly; others contact a more adhesive substratum and persist longer. It appears as though the microspikes serve as feelers to test the surfaces in the neighborhood of the growth cone and to steer it along the most adhesive track.

The stickiness of the substratum is not the only guiding influence to which a growth cone responds, however. The shape of the terrain is also important: growth cones clinging to fibers, for example, will tend to follow their orientation—a phenomenon known as *contact guidance*. Substances dissolved in the extracellular fluid also seem to play a part. When the embryonic ganglion containing the sensory neurons that will innervate the jaw, for example, is placed in culture at a distance of about a millimeter from the embryonic jaw rudiment, neurites grow out predominantly in the direction of the jaw rudiment, suggesting that this target tissue secretes molecules that have a chemotactic effect. Electric fields also have a powerful orienting action, causing growth cones in nerve cell cultures to move toward the negative electrode; a field as small as 7 mV/mm is effective.

Although such culture experiments show what types of factors can guide growth cones, they do not indicate which influences are important in the developing animal. Are growth cones normally constrained to follow specific paths, or do they go exploring at random? Studies of the behavior of growth cones in their natural environments provide some answers.

The Growth Cone Pilots the Developing Neurite Along a Precisely Defined Path *in Vivo*: The Doctrine of Pathway Guidance[55]

In general, growth cones in living animals travel toward their targets along precisely specified routes. The detailed mechanism of such **pathway guidance** is difficult to study in most vertebrates; it is somewhat easier to examine in certain invertebrates, such as the grasshopper, where the innervation of the developing limb has been analyzed in detail (Figure 19–65).

The sensory neurons in a grasshopper limb originate from cells in the embryonic limb epithelium; the nerve cell bodies remain in the periphery and send axons back toward the central nervous system along precisely defined zigzag paths. These paths are initially followed in each limb bud by one or two "pioneer" axons, which can be selectively stained by antibodies or by injecting the fluorescent dye lucifer yellow into their cell bodies. At each turning in the path, the pioneer growth cone is seen to make contact with a specific "guidepost" or "stepping-stone" cell (Figure 19–66), with which it transiently forms gap junctions: if lucifer yellow has been injected into the axon, the stepping-stone cell will also become brightly stained. A pioneer growth cone extends microspikes that are as much as 50 or even 100 μm long—long enough to reach the next stepping-stone cell along the pathway. Microspikes that make contact with this particular cell are stabilized, while others are withdrawn. In this way the growth cone is guided step by step toward the central nervous system. If a specific stepping-stone cell is destroyed by a laser beam before the pioneer growth cone has reached it, the growth cone goes astray at that point in its pathway (see Figure 19–66). In those places where there are no stepping-stone cells along the normal pathway, the pioneer growth cone is guided instead by graded differences in the adhesivity of the basal lamina underlying the limb epithelium (see p. 819). In all its travels, a growth cone depends on specific molecules on its surface that enable it to adhere to the appropriate substratum. Some of these adhesion molecules are beginning to be identified.

Figure 19–65 Scanning electron micrograph of a grasshopper embryo seen from the ventral side, showing the leg buds (*arrow*). From D. Bentley and H. Keshishian, *Science* 218:1082–1088, 1982. Copyright 1982 by the AAAS.)

Growth Cones Use Specific Adhesion Molecules to Adhere to Cell Surfaces and to Extracellular Matrix[56]

Once the first neurites have pioneered a given route, others follow by contact guidance: growth cones cling to the existing neurites and advance along them. This is a universal tendency, in both vertebrates and invertebrates. Since there is a strong cohesion between neurite and neurite as well as between neurite and growth cone, the consequence is that nerve fibers in a mature animal are usually

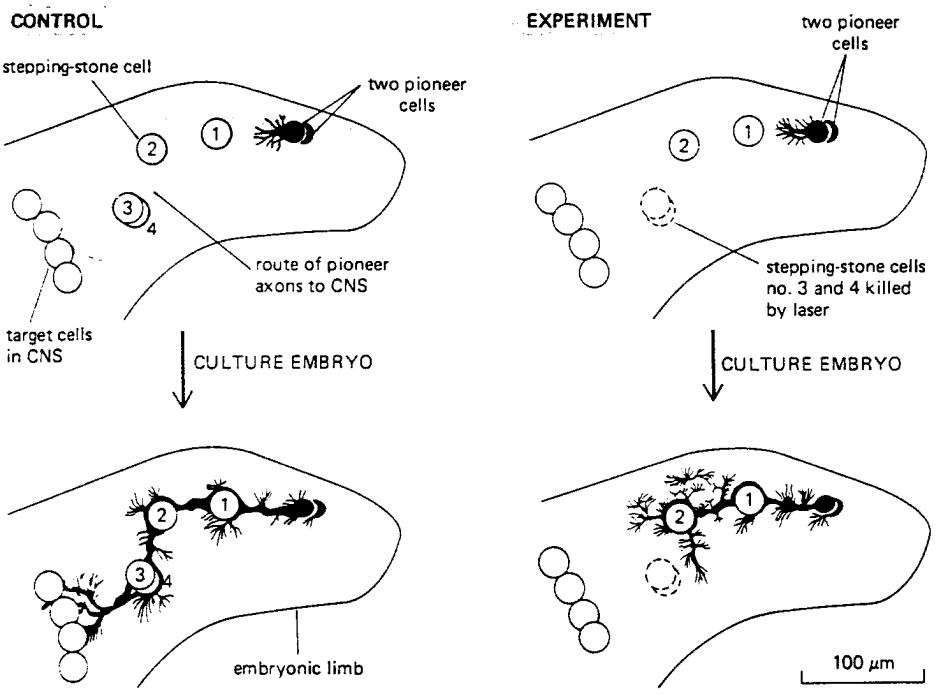


Figure 19–66 The guidance of pioneer axons by specific "stepping-stone" cells in the epithelium of an embryonic grasshopper limb. If stepping-stone cells are destroyed, the pioneer axons fail to advance along their normal path. The stepping-stone cells themselves are destined later to differentiate into neurons whose axons will travel to the CNS by following the routes taken by the pioneers. (After D. Bentley and M. Caudy, *Nature* 304:62–65, 1983.)

found grouped together in tight parallel bundles, or *fascicles*. The large peripheral nerves of a vertebrate, which are visible to the naked eye, originate in this way (although subsequently the axons become individually enveloped and insulated from one another by Schwann cells). Specific integral membrane glycoproteins that mediate this cohesion of neurites have been identified in vertebrates. Two widely studied examples are the so-called *neural cell-adhesion molecule*, or *N-CAM* (see p. 829), and the *L1 glycoprotein*, also known as the *neuron-glia cell-adhesion molecule*, or *Ng-CAM*. Antibodies directed against these glycoproteins, both of which are members of the immunoglobulin superfamily (see p. 830 and p. 822), inhibit the tendency of developing neurites to form fascicles and can be shown to disrupt the normal pattern of axon outgrowth (to varying degrees in different parts of the nervous system). N-CAM is found not only on neurons but also on glial cells and, during development and regeneration, on many nonneural cells, including muscle cells. In the latter case it may help to entice the growth cones of motor neurons into the regions where they are to form synapses.

Growth cones are guided not only by their adhesion to the surfaces of other cells but also by their adhesion to various components of the extracellular matrix. An important example is seen in nerve regeneration. When a peripheral nerve is severed, the axons will commonly regenerate by forming growth cones at their cut ends. These growth cones crawl down tunnels of basal lamina, secreted originally by the Schwann cells that ensheathed the axons in the degenerated distal portion of the nerve. There is evidence that *laminin* (see p. 819), or a complex of laminin and heparan sulfate proteoglycan, plays a crucial part in such guidance by binding to matrix receptors of the *integrin* family (see p. 822) in the growth cone membrane. Both materials promote neurite outgrowth *in vitro*, and antibodies that bind to the laminin/proteoglycan complex inhibit nerve regeneration in intact animals.

While proteins such as N-CAM, Ng-CAM, and laminin appear to play an important part in promoting cell adhesion and guiding growth cone migration, they do not provide an obvious answer to the crucial question of why some growth cones take one route while others take another.

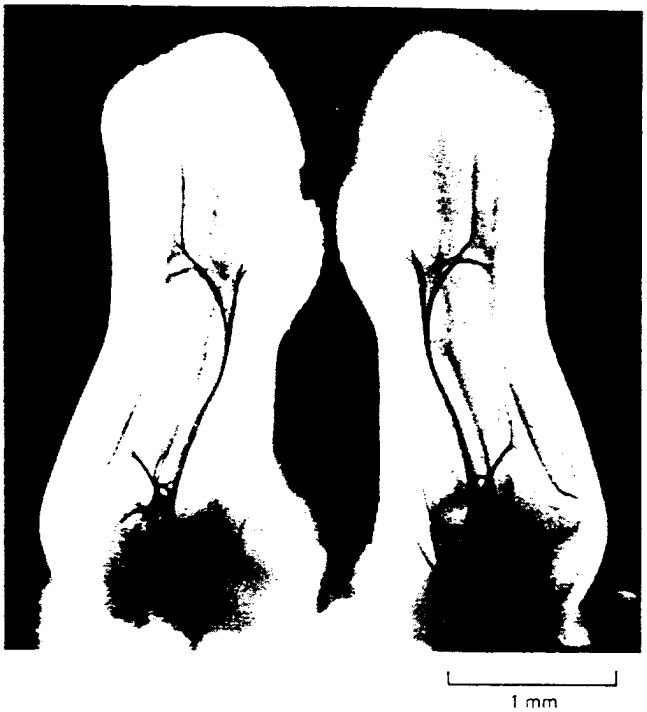


Figure 19–67 Light micrograph of the wings of an 8-day chick embryo, silver-stained to show the patterns of nerves. Compare the right wing with the left: the routes followed by the nerves are almost exactly symmetrical on the two sides of the body, implying the existence of a precise guidance system for nerve outgrowth. Individual growth cones face choices at points where the routes branch; the choices are made according to strict rules.

The Pattern of Nerve Connections Is Governed by the Nonequivalent Characters of Neurons: The Doctrine of Neuronal Specificity[37]

In the developing limb of a chick embryo, as in other systems, the growing axons are confined to a precisely defined set of paths (Figure 19–67). These paths branch along their course, with different branches leading to different targets. Thus individual growth cones face a series of choices at successive branch points. The choices are made according to precise rules, with the consequence that a highly ordered system of connections is set up between neurons and their target cells. The connections can be traced by the horseradish peroxidase technique (see p. 1064), which reveals that the cell bodies of the motor neurons innervating a given muscle lie clustered at a site in the spinal cord that is the same in every animal but different for different muscles (Figure 19–68).

How is the choice of pathway made? Are the growth cones somehow channeled to their different destinations as a direct consequence of their different starting positions, like drivers on a multilane highway where it is forbidden to change lanes? This possibility was tested by cutting out a short portion of the neural tube of an embryo at an early stage, before any axons had begun to grow out, and replacing it with its anteroposterior axis reversed. Thus the precursors

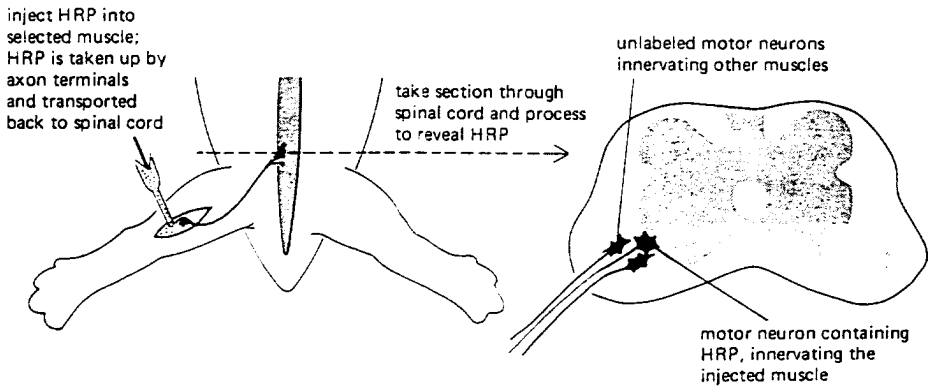


Figure 19–68 The use of retrograde transport of horseradish peroxidase (HRP) to identify which motor neurons in the spinal cord innervate a particular muscle. For clarity, the sizes of the motor neurons are exaggerated in the cross-section, and only three are shown; in reality each muscle is supplied by a nerve that contains the processes of many individual nerve cells (typically a few hundred) whose cell bodies are clustered together in the spinal cord.

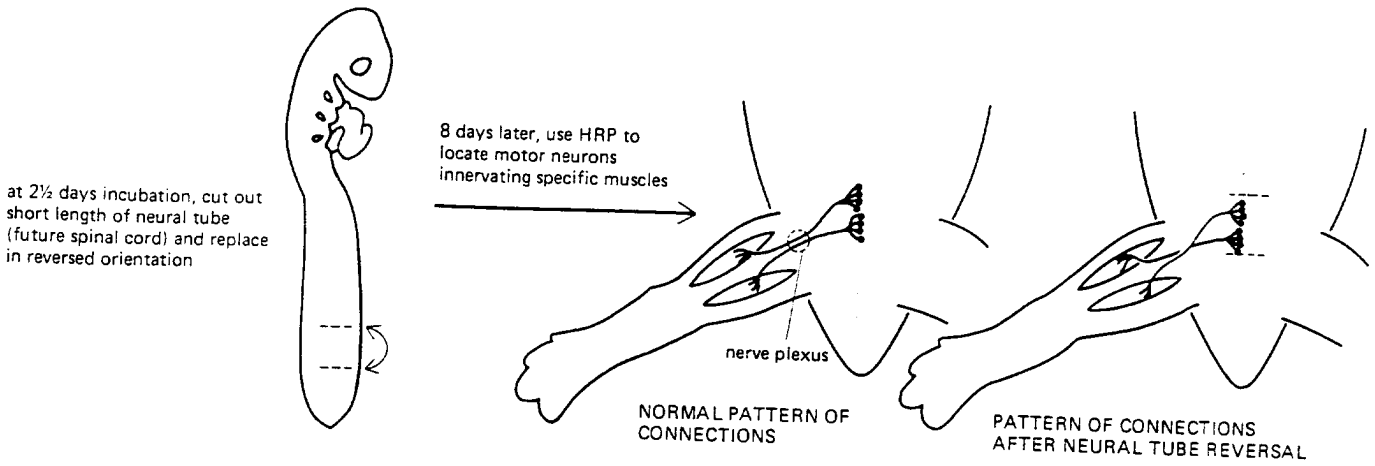


Figure 19–69 An experiment on a chick embryo demonstrating that motor neurons, even when misplaced, nevertheless send their axons to the muscles appropriate to their original positions in the embryonic spinal cord. Note that the axons from motor neurons at different levels along the spinal cord are funneled together into a *plexus* at the base of the limb and then separate again to innervate their separate targets. A growth cone passing through the region of the plexus has a large choice of targets available to it.

of the neurons originally destined to innervate muscle A were put in the place of those originally destined to innervate muscle B, and vice versa. Provided that the shift of position was not too extreme, the growth cones of the misplaced neurons traveled out by altered routes to connect with the muscle appropriate to their *original* position in the neural tube (Figure 19–69). This implies that the motor neurons destined to innervate different muscles are nonequivalent (see p. 915): like the neurons in the *reeler* mouse cerebral cortex, they are distinguished from one another not simply by their positions but by their intrinsic chemical characters. Such nonequivalence among neurons is commonly referred to as **neuronal specificity.** As discussed in Chapter 16, connective tissue cells in different regions of the limb bud are also nonequivalent and may provide the markers that enable a specific growth cone to select a specific branch of the highway system.

In the central nervous system there is also evidence, from both vertebrates and invertebrates, that particular subsets of neurons or glial cells display specific labels that are recognized by other neurons and so help to guide the formation of selective nerve connections. But so far little is known about the molecules involved in either the central or peripheral nervous system.

Target Tissues Release Neurotrophic Factors That Control Nerve Cell Growth and Survival[58]

During the initial part of its journey, the growth cone is generally guided by the tissues through which it is passing; as it nears its destination, it comes under the influence of the target itself, often even before cell-to-cell contact has been made, through the action of *neurotrophic factors* that emanate from the target cells. As we have seen in the example of the trigeminal ganglion innervating the embryonic jaw, such factors may serve as chemotactic attractants for growth cones. More fundamentally, however, they control the *survival* of growth cones, of axon branches, and of entire neurons.

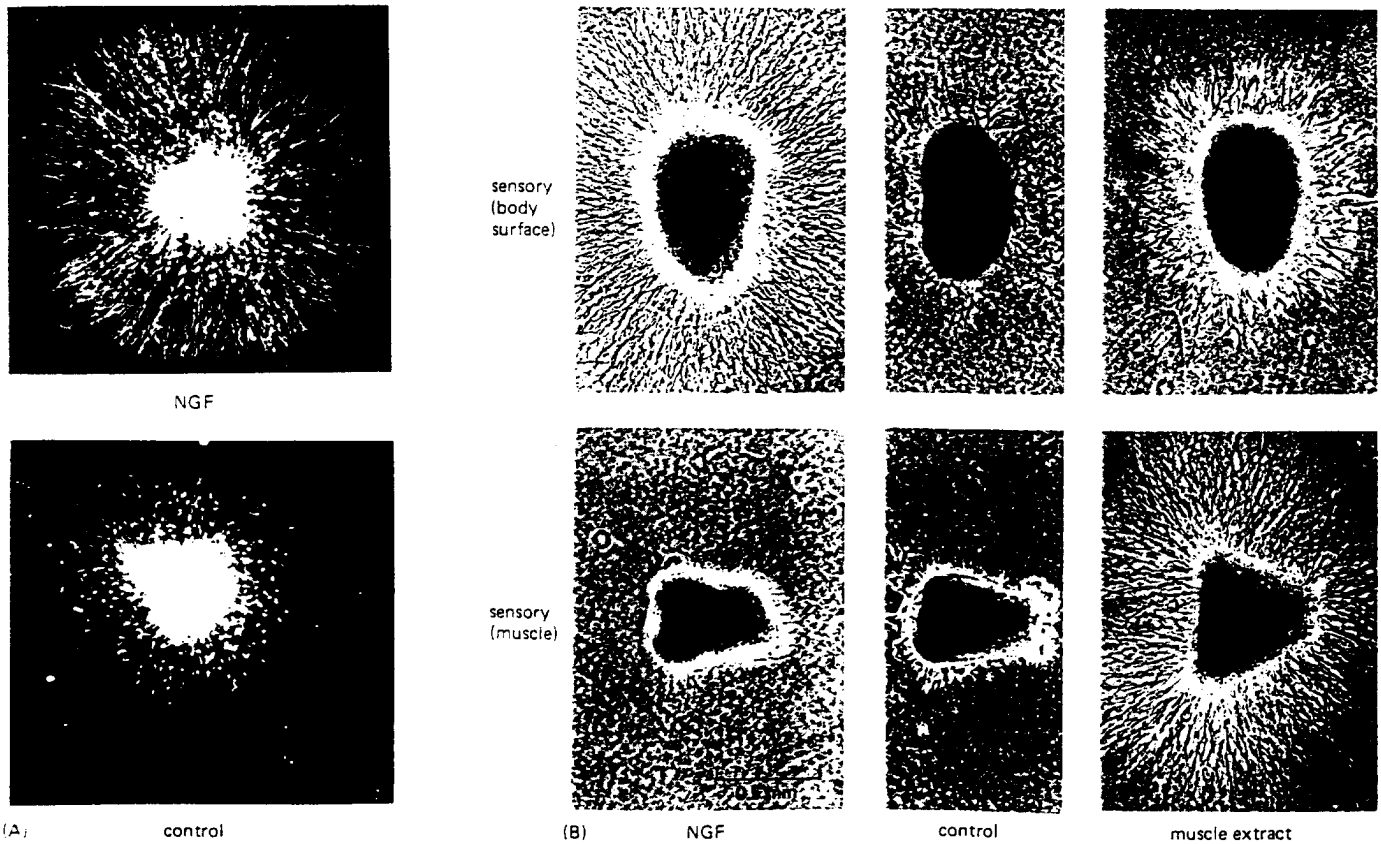
The first neurotrophic factor to be identified, and by far the best characterized, is known simply as **nerve growth factor,** or **NGF.** It was discovered by accident in the course of experiments in which foreign tissues and tumors were transplanted into chick embryos. Transplants of one particular tumor became exceptionally densely innervated and caused a striking enlargement of certain groups of peripheral neurons in the vicinity of the graft. Just two classes of neurons were affected: *sensory neurons* and *sympathetic neurons* (a subclass of the peripheral autonomic neurons that control contractions of smooth muscle and secretion from exocrine glands). Soluble extracts from the tumor also stimulated neurite outgrowth from these neurons in culture. Further work showed that one particular tissue, the salivary gland of the male mouse, produced the same factor in enormous quantities. This quirk of nature is still puzzling, since bulk production of NGF by male mouse salivary gland cells bears no obvious relation to the major functions of the factor, but it made it possible to purify NGF in large enough quantities to discover its chemistry and explore its functions. The activity was

found to lie in a protein dimer composed of two identical polypeptide chains 118 amino acids long. Once NGF had been purified, it was possible to raise antibodies that would block its activity. If anti-NGF antibodies are administered to mice while the nervous system is still developing, most sympathetic neurons and some sensory neurons die.

Likewise in culture, sympathetic neurons and some sensory neurons die in the absence of NGF; if NGF is present, they survive and send out neurites (Figure 19–70). Neuronal survival and neurite production represent two distinct effects of NGF. This has been neatly shown by placing the cells in the central compartment of a three-chambered culture dish whose two side compartments are separated from the central one by barriers that prevent mixing of the media in the three compartments but allow neurites to pass (Figure 19–71). If NGF is present in all three compartments, neurites extend into all three. If NGF is absent from one of the side compartments, no neurites will extend into it; and if all NGF is removed from one of the side compartments when neurites are already there, they will wither and retract as far as the barrier. The cells in the central compartment will not survive or send out neurites unless NGF is present there initially; but if NGF is provided initially in all three compartments and then withdrawn from the central one after the neurites have extended into the side compartments, the cells survive and neurite outgrowth continues in the side compartments.

Thus NGF acts both locally at the periphery of the cell, maintaining and stimulating those neurites and growth cones that are exposed to it, and centrally as a survival factor for the cell as a whole. The local effect on growth cones is direct, rapid, and independent of communication with the cell body; when medium devoid of NGF is substituted for medium containing NGF, the deprived growth cones halt their movements within a minute or two. Besides responding directly to NGF, the growth cones of NGF-sensitive cells take up NGF by endocytosis into vesicles, which are carried by retrograde transport back to the cell body, where the NGF (or some intracellular messenger) presumably exerts its effect on cell survival.

Figure 19–70 (A) Dark-field photomicrographs of a sympathetic ganglion cultured for 48 hours with (*above*) or without (*below*) NGF. Neurites grow out from the sympathetic neurons only if NGF is present in the medium. Each culture also contains Schwann cells that have migrated out of the ganglion; these are not affected by NGF. (B) Phase-contrast photomicrographs showing the behavior of sensory neurons cultured for 24 hours either with NGF (*left*), or in control medium without NGF (*center*), or in medium containing an extract of skeletal muscle (*right*). The sensory neurons in the upper row of pictures are from a group that normally innervates chiefly the body surface (but also sends a few fibers to skeletal muscle and elsewhere); most of these cells respond to NGF like the sympathetic neurons in (A). The sensory neurons in the lower row are from a group that normally innervates skeletal muscle (to provide sensory feedback); these are not responsive to NGF but respond strongly to an extract prepared from skeletal muscle. (A, courtesy of Naomi Kleitman; B, from A.M. Davies, *Dev. Biol.* 115:56–67, 1986.)



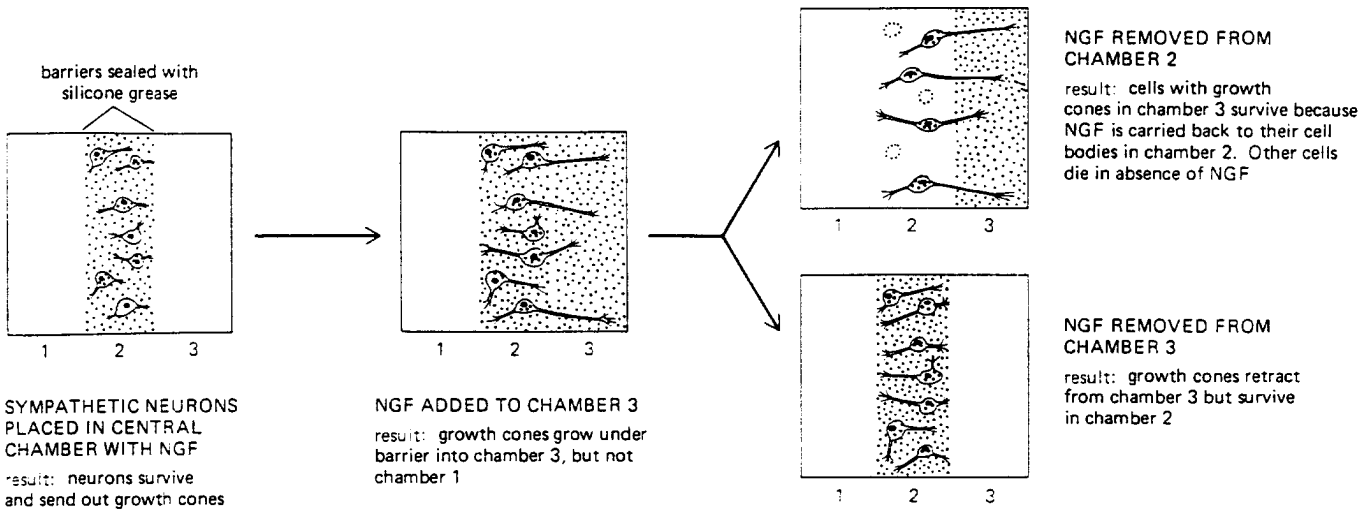


Figure 19–71 Schematic diagram of tissue-culture experiments showing that sympathetic neurons, besides requiring NGF in order to survive, can send out growth cones and maintain neurites only in regions where NGF is present. Note that cell survival does not require NGF in the neighborhood of the cell body as long as the cell has a neurite extending into a region that contains NGF.

Cell Death Adjusts the Number of Surviving Neurons According to the Amount of Target Tissue[39]

In a vertebrate the spinal sensory ganglia are generated in a regular segmental pattern corresponding to the series of vertebrae. Each ganglion consists of a cluster of sensory neurons derived from the neural crest, each of which sends one neurite outward to the periphery of the body and one neurite inward to the spinal cord. The rudiments of the ganglia at first are all similar in size, but in the mature animal the ganglia that innervate the body segments that have limbs attached are much bigger and contain more neurons than the ganglia that innervate the thoracic segments, where there are no limbs (Figure 19–72). This disparity is brought about chiefly by cell death: a larger proportion of the ganglion neurons at the thoracic levels die. If a limb bud is cut off at an early stage, the adjacent ganglia are reduced to the size of thoracic ganglia; conversely, if an extra limb bud is grafted onto the embryonic thorax, it becomes innervated and an abnormally large number of ganglion neurons survive at that level. The control of the survival of ganglion neurons according to the quantity of target tissue is thought to be mediated in large part by NGF secreted by the target. If extra NGF is injected into the embryo during the appropriate period of development, a large proportion of the thoracic ganglion neurons that would ordinarily die are saved, as are ganglion neurons adjacent to an amputated limb bud.

It may seem wasteful to generate excess neurons and then adjust the numbers by cell death according to the amount of the target tissue. Yet this strategy is

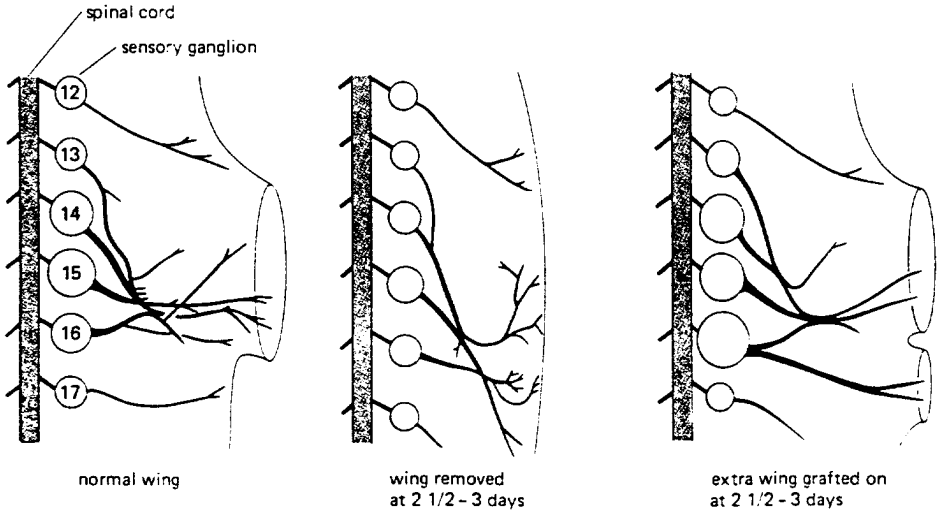


Figure 19–72 The control of nerve cell survival in the spinal sensory ganglia of a chick embryo. The spinal ganglia (*colored*) and nerves (*black*) are sketched from embryos 8–9 days old. The size of each ganglion reflects the number of neurons that have survived, which in turn is governed by the quantity of tissue that is available for the ganglion to innervate. (After V. Hamburger, *J. Exp. Zool.* 68:449–494, 1934 and *J. Exp. Zool.* 80:347–389, 1939.)

commonplace throughout the nervous system in vertebrates—both in sensory and in motor cell groups, and in the central nervous system as well as in the periphery. About 50% of all the motor neurons that send axons to skeletal muscles, for example, die in the course of embryonic development within a few days after making contact with their target muscles. A variety of target-cell-derived trophic factors analogous to NGF appear to regulate neuronal survival in these systems. The strategy has several major advantages. First, it provides an automatic device to correct for variations in the relative sizes of different parts of the body. Second, it facilitates evolution: if mutations alter the size of one part of the body, the numbers of neurons connecting with it will be automatically adjusted without other mutations being needed to alter the programs that generate the neurons. Finally, a small number of neurotrophic factors such as NGF can regulate the individual quantitative matching of a large number of paired targets and sources of innervation, even if the system of connections is very intricate. Through axonal transport, the factor produced by a given target is delivered selectively to the neurons that innervate that target and not to other neurons that may have similarly located cell bodies and similar receptors but send their axons elsewhere. Thus cell death regulated by neurotrophic growth factors can help to set up precise and detailed correspondences between the numbers of cells in different parts of the nervous system.

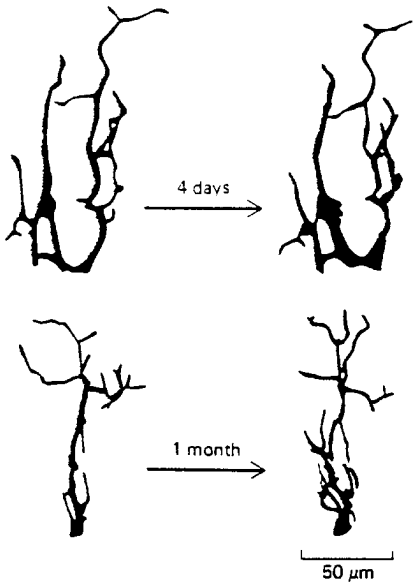


Figure 19–73 Remodeling of the dendrites of neurons in the superior cervical (autonomic) ganglion of a mouse. The ganglion is exposed by careful dissection in the anesthetized animal, and a fluorescent dye is microinjected into one of the nerve cell bodies so as to make its dendrites visible. The wound is then stitched up, and after an interval of some days or weeks, the dissection is repeated and the dye is again injected into the same neuron. The upper and lower pictures show two neurons left for different periods of time. The longer the interval between the first and second injections, the greater is the change seen in the pattern of the cell's dendrites. (Reprinted by permission from D. Purves and R.D. Hadley, *Nature* 315:404–406, 1985. Copyright © 1985 Macmillan Journals Limited.)

Neural Connections Are Made and Broken Throughout Life[60]

Even in normal, undamaged nervous tissue, there is evidence that dendrites and axon terminals continually retract and regrow. In a mature autonomic ganglion of a mouse, for example, identified individual neurons can be seen to withdraw some dendrite branches and to sprout others over the course of a month (Figure 19–73). Such remodeling occurs slowly and on a limited scale in normal circumstances, but it is called into play in a striking way when a proportion of the target cells in a tissue are deprived of innervation. In the case of a skeletal muscle, this can be done by cutting some but not all of the axons that innervate it. The denervated muscle fibers then apparently secrete a diffusible "sprouting factor," which stimulates profuse sprouting of new growth cones from the surviving axon terminals on neighboring innervated muscle fibers (Figure 19–74). The sprouting factors produced by denervated skeletal muscle have not yet been identified, but for smooth muscle, NGF has been shown to play an exactly analogous role. Denervation leads to an increase in the amount of NGF available from the smooth muscle (at least in part because there are fewer nerve terminals transporting the NGF away), and the excess NGF stimulates growth of axons toward the muscle so as to restore its innervation.

Evidently NGF acts in the intact animal just as it does in a culture dish, both as a survival factor, to determine whether cells shall live or die, and as a local stimulus for growth cone activity, to control the sprouting of axon terminals. The first action is prominent during development; the second is important throughout life. But both actions contribute to the same end: they adjust the supply of innervation according to the requirements of the target. Evidence for the existence of other neurotrophic growth factors, performing similar functions in relation to

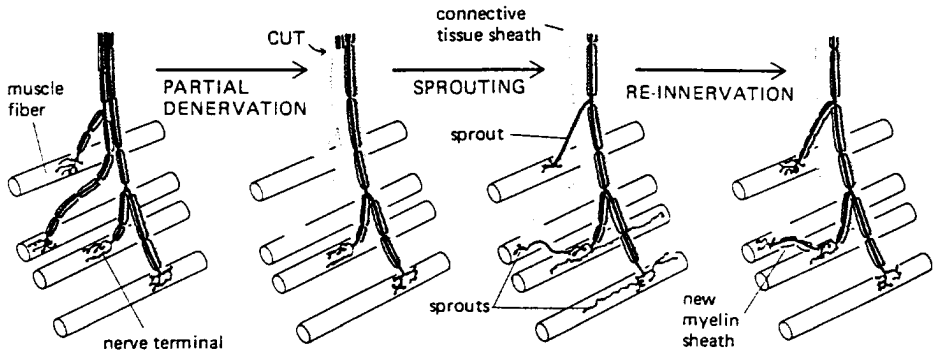


Figure 19–74 By cutting some of the axons that innervate a skeletal muscle, it is possible to deprive some of the muscle cells of innervation while leaving other, adjacent muscle cells with their innervation intact. The cut axons degenerate; the surviving axons, although they have suffered no direct disturbance, are provoked to sprout where they lie close to denervated muscle fibers. Within a month or two, those sprouts that have found their way to vacated sites on the denervated muscle fibers have formed stable synapses on them, restoring their innervation, and the other sprouts have been retracted. Such phenomena suggest that denervated muscle fibers release a diffusible "sprouting factor." (Reproduced with permission from M.C. Brown, R.L. Holland, and W.G. Hopkins, *Ann. Rev. Neurosci.* 4:17–42, 1981. © 1981 by Annual Reviews Inc.)

other classes of nerve cells, is rapidly accumulating (see Figure 19–70). In the next section we shall see that such factors may well mediate some of the important effects of electrical activity on the developing pattern of nerve connections.

Summary

The development of a nervous system can be conveniently divided into three phases, which partly overlap. In the first phase, the neurons are generated by finite programs of cell proliferation and the newborn cells migrate from their birthplaces to settle in an orderly fashion in other locations. In the second phase, axons and dendrites extend from the cell bodies by means of growth cones. The growth cones travel along precisely specified paths, guided for the most part by contact interactions with other cell surfaces or with components of the extracellular matrix. Neurons destined to connect with different targets behave as though they have intrinsically different characters (neuronal specificity), expressed in distinctive surface characteristics that enable their growth cones to select different paths. At the end of its path, a growth cone encounters the target cell with which it is to synapse and falls under the influence of target-derived neurotrophic factors. These govern the sprouting and movement of the growth cone in the neighborhood of the target and also control the survival of the neuron from which the growth cone originates. In these two ways neurotrophic factors, such as nerve growth factor (NGF), regulate the density of innervation of target tissues. In the third phase of neural development, to be discussed in the next section, synapses are formed and the pattern of connections is adjusted by mechanisms that depend on electrical activity.

Synapse Formation and Elimination[61]

The encounter of a growth cone with its target cell is a crucial moment in neuronal development: both the growth cone and the target cell undergo a transformation, and synaptic communication can begin. But the developmental process does not end there: many of the synapses formed initially are later eliminated, and new synapses form elsewhere on the same target cell. This local remodeling of the pattern of synaptic connections provides an opportunity for error correction and fine tuning: first the system is roughed out through pathway guidance as growth cones migrate along specific routes to the vicinity of their target cells: then tentative synaptic connections are made, allowing pre- and postsynaptic cells to communicate: and lastly, the initial connections are revised and adjusted by mechanisms that involve both neurotrophic factors and electrical signals in the form of action potentials and synaptic transmission. Thus external stimuli that excite electrical activity in the nervous system can influence the development of the pattern of nerve connections.

In this section we examine the molecular events of synapse formation, the rules that determine whether synapses are to be formed or eliminated, and the part played by electrical activity in controlling these processes. We begin with synapses between motor neurons and skeletal muscle cells because most is known about them.

Synaptic Contact Induces Specializations for Signaling in Both the Growing Axon and the Target Cell[62]

The early events of neuromuscular synapse formation can be observed best in culture. Here it can be seen that much of the molecular machinery for synaptic transmission is present even before a growth cone has made contact with a muscle cell. As the growth cone crawls forward, it releases tiny pulses of acetylcholine in response to electrical excitation of the nerve cell body (Figure 19–75). Its membrane already contains voltage-gated Ca^{2+} channels to couple excitation to secretion, and these channels also serve to propagate action potentials along the embryonic neurite (which at first lacks Na^+ channels). Before the muscle cell is innervated,

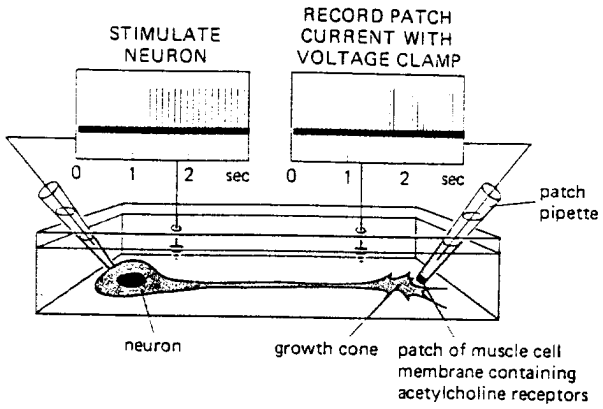


Figure 19–75 An experiment showing that a growing motor neuron in culture discharges pulses of acetylcholine from its growth cone in response to stimulation of the cell body. The minute quantities of acetylcholine released are detected by measuring their effect on the current through a detached patch of muscle cell membrane, rich in acetylcholine receptors, covering the mouth of a patch pipette. The release of acetylcholine from the growth cone is much less plentiful and less reliable than the release from a mature synaptic terminal.

it already has acetylcholine receptors (of an embryonic type) and responds to acetylcholine by depolarization and contraction.

A relatively inefficient form of synaptic transmission can be demonstrated within minutes after the first contact of growth cone with muscle cell. To form a mature synapse, however, both the growth cone and the target cell must develop structural and biochemical specializations—a process that typically takes several days. The growth cone halts its movements, accumulates synaptic vesicles in its interior, and constructs "active zones" for rapid and localized release of acetylcholine (see p. 1078). The muscle cell concentrates its acetylcholine receptors at the synapse and removes them from other regions of its plasma membrane. How is this rearrangement of neurotransmitter receptors achieved? The question is as relevant to neurons as it is to muscle cells, since neurons also, as we have seen, must be able to concentrate particular classes of receptors and ion channels in specific regions of their plasma membranes in order to function in signaling and computation.

Acetylcholine Receptors Diffuse in the Muscle Cell Membrane and Become Tethered at the Forming Synapse[63]

In an adult muscle cell the concentration of acetylcholine receptors at the synapse is more than a thousand times greater than elsewhere in the plasma membrane. Fluorescence bleaching experiments (see p. 295) show that the receptors at the synapse are tethered in place and not free to diffuse in the plane of the membrane. In the uninnervated embryonic muscle cell, by contrast, the receptors are initially spread out over the whole surface and diffuse more freely. When a motor axon makes contact with the muscle cell, these acetylcholine receptors begin to aggregate beneath the axon terminal; moreover, newly synthesized acetylcholine receptors are now preferentially inserted at the developing synapse (Figure 19–76). The receptors become locked into place—perhaps by adhering to one another, perhaps by anchoring to the underlying cytoskeleton or to the overlying extracellular matrix. Some important clues as to how the axon terminal marks out the site of the synapse come from studies of the regeneration of neuromuscular connections.

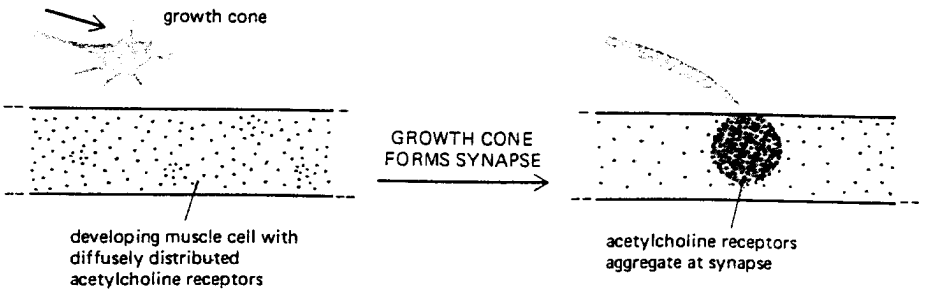


Figure 19–76 The aggregation of acetylcholine receptors in the membrane of a developing muscle cell at the site where a motor axon terminal makes contact to form a synapse. The aggregation depends partly on diffusion of receptors toward that site from neighboring regions of the muscle cell membrane and partly on the insertion of newly synthesized receptors into the membrane there. The aggregation at the synapse seems to be independent of neurotransmitter release from the nerve terminal, for it occurs even in the presence of agents that block action potentials in the nerve cells and even when the extracellular medium contains high concentrations of α-bungarotoxin, a poison from snake venom that binds to the acetylcholine receptors and blocks their interaction with acetylcholine. The receptors aggregated at the synapse are somehow trapped there; they have a much slower rate of turnover than receptors elsewhere in the membrane, surviving for 5 days or more before they are degraded and replaced.

The Site of a Neuromuscular Synapse Is Marked by a Persistent Specialization of the Basal Lamina[64]

Each muscle cell in an adult muscle is enveloped in a basal lamina (see Figures 19–16 and 19–18A). If the muscle is badly damaged, it degenerates and dies, and macrophages move in to clear away the debris. The basal lamina, however, remains and provides a scaffolding within which new muscle fibers can be constructed from surviving stem cells (see p. 986). Moreover, even if a muscle fiber and its axon terminal have both been destroyed, the site of the old neuromuscular junction is still recognizable from the corrugated appearance of the basal lamina there. This *junctional basal lamina* has a specialized chemical character, and it is possible to make antibodies that bind selectively to it. Remarkably, it is the junctional basal lamina that controls the localization of the other components of the synapse.

The importance of the basal lamina at the neuromuscular junction has been demonstrated in a series of experiments on amphibians. By destroying both the nerve and the muscle cells, leaving only empty shells of basal lamina, it is easily shown that the acetylcholinesterase molecules that hydrolyze the acetylcholine released by the axon terminal are tethered in the junctional basal lamina. Moreover, the junctional basal lamina holds the nerve terminal in place: if the muscle cell but not the nerve is destroyed, the nerve terminal remains attached to the basal lamina for many days. On the other hand, removing the basal lamina with collagenase causes the nerve terminal to detach even if the muscle cell is still present.

Indeed, it appears that the basal lamina by itself can guide the regeneration of an axon terminal. This has been demonstrated by destroying both the muscle and the nerve and then allowing the nerve to regenerate while the basal lamina remains empty: a regenerating axon regularly seeks out the original synaptic site and differentiates there into a synaptic ending. The junctional basal lamina also controls the localization of the acetylcholine receptors at the junctional region. If the muscle and the nerve are both destroyed, but now the muscle is allowed to regenerate while the nerve is prevented from doing so, the acetylcholine receptors synthesized by the regenerated muscle localize predominantly in the region of the old junctions, even though the nerve is absent (Figure 19–77). As might be expected, extracts prepared from junctional basal lamina contain a protein, called *agrin*, that promotes receptor clustering in cultured muscle cells.

Evidently, where an axon terminal contacts a muscle cell, it deposits, or causes the muscle cell to deposit, specialized macromolecules, including agrin, that sta-

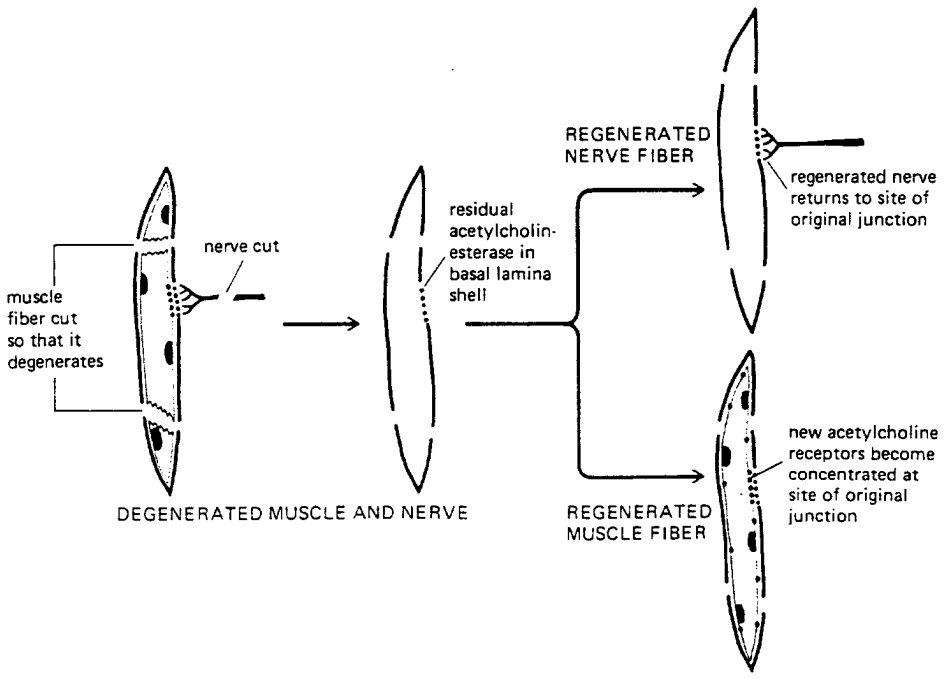


Figure 19–77 Experiment showing that the specialized character of the basal lamina at the neuromuscular junction controls the localization of the other components of the synapse.

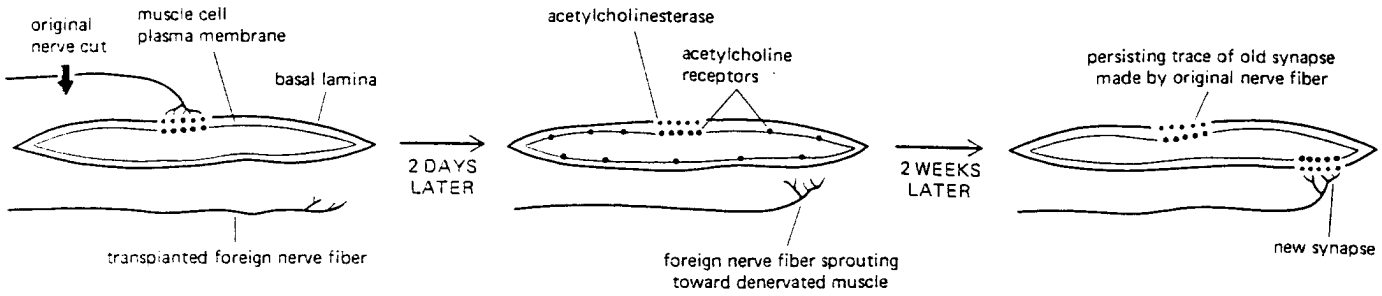


Figure 19–78 An experiment on the soleus muscle of a rat, showing how a muscle cell becomes receptive to synapse formation by a transplanted foreign nerve fiber when (and only when) the original nerve is cut. Note that the distribution of acetylcholine receptors in the muscle cell membrane changes as a result of denervation: new extrajunctional receptors become distributed over the cell's entire surface, although the concentration of receptors remains especially high at the site of the old neuromuscular junction. The electrical excitability of the membrane also changes following denervation, through the appearance in the membrane of a new class of voltage-gated channels that are relatively resistant to tetrodotoxin.

bilize the synaptic connection. The role of the basal lamina, however, is only part of the story of neuromuscular synapse formation. For not all encounters between axon and muscle cell lead to formation of a synapse, nor are all newly formed synapses absolutely stable.

The Receptivity of a Muscle Cell Is Controlled by Its Electrical Activity[63,65]

If a nerve in a rat is cut and the cut end is deflected so that it lies over an adjacent normal healthy muscle, the severed axons will regenerate and grow over the surface of this muscle; but as long as the muscle's own nerve supply is intact, these foreign axons do not make contact with the individual muscle cells or form synapses on them. If now the normal nerve to the muscle is cut, striking changes occur. Within a few days the muscle cells alter their membrane properties and metabolism: in particular, large quantities of new acetylcholine receptors are synthesized and inserted in the membrane of each muscle cell over its whole surface, making it supersensitive to acetylcholine. At the same time the muscle cells become receptive to new synapse formation by the foreign axons that had grown over the surface of the muscle. Although the axons show a preference for sites where synapses existed before, they can also make synapses at new locations on the muscle cells. Once synapses have formed, the diffuse distribution of acetylcholine receptors disappears, as in embryonic development, leaving a high concentration of receptors only at the sites of the synapses (Figure 19–78).

A denervated muscle cell is deprived of stimulation from its nerve, and it is principally the lack of electrical activity in the muscle cell that brings about the changes described above, as well as evoking release of the "sprouting factor" mentioned previously (see p. 1121). All of these effects of denervation, which make the muscle more receptive to synapse formation, can be mimicked by applying a local anesthetic to the intact nerve, thereby blocking the stimulation of the muscle. Conversely, if a denervated muscle is stimulated artifically through implanted electrodes, the extrajunctional sensitivity to acetylcholine is suppressed and new synapses are prevented from forming. Normally the electrical activity triggered by a neuron that has already established a synapse prevents the muscle cell from receiving unwanted additional innervation.

In a related fashion, electrical activity regulates the elimination of synapses during development. In the vertebrate embryo, where many nerve terminals encounter an uninnervated muscle cell more or less simultaneously, many superfluous nerve connections are initially formed. The adult pattern, in which each muscle cell normally receives only one synapse, is achieved in two distinct steps separated in time. The first involves the death of surplus motor neurons (*neuronal death*); the second involves pruning of axon branches (*synapse elimination*).

Electrical Activity in Muscle Influences the Survival of Embryonic Motor Neurons[59,66]

As mentioned earlier, about 50% of embryonic motor neurons die shortly after making synaptic contact with muscle cells. This death of surplus neurons is prevented if neuromuscular transmission is blocked by means of a toxin such as

α-bungarotoxin and is increased if the muscle is given direct electrical stimulation. These findings suggest that electrical activity in the muscle controls production of a muscle-derived neurotrophic factor necessary for survival of embryonic motor neurons. By analogy with NGF, this might be identical with the "sprouting factor" thought to cause sprouting of axon terminals toward a muscle cell that is denervated. A muscle that is inactive, either because of a block of synaptic transmission or because it is not innervated, would produce the factor in large quantities as a signal of its need for innervation; electrical activation of the muscle, either by artificial stimulation or by the normal spontaneous firing of motor neurons that innervate it, would depress production of the factor, and in the embryo some of the young motor neurons would die in the competition for what little there was.

Electrical Activity Regulates the Competitive Elimination of Synapses According to a Temporal Firing Rule[61,67]

Even after half the embryonic motor neurons have died, the developing muscles are left with a large excess of synaptic inputs. Each motor neuron branches profusely, making synapses on many muscle cells; and a typical muscle cell becomes innervated by branches from several neurons. To attain the adult configuration, all but one of the synapses on each muscle cell must be eliminated. The process of **synapse elimination** during development has been well studied in the soleus muscle of the rat leg, where about three motor neurons on average innervate each muscle cell at birth. During the next 2 or 3 weeks, each neuron retracts a large proportion of its terminal branches until each muscle cell is innervated by a single branch of one motor axon (Figure 19–79).

If the surplus axon branches were eliminated at random, some muscle cells would be left with no synapse at all while others would retain several. The fact that each muscle cell retains exactly one synapse implies that the process of synapse elimination is competitive. Indeed, competitive synapse elimination throughout the nervous system is one of the most important processes governing the development of neural connections and, as we shall see later, their subsequent modification by environmental input. Although the molecular mechanisms of competitive synapse elimination are not understood, the competition in most cases seems to be governed by a simple and general set of principles, applicable both to neuromuscular synapses and to synapses of neuron on neuron.

First, when competition occurs, it involves an element of chance; but the final outcome is clear-cut, and each synapse either survives or is completely eliminated. Second, competition generally occurs only between synapses that are relatively close together and on the same target cell. Thus, in the normal development of a typical mammalian skeletal muscle cell, the incoming nerve terminals all initially synapse in the same small "end-plate" region, and they then compete until only

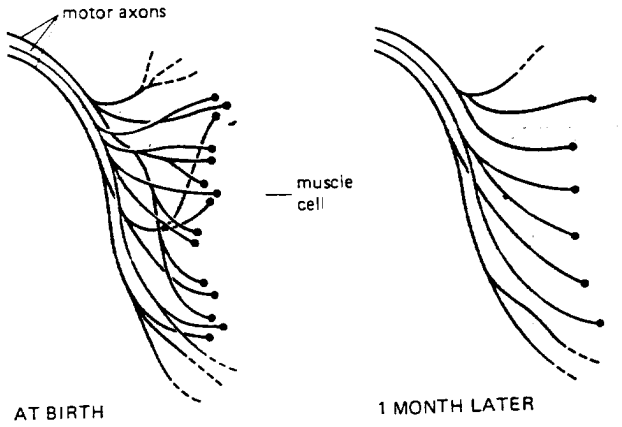
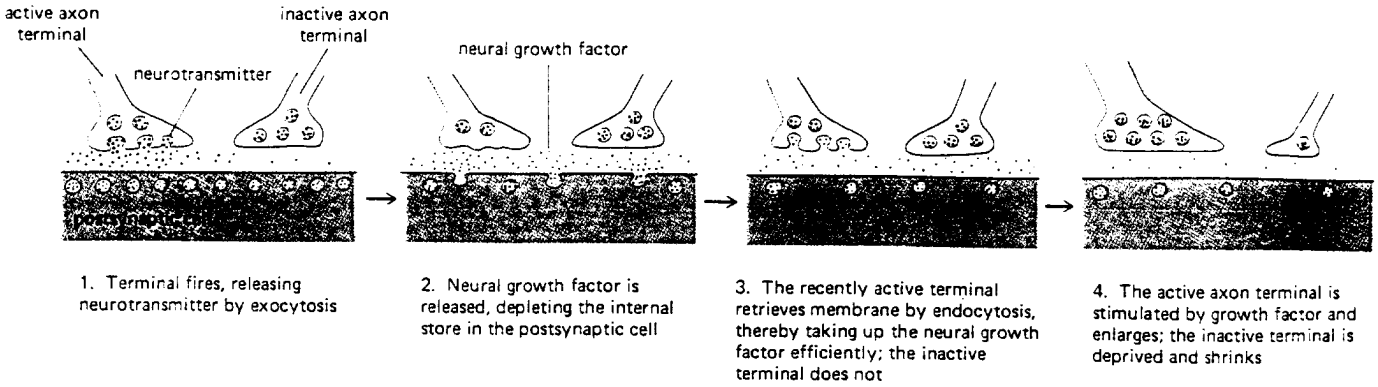


Figure 19–79 Elimination of surplus synapses in a mammalian skeletal muscle in the period after birth. In this schematic diagram the number of terminal branches of each motor axon is underrepresented for the sake of clarity; in reality a single motor axon in a mature muscle typically branches to innervate several hundred muscle cells. All the axon branches innervating one immature muscle cell normally make their synapses in the same small neighborhood on the muscle cell and compete at close range until only one synapse is left.



one is left. When multiple synapses are artificially induced to form on the same cell at sites separated by 1 mm or more, the multiple innervation is retained.

Third, and most important to the theme of this section, the competitive elimination of synapses depends on electrical activity in both the axons and the target cells that they innervate. For example, synapse elimination is delayed if excitation of a developing muscle is blocked by applying local anesthetic to the nerve or α-bungarotoxin to the neuromuscular junction. And in most systems that have been studied, if some of the innervating axons are paralyzed while others remain active, the active axons gain control of more target cells. It seems as though in the neighborhood of an active synapse, the stimulated target cell either produces something that tends to destroy other synapses on that region of its surface or fails to produce something that is required for their maintenance.

But this presents a paradox. If synaptic stimulation of the target cell drives off synaptic contacts, how can the synapse through which the cell gets its stimulation remain and consolidate itself? The answer seems to lie in the following **firing rule:**

> Each excitation of the target cell tends to consolidate any synapse where the presynaptic axon terminal has just been active and to cause rejection of any synapse where the presynaptic axon terminal has just been quiet.

Thus the relative timing of activity is all-important, and where several independently active neurons make neighboring contacts with a single target cell, each of them tends to consolidate its own synapse while promoting elimination of the synapses made by the others.

The molecular mechanisms underlying the firing rule are unknown; Figure 19–80 outlines one speculative suggestion that has been put forward, and the legend mentions another. Nonetheless, there is evidence for the firing rule from many different systems, and we shall now examine some of its applications to synapses other than the neuromuscular junction.

Synchronously Firing Axon Terminals Make Mutually Supportive Synapses[68]

One of the corollaries of the firing rule is illustrated in the submandibular ganglion of the rat, where each neuron is innervated at birth by axons from about five presynaptic neurons located in the brainstem. By the end of the first month of postnatal life, through competitive synapse elimination, each neuron in the ganglion is innervated by only one such axon. But meanwhile that axon has formed many new terminal branches, synapsing on the same cell at many sites, so that the total number of synapses is larger finally than it was initially (Figure 19–81). The branches of a single axon have one obvious property in common that distinguishes them from branches of other axons of the same type: they all fire at the same time. In accordance with the firing rule, neighboring axon terminals that fire synchronously have collaborated in forming synapses, whereas terminals that fire asynchronously have competed.

Figure 19–80 One of several molecular mechanisms that have been tentatively suggested to underlie the firing rule. In this view, maintenance of a synapse depends on a neural growth factor released from the postsynaptic cell. Release is triggered locally by the electrical stimulation that follows delivery of an action potential at the synapse; at other times there is some spontaneous release, at a lower rate. The factor is taken up into recently active axon terminals during the endocytic retrieval of membrane that immediately follows the exocytosis of neurotransmitter (see p. 1079). Those axon terminals that endocytose the factor respond by enlarging and depositing materials that reinforce the synaptic bond. The more frequently the muscle is stimulated, the more its internal store of the factor is depleted and the lower is the rate of spontaneous release in the absence of stimulation. Thus inactive axon terminals competing with active ones fail to get adequate growth factor and consequently shrink and are eventually withdrawn. Two axon terminals that are active at different times will compete for the limited amount of growth factor that the postsynaptic cell contains. If large terminals take up more growth factor, which in turn makes them larger still, the outcome of such competition may depend on slight differences in initial size.

An alternative hypothesis proposes that stimulation of the postsynaptic cell causes local release or activation of a protease that tends to destroy inactive synapses but against which recently active synapses are somehow protected.

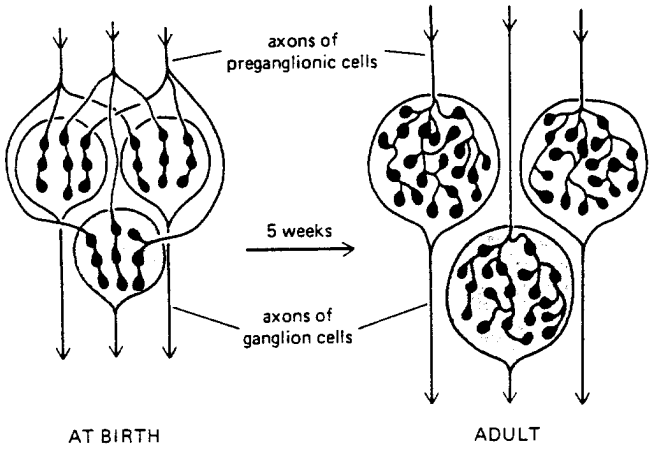


Figure 19–81 The changes that occur soon after birth in the pattern of synapses on neurons in the submandibular ganglion of a rat. Initially each cell is innervated by several axons. These compete until, by synapse elimination, one axon is left in sole command; this one axon, meanwhile, increases the number of its synapses on the cell, which show no sign of competing with one another. (After D. Purves and J.W. Lichtman, *Physiol. Rev.* 58:821–862, 1978.)

The Number of Surviving Inputs Depends on the Number of Dendrites on the Postsynaptic Neuron[69]

Because the competition among synapses for survival depends in part on the distances between them, the final outcome depends on the structure of the postsynaptic cell. The submandibular ganglion neuron is a somewhat atypical neuron in that it has no dendrites and, following a synaptic competition fought out at close quarters on the cell body, retains input from only one axon. Most other neurons have multiple dendrites and continue in adult life to receive inputs from multiple sources, this being essential for their integrative function. The role of dendrites in regulating synapse elimination is illustrated in the ciliary ganglion of a rabbit, where some of the neurons have many dendrites while others have few or none (Figure 19–82). At birth all the neurons are similarly innervated by about four or five presynaptic axons. But in the adult the cells without dendrites receive input from just one axon, while the number of axons providing input to the other cells increases in direct proportion to the number of main dendrites. The synapses on a single dendrite, however, tend to be made by branches of a single axon. Thus

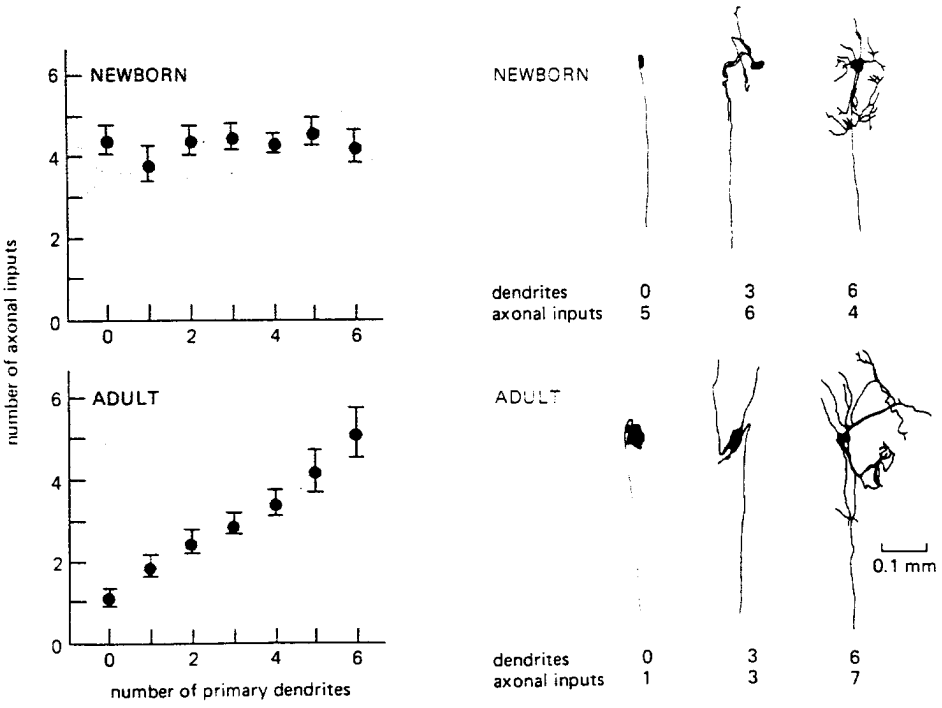


Figure 19–82 The developmental relationship between the number of primary dendrites and the number of axonal inputs to individual cells in the ciliary ganglion of a rabbit. At birth the average number of inputs is independent of the number of dendrites; in the adult the average number of inputs that have survived the period of competitive synapse elimination is proportional to the number of dendrites. At the right the relationship is illustrated by drawings of selected individual ganglion cells. (After D. Purves and R.I. Hume, *J. Neurosci.* 1:441–452, 1981; and R.I. Hume and D. Purves, *Nature* 293:469–471, 1981.)

it seems that each dendrite provides a separate and independent territory, such that synapses on one dendrite do not compete with those on another. As in skeletal muscle, the competition is local, and it obeys the predictions of the firing rule.

Perhaps the most profound implications of the firing rule, however, concern the ways in which stimuli from the external world control adjustments of the anatomical connections between neurons. This is especially clear from studies on the development of the vertebrate visual system. We shall concentrate here on the evidence from mammals.

Visual Connections in Young Mammals Are Adjustable and Sensitive to Visual Experience[70]

The visual system of a mammal is not mature at birth. The first few years of postnatal life (in humans), or the first few months (in cats or monkeys), are a *sensitive* (or *critical*) *period*, during which the pattern of neural connections is still adjustable, and abnormal visual experience can have drastic and irreversible consequences. A common example is the "lazy eye" that can result from a childhood squint. Children with a squint frequently fall into the habit of using one eye only and neglecting the input from the other eye, which is perpetually misdirected and rarely receives a sharply focused image on its retina. If the squint is corrected early and the child is taught to use both eyes, both eyes will continue to function normally. But if the squint goes uncorrected throughout childhood, the unused eye becomes almost completely blind in a permanent way that no lens can correct—a condition known as *amblyopia*. The eye itself remains normal: the defect lies in the brain. Before explaining the nature of the defect, we must outline some of the anatomy of the adult mammalian visual system.

Active Synapses Tend to Displace Inactive Synapses in the Mammalian Visual System[71]

Each eye in a mammal such as a human or a cat sees almost the same visual field, and the two views are combined in the brain to provide binocular stereoscopic vision. This is possible because axons relaying input from equivalent regions in the two retinas make synapses in the same region of the brain (Figure 19–83). Thus, on the *primary visual cortex* of the left side of the adult brain, there are two orderly maps of the right half of the visual field—one from the left eye, the other from the right eye. These two maps, however, are not precisely superimposed on

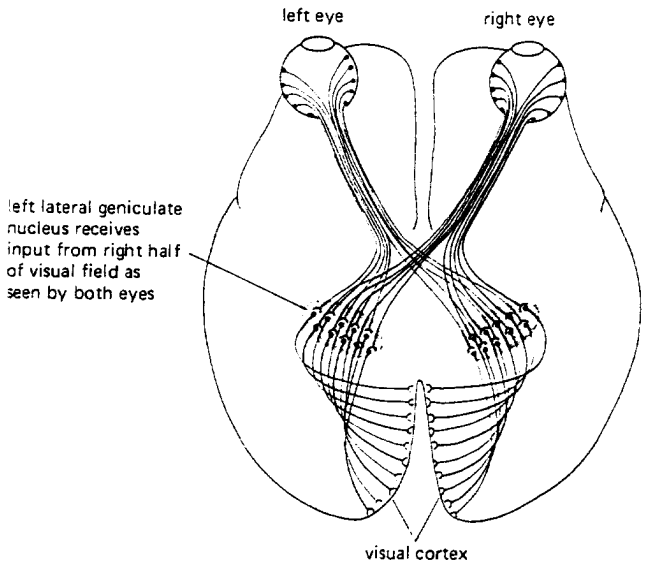


Figure 19–83 The major human visual pathway, showing how the inputs from the right and left eyes are distributed so that related streams of information are brought together in the same region of the brain. Note that all the information obtained by the left side of each eye (relating to the right side of the visual field) is relayed to the left side of the brain, and vice versa.

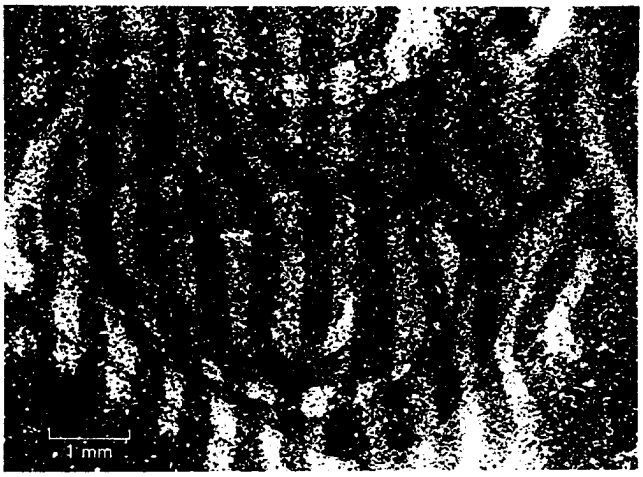


Figure 19–84 Ocular dominance columns in the visual cortex of a normal monkey. Radioactive proline is injected into one eye and the animal is then allowed to survive for 10 days, during which the radioactive label is transported to the parts of the cortex that receive their input from that eye. Sections of the cortex are cut tangentially to its surface, and autoradiographs are prepared. With dark-field illumination the silver grains covering the radioactive regions appear bright against a dark background. The picture is a montage composed of photographs of several successive sections cut at slightly different depths through the thickness of the cortex. The ocular dominance columns connected to the labeled eye (*bright bands*) are of the same width as those connected to the unlabeled eye (*dark bands*). (From D.H. Hubel, T.N. Wiesel, and S. Le Vay, *Philos. Trans. R. Soc. [Biol.]* 278:377–409, 1977.)

each other: instead the inputs arriving from the two eyes are segregated in a pattern of narrow, alternating stripes known as **ocular dominance columns.** This arrangement is represented schematically in Figure 19–83 and can be demonstrated directly by a labeling technique that involves injecting radioactive amino acids into one eye. The labeled molecules are taken up by the retinal neurons and incorporated into proteins, which are carried by axonal transport toward the visual cortex; the labeled proteins somehow pass from one neuron to the next at a synaptic relay station (the lateral geniculate nucleus) on the way. Autoradiographs of sections of the visual cortex of the adult monkey brain, for example, show labeled bands about half a millimeter wide, receiving their input from the labeled eye, alternating with unlabeled bands of equal width, receiving their input from the unlabeled eye (Figure 19–84).

During development, however, when the visual connections are first made, no ocular dominance columns can be seen: the projections from the two eyes overlap completely. It is only later (typically during the first few weeks after birth) that the projections segregate into the adult pattern of alternating stripes by means of the competitive elimination of overlapping axon terminals. The pattern apparently develops through the operation of the firing rule: axons relaying excitations from adjacent sites in one eye tend to fire in close synchrony with one another but often out of synchrony with axons relaying excitations from the other eye. The axons that fire in synchrony with one another collaborate in establishing their own set of synapses on a given cortical cell while driving off the synapses from other axons. The segregation into alternating stripes can be halted either by artificially stimulating both optic nerves so as to force the axons from the left eye to fire in strict synchrony with those from the right, or by suppressing electrical activity with injections of tetrodotoxin (which blocks voltage-gated Na^+ channels) into both eyes.

The most striking effects, from a functional point of view, are seen when one eye is simply kept covered and thereby deprived of visual stimulation during the sensitive period. When the cover is removed, the animal behaves as though blind or semiblind in the deprived eye. Autoradiographic tracing shows that the ocular dominance columns connected to the deprived eye have shrunk drastically, while those connected to the experienced eye have widened to occupy the vacated space (Figure 19–85). Again in accordance with the firing rule, synapses made by inactive axons have been eliminated, whereas active axons have consolidated their synapses and made more. In this way cortical territory is allocated to axons that carry information and is not wasted on those that are silent. The effect is irreversible once the sensitive period has ended. Thus the stimulation that a sensory pathway receives in early life determines the amount of cortical machinery—the number of neurons and synapses—that will be available to deal with that input in the adult.

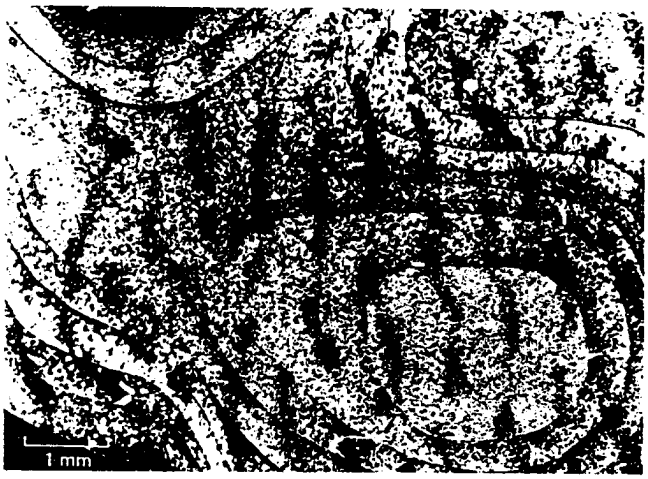


Figure 19–83 Ocular dominance columns in the visual cortex of a monkey that has had one eye covered during the sensitive period of development. The other eye has received an injection of radioactive proline, and autoradiographs have been prepared as described in the caption to Figure 19–84. The ocular dominance columns connected to the eye deprived of visual experience (*dark bands*) are abnormally narrow, whereas those connected to the other eye are abnormally wide. If the deprived eye is labeled, a converse picture is seen, with narrow bright bands alternating with broad dark bands. (From D.H. Hubel, T.N. Wiesel, and S. Le Vay, *Philos. Trans. R. Soc. [Biol.]* 278:377–409, 1977.)

Convergent Binocular Visual Connections Depend on Synchronized Binocular Stimulation[70,71,72]

Early visual experience is also important is subtler ways in establishing the nerve connections that enable us to see. For example, some children with an uncorrected squint, instead of neglecting one eye, will use both eyes but in alternation rather than together. Both eyes then remain functional, but the ability to see depth (stereopsis) is permanently lost. Studies of the behavior of single cells in the brain show that this phenomenon too can be explained by the firing rule.

Stereopsis depends on *binocularly driven* neurons—that is, neurons that receive and respond to convergent synaptic inputs from both eyes at once. Such neurons can be identified in experimental animals by inserting a recording microelectrode into the visual cortex of the brain and observing the firing of single cells in response to visual stimuli presented to the two eyes. Such cells are found in particular layers of the visual cortex, above and below the layer containing monocularly driven neurons arranged in clear-cut ocular dominance columns. In a normal animal, binocularly driven neurons are plentiful. But there are scarcely any to be found in an animal that has been specifically deprived of synchronous binocular stimulation during the sensitive period (either by covering different eyes on alternate days or as a result of a severe squint). Evidently, the inputs from each eye to a binocularly driven neuron are maintained only if the two inputs frequently fire in synchrony. When synchronous stimulation is prevented, the axons bringing input from one eye compete with those bringing input to the same neuron from the other eye, in accordance with the firing rule for synapse elimination and maintenance, until one eye gains sole control of that neuron and the possibility of stereopsis is lost.

The Firing Rule Guides the Organization of the Nervous System in the Light of Experience[73]

The development of binocular vision illustrates a general organizing principle: synchronous firing tends to establish convergent connections. This principle, which follows from the firing rule, may help to explain how the brain comes to contain neurons that are specifically responsive to particular complex combinations of sensations that are repeatedly evoked by common objects in the world around us. The brains of primates, for example, contain neurons that appear to fire specifically in response to the sight of a particular face. More generally, one can begin to understand how the brain may be adjusted in the light of experience so as to represent in its own structure and function the existence of relationships between one external phenomenon and another. In this sense the rules for synapse formation and elimination early in life provide a basis for early learning and memory.

It was suggested earlier in the chapter that memory in the adult involves modulation of synaptic transmission through long-lasting chemical changes initiated by neurotransmitters binding to certain types of receptors. Is there any connection between this and the developmental phenomena we have just described, involving relatively gross structural changes in the pattern of synaptic connections? In some cases at least, chemical and structural synaptic changes seem to be intimately related. For example, when *Aplysia* is subjected to long-term habituation or sensitization (see p. 1095) by repeated training on successive days, the chemical modulation of synaptic transmission becomes reinforced by alterations in the size of the presynaptic structures. It is noteworthy also that ocular dominance columns in a frog are altered by exposure to agonists or antagonists of the NMDA receptor, which is thought to play a part in memory in the adult hippocampus (see p. 1100).

Beyond such hints, however, one can only speculate; memory and the mechanisms of synapse formation and elimination are not yet adequately understood. But it is clear that they are among the problems that go to the heart of nerve cell biology and whose solutions promise to illuminate and unify our understanding of the brain at almost every level.

Summary

Synapses first form early in development, but the initial pattern of connections undergoes prolonged remodeling through the elimination of old synapses and the creation of new ones. The creation of a synapse between a motor neuron and a muscle cell involves changes in both cells and the deposition of specialized components in the basal lamina that lies between them. The specialized junctional basal lamina survives the destruction of both motor axons and muscle cells and controls the location of synaptic specializations in both muscle and axon terminal when they regenerate.

In a mammal at the time of birth, each muscle cell generally has several synapses on it, all but one of which are subsequently eliminated by a competitive mechanism. Activation of a muscle cell at a synapse tends to cause disappearance of other synapses in the neighborhood of the one that is active if these synapses are not themselves active at the same time; but where pre- and postsynaptic activity occur synchronously, the synapse tends to be consolidated. This "firing rule," whose molecular mechanism is unknown, appears to explain the phenomena of synapse formation and elimination in many different parts of the developing nervous system. In particular, it helps to explain how synaptic connections in the brain become adjusted according to an animal's experience of the world.

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