9 Principles of Direct Synaptic Transmission

SYNAPSES ARE POINTS OF CONTACT between nerve cells and their targets where signals are handed on from one cell to the next. At electrical synapses, current flows from the presynaptic nerve terminal into the postsynaptic cell to alter its membrane potential. Electrical transmission is prevalent in the nervous systems of invertebrates and also occurs at synapses in the mammalian CNS. At chemical synapses, the arrival of the action potential in the presynaptic nerve terminal causes neurotransmitter molecules to be released. At direct chemical synapses, the transmitter binds to ionotropic receptors in the membrane of the postsynaptic cell that are themselves ion channels. As a result, the conformation of the receptor changes, the channel opens, ions flow, and the membrane potential changes. At indirect chemical synapses, metabotropic receptors and intracellular second messengers are involved (Chapter 10).

The channels opened at excitatory synapses allow cations to enter, driving the membrane potential toward threshold. At inhibitory synapses, transmitters open channels that are permeable to anions, tending to keep the membrane potential negative to threshold. At both excitatory and inhibitory synapses, the direction of current flow is determined by the balance of concentration and electrical gradients acting on the permeant ions.

Synapses between motor nerves and muscle fibers provided important preparations for understanding the mechanisms of direct chemical synaptic transmission. In the mammalian central nervous system, directly mediated excitatory and inhibitory transmission occurs at synapses where acetylcholine, glutamate, γ-aminobutyric acid, serotonin, and purines are released to activate ionotropic receptors.

More than one type of transmitter may be released at a single chemical synapse, and many transmitters act both rapidly, by binding to and opening ion channels directly, and more slowly through indirect mechanisms.

Information is transmitted from one neuron to another, or from a neuron to an effector cell such as a muscle fiber, at a specialized point of contact, the **synapse**. In this chapter we consider the principles of **direct synaptic transmission.** Direct, or "fast," synapses include electrical synapses, at which transmission is mediated by the flow of current from the pre- to the postsynaptic cell. More common are direct chemical synapses, at which a neurotransmitter is released from the axon terminal and binds to receptors on the target cell that are ion channels.

Subsequent chapters describe how chemical neurotransmitters influence target cells indirectly by binding to receptors that trigger intracellular signaling cascades (Chapter 10), how neurotransmitters are released (Chapter 11), how neurotransmitters are synthesized and stored within axon terminals (Chapter 13), and how the efficacy of transmission can be dramatically altered by repetitive activity (Chapter 12). Because of the variety and complexity of synaptic interactions, it is useful to begin by reviewing the history of some of the fundamental ideas.

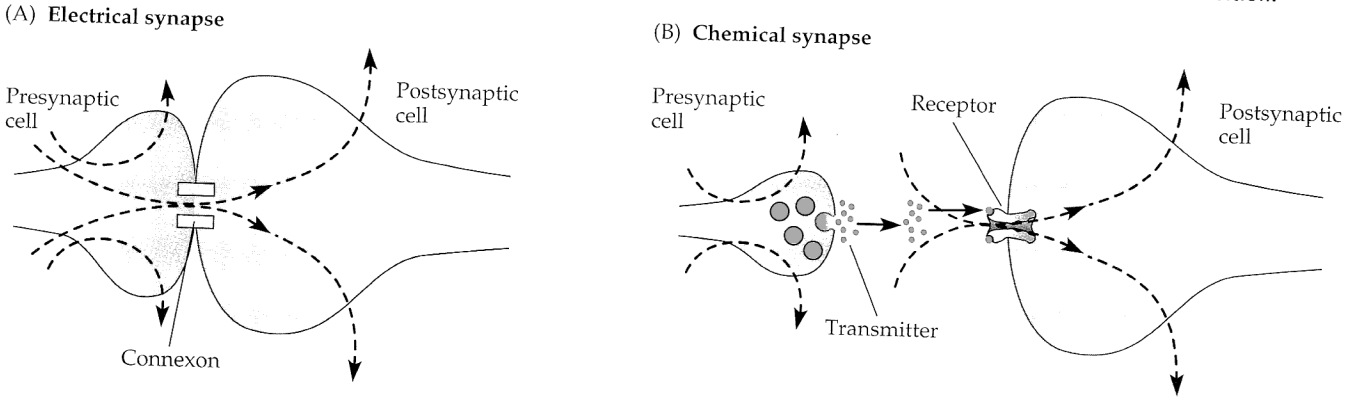
NERVE CELLS AND SYNAPTIC CONNECTIONS

It was not always clear that the two main components of the synapse—presynaptic terminal and postsynaptic cell—are morphologically distinct. In the second half of the nineteenth century there was vigorous debate between proponents of the **cell theory**, who considered that neurons were independent units, and those who thought that nerve cells were a **syncytium**, interconnected by protoplasmic bridges. Not until the late nineteenth century did it become generally accepted that nerve cells are independent units. It remained for electron microscopy to obtain definitive evidence that each neuron is surrounded completely by its own plasma membrane. Even so, electron microscopy and other modern techniques eventually revealed that some neurons are, in fact, connected by channels, called connexons, that permit direct intercellular flow of ions and other small molecules (Chapter 7).

The disagreement about synaptic structure was accompanied by a parallel disagreement about function. In 1843, Du Bois-Reymond showed that flow of electrical current was involved in both muscle contraction and nerve conduction, and it required only a small extension of this idea to conclude that transmission of excitation from nerve to muscle was also due to current flow (Figure 9.1A).[1] Du Bois-Reymond himself favored an alternative explanation: the secretion by the nerve terminal of an excitatory substance that then caused muscle contraction (Figure 9.1B). However, the idea of animal electricity had such a potent hold on people's thinking that it was more than 100 years before contrary evidence finally overcame the assumption of electrical transmission between nerve and muscle and, by extension, between nerve cells in general.

[1]Du Bois-Reymond, E. 1848. *Untersuchungen über thierische Electricität*. Reimer, Berlin.

FIGURE 9.1 Electrical and Chemical Synaptic Transmission. (A) At electrical synapses, current flows directly from one cell to another through connexons, intercellular channels that cluster to form gap junctions. (B) At a chemical synapse, depolarization of the presynaptic nerve terminal triggers the release of neurotransmitter molecules, which interact with receptors on the postsynaptic neuron, causing excitation or inhibition.



Chemical Synaptic Transmission in the Autonomic Nervous System

One reason that the idea of chemical synaptic transmission seemed unattractive is the speed of signaling between nerve cells or between nerve and muscle. The fraction of a second that intervenes between stimulation of a motor axon and contraction of the corresponding muscle did not appear to provide sufficient time for a chemical neurotransmitter to be released from the nerve terminal and interact with receptors on the postsynaptic target to cause excitation. This difficulty did not exist in the autonomic nervous system that controls glands and blood vessels. There the effects of nerve stimulation are slow and prolonged, waxing and waning over the course of many seconds (Chapter 16).

In 1892, Langley proposed that synaptic transmission in mammalian autonomic ganglia was chemical, rather than electrical, based on the observation that transmission through the mammalian ciliary ganglion was blocked selectively by nicotine.[2] About a decade later, Elliot pointed out that an extract from the adrenal gland, adrenaline (epinephrine), mimicked the action of sympathetic nerve stimulation when applied directly to the target tissues and suggested that it might be secreted by nerve terminals as a transmitter.[3] It was not until 1921, however, that Otto Loewi did a direct and simple experiment that established the chemical nature of transmission at autonomic synapses between the vagus nerve and the heart.[4] He perfused the heart of a frog and stimulated the vagus nerve, thereby slowing the heartbeat. When the fluid from the inhibited heart was transferred to a second unstimulated heart, it too began to beat more slowly. Apparently stimulation of the vagus nerve had caused an inhibitory substance to be released into the perfusate. Loewi and his colleagues demonstrated in subsequent experiments that the substance was mimicked in every way by acetylcholine (ACh).

It is an amusing sidelight that Loewi had the idea for his experiment in a dream, wrote it down in the middle of the night, but could not decipher his writing the next morning. Fortunately the dream returned, and this time Loewi took no chances; he rushed to the laboratory and performed the experiment. Later he reflected:

> On mature consideration, in the cold light of morning, I would not have done it. After all, it was an unlikely enough assumption that the vagus should secrete an inhibitory substance; it was still more unlikely that a chemical substance that was supposed to be effective at very close range between nerve terminal and muscle be secreted in such large amounts that it would spill over and, after being diluted by the perfusion fluid, still be able to inhibit another heart.[4]

Subsequently, in the early 1930s, the role of ACh in synaptic transmission in ganglia in the autonomic nervous system was firmly established by Feldberg and his colleagues.[5] Highlights of such experiments and ideas from the beginning of the twentieth century are contained in the writings of Dale, for several decades one of the leading figures in British physiology and pharmacology.[6] Among his many contributions are the clarification of the action of acetylcholine at synapses in autonomic ganglia and the establishment of its role in neuromuscular transmission.

Henry Dale (left) and Otto Loewi, mid-1930s. (Kindly provided by Lady Todd and W. Feldberg.)

Chemical Synaptic Transmission at the Vertebrate Skeletal Neuromuscular Junction

In 1936, Dale and his colleagues demonstrated that stimulation of motor nerves to vertebrate skeletal muscle caused the release of ACh.[7] In addition, when ACh was injected into arteries supplying the muscle, it caused a large synchronous contraction of the muscle fibers. Later, electrical recording techniques were used to characterize the change in muscle fiber membrane potential evoked by stimulation of the motor axon and demonstrate that it could be mimicked by application of ACh. It was also shown that the responses to both nerve stimulation and direct application of ACh were antagonized by curare, a South American Indian arrow poison that blocks the ACh receptor, and potentiated by eserine, a drug that prevents the hydrolysis of ACh by the enzyme acetylcholinesterase. Such experiments served to establish firmly the idea of chemical synaptic transmission at nerve–muscle synapses. As we shall see, elaborate pre- and postsynaptic specializations allow chemical synaptic transmission to take place on a millisecond timescale. Thus, the

[2]Langley, J. N., and Anderson, H. K. 1892. *J. Physiol.* 13: 460–468.

[3]Elliot, T. R. 1904. *J. Physiol.* 31: (Proc.) xx–xxi.

[4]Loewi, O. 1921. *Pflügers Arch.* 189: 239–242.

[5]Feldberg, W. 1945. *Physiol. Rev.* 25: 596–642.

[6]Dale, H. H. 1953. *Adventures in Physiology*. Pergamon, London.

[7]Dale, H. H., Feldberg, W., and Vogt, M. 1936. *J. Physiol.* 86: 353–380.

long-championed hypothesis of electrical transmission, which had been accepted almost universally for about 100 years on inadequate evidence, was finally forsaken for good experimental reasons, only to be shown valid after all at a different kind of synapse.

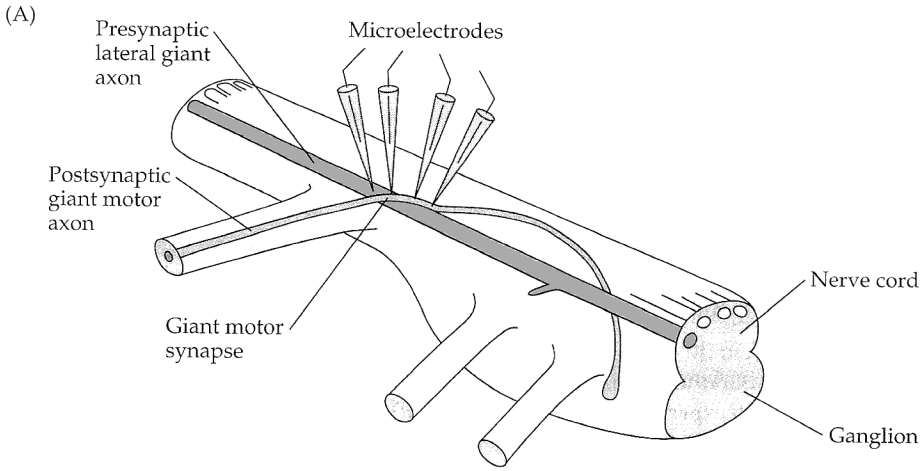
ELECTRICAL SYNAPTIC TRANSMISSION

Identification and Characterization of Electrical Synapses

In 1959, Furshpan and Potter, using intracellular microelectrodes to record from nerve fibers in the abdominal nerve cord of the crayfish, discovered an **electrical synapse** between neurons that mediated the animal's escape reflex (Figure 9.2A).[8] They demonstrated that an action potential in a lateral giant fiber led, by direct intercellular current flow, to depolarization of a giant motor fiber leaving the cord (Figure 9.2B). The depolarization was sufficient to initiate an action potential in the postsynaptic fiber. The electrical coupling was in one direction only; depolarization of the postsynaptic fiber did not lead to presynaptic depolarization (Figure 9.2C). In other words, the synapse **rectified**.

[8]Furshpan, E. J., and Potter, D. D. 1959. *J. Physiol.* 145: 289–325.

Unlike the crayfish giant synapse, most electrical synapses do not exhibit rectification, but conduct equally well in both directions. We now know that the morphological spe-



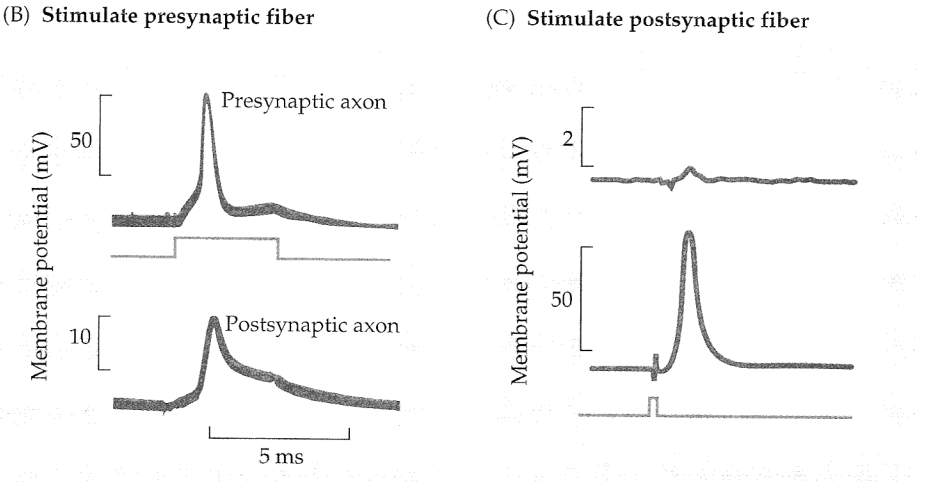


FIGURE 9.2 Electrical Synaptic Transmission at a Giant Synapse in the crayfish central nervous system. (A) The experimental preparation. The presynaptic lateral giant axon makes an electrical synapse with the postsynaptic giant motor axon in the abdominal nerve cord. (B) Depolarization of the presynaptic axon spreads immediately to the postsynaptic fiber. In this case each cell reaches threshold and fires an action potential. (C) When the postsynaptic axon is stimulated directly to give an action potential, depolarization spreads poorly from the postsynaptic to the presynaptic axon. The synapse is said to rectify. (After Furshpan and Potter, 1959.)

cialization for electrical coupling at the crayfish giant synapse and other electrical synapses is the **gap junction**.[9] Gap junctions are formed by an assembly of connexons, which allows current to pass from one cell to the next (Chapter 7).

Electrical transmission has been demonstrated at a wide variety of synapses,[10,11] such as those between motoneurons in the spinal cord of the frog,[12] sensory neurons in the mesencephalic nucleus of the rat,[13] pyramidal cells in the rat hippocampus,[14] and horizontal cells in the zebra fish retina.[15] In the leech, electrical coupling between pairs of sensory neurons has the remarkable property that depolarization spreads readily from either cell to the other, but hyperpolarization spreads poorly; that is, the electrical connections are doubly rectifying.[16,17]

The degree of electrical coupling between cells is usually expressed as a **coupling ratio**; a ratio of 1:4 means that one-fourth of the presynaptic voltage change appears in the postsynaptic cell. For cells to be strongly electrically coupled, the resistance of the junction between the cells must be very low and there must be a reasonable match between the sizes of the presynaptic and postsynaptic elements (Chapter 7).

Electrical and chemical transmission often coexist at a single synapse. Such combined electrical and chemical synapses were first found between cells of the avian ciliary ganglion, where a chemical synaptic potential (produced by ACh) is preceded by an electrical coupling potential (see Figure 9.3).[18] Similar synapses occur widely in vertebrates—for example, onto spinal interneurons of the lamprey[19] and spinal motoneurons of the frog.[20] Postsynaptic cells also may receive separate chemical and electrical synaptic inputs from different sources. For example, in leech ganglia (Chapter 15) motor neurons receive three distinct types of synaptic input from sensory neurons signaling three different modalities; one input is chemical, one electrical, and one combined electrical and chemical.[21]

Synaptic Delay at Chemical and Electrical Synapses

One characteristic of electrically mediated synaptic transmission is the absence of a **synaptic delay**. At chemical synapses there is a pause of approximately 1 ms between the arrival of an impulse in the presynaptic terminal and the appearance of an electrical potential in the postsynaptic cell. The delay is due to the time taken for the terminal to release transmitter (Chapter 11). At electrical synapses there is no such delay; current spreads instantaneously from one cell to the next.

The presence of both electrical and chemical transmission at the same synapse provides a convenient means of comparing the two modes of transmission. This is illustrated in Figure 9.3, which shows intracellular records from a cell in the ciliary ganglion of the chick. Stimulation of the preganglionic nerve leads to an action potential in the postsynaptic cell, with very short latency (Figure 9.3A). When the cell is hyperpolarized slightly (Figure 9.3B), the action potential arises at a later time, revealing an early, brief depolarization that, because the cell has been hyperpolarized, is now subthreshold. This depolarization is an electrical coupling potential, produced by current flow from the presynaptic nerve terminal into the cell. Further hyperpolarization (Figure 9.3C) blocks the initiation of the action potential altogether, revealing the underlying chemical synaptic potential. The cells, then, have the property that, under normal conditions, initiation of a postsynaptic action potential by chemical transmission is preempted by electrical coupling. In this example the coupling potential precedes the chemical synaptic potential by about 2 ms, giving us a direct measure of the synaptic delay. Additional experiments on these cells have shown that the electrical coupling is bidirectional; that is, the synapses do not rectify.

There are several advantages to electrical transmission. One is that electrical synapses are more reliable than chemical synapses; transmission is less likely to fail because of synaptic depression or to be blocked by neurotoxins.[22,23] A second advantage is the greater speed of electrical transmission. Speed is important in rapid reflexes involving escape reactions, in which the saving of a millisecond may be crucial for surviving attack by a predator. Other functions include the synchronization of electrical activity of groups of cells[24,25] and intercellular transfer of key molecules such as calcium, ATP, and cAMP.[26] Dopamine has been shown to modulate the activity of gap junctions between cells in the retina.[27,28] Thus, gap junctions do not function merely as passive connections, but can be dynamic components of neuronal circuits.

[9]Bennett, M. V. 1997. *J. Neurocytol.* 26: 349–366.

[10]Loewenstein, W. 1981. *Physiol. Rev.* 61: 829–913.

[11]Llinás, R. 1985. In *Gap Junctions*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 337–353.

[12]Grinnell, A. D. 1970. *J. Physiol.* 210: 17–43.

[13]Baker, R., and Llinás, R. 1971. *J. Physiol.* 212: 45–63.

[14]MacVicar, B. A., and Dudek, F. E. 1981. *Science* 213: 782–785.

[15]McMahon, D. G. 1994. *J. Neurosci.* 14: 1722–1734.

[16]Baylor, D. A., and Nicholls, J. G. 1969. *J. Physiol.* 203: 591–609.

[17]Acklin, S. E. 1988. *J. Exp. Biol.* 137: 1–11.

[18]Martin, A. R., and Pilar, G. 1963. *J. Physiol.* 168: 443–463.

[19]Rovainen, C. M. 1967. *J. Neurophysiol.* 30: 1024–1042.

[20]Shapovalov, A. I., and Shiriaev, B. I. 1980. *J. Physiol.* 306: 1–15.

[21]Nicholls, J. G., and Purves, D. 1972. *J. Physiol.* 225: 637–656.

[22]Brodin, L., et al. 1994. *J. Neurophysiol.* 72: 592–604.

[23]Dityatev, A. E., and Clamann, H. P. 1996. *J. Neurophysiol.* 76: 3451–3459.

[24]Draguhn, A., et al. 1998. *Nature* 394: 189–192.

[25]Brivanlou, I. H., Warland, D. K., and Meister, M. 1998. *Neuron* 20: 527–539.

[26]Loewenstein, W. R. 1999. *The Touchstone of Life*. Oxford University Press, New York.

[27]DeVries, S. H., and Schwartz, E. A. 1989. *J. Physiol.* 414: 351–375.

[28]Hampson, E. C. G. M., Vaney, D. I., and Weiler, R. 1992. *J. Neurosci.* 12: 4911–4922.

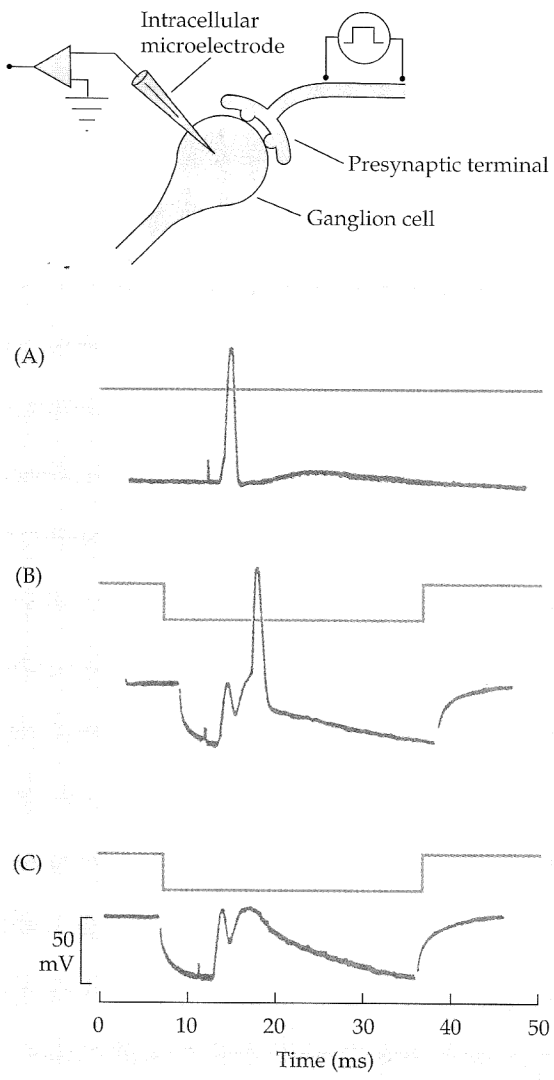


FIGURE 9.3 Electrical and Chemical Synaptic Transmission in a chick ciliary ganglion cell, recorded with an intracellular microelectrode. (A) Stimulation of the preganglionic nerve produces an action potential in the ganglion cell (lower trace). (B) When the ganglion cell is hyperpolarized by passing current through the recording electrode (upper trace), the cell reaches threshold later, revealing an earlier, transient depolarization. This depolarization is an electrical synaptic potential (coupling potential), caused by current flow into the ganglion cell from the presynaptic terminal. In A, the electrical synaptic potential depolarized the ganglion cell to threshold, initiating an action potential. (C) Slightly greater hyperpolarization prevents the ganglion cell from reaching threshold, exposing a slower chemical synaptic potential. The chemical synaptic potential follows the coupling potential with a synaptic delay of about 2 ms at room temperature. (After Martin and Pilar, 1963.)

CHEMICAL SYNAPTIC TRANSMISSION

Certain obvious questions arise when one considers the elaborate scheme for **chemical synaptic transmission**, which entails the secretion of a specific chemical by a nerve terminal and its interaction with postsynaptic receptors (see Figure 9.1B). How does the terminal liberate the chemical? Is there a special feature of the action potential mechanism that causes secretion? How is the interaction of a transmitter with its postsynaptic receptor rapidly converted into excitation or inhibition? The release process will be considered in detail in Chapter 11; the present discussion is concerned with the question of how transmitters act on the postsynaptic cell at direct chemical synapses.

Many of the pioneering studies of chemical synaptic transmission were done on relatively simple preparations, such as the skeletal neuromuscular junction of the frog. At the time, this particular preparation had the advantage that the neurotransmitter (ACh) had been definitively identified. (Many years later was it shown that ATP is co-released with acetylcholine by motor nerve endings to act as a second transmitter.[29])

Synaptic Structure

Chemical synapses are complex in structure. Figure 9.4 illustrates the morphological features of the neuromuscular junction of the frog. Individual axons branch from the incom-

[29]Silinsky, E. M., and Redman, R. S. 1996. *J. Physiol.* 492: 815–822.

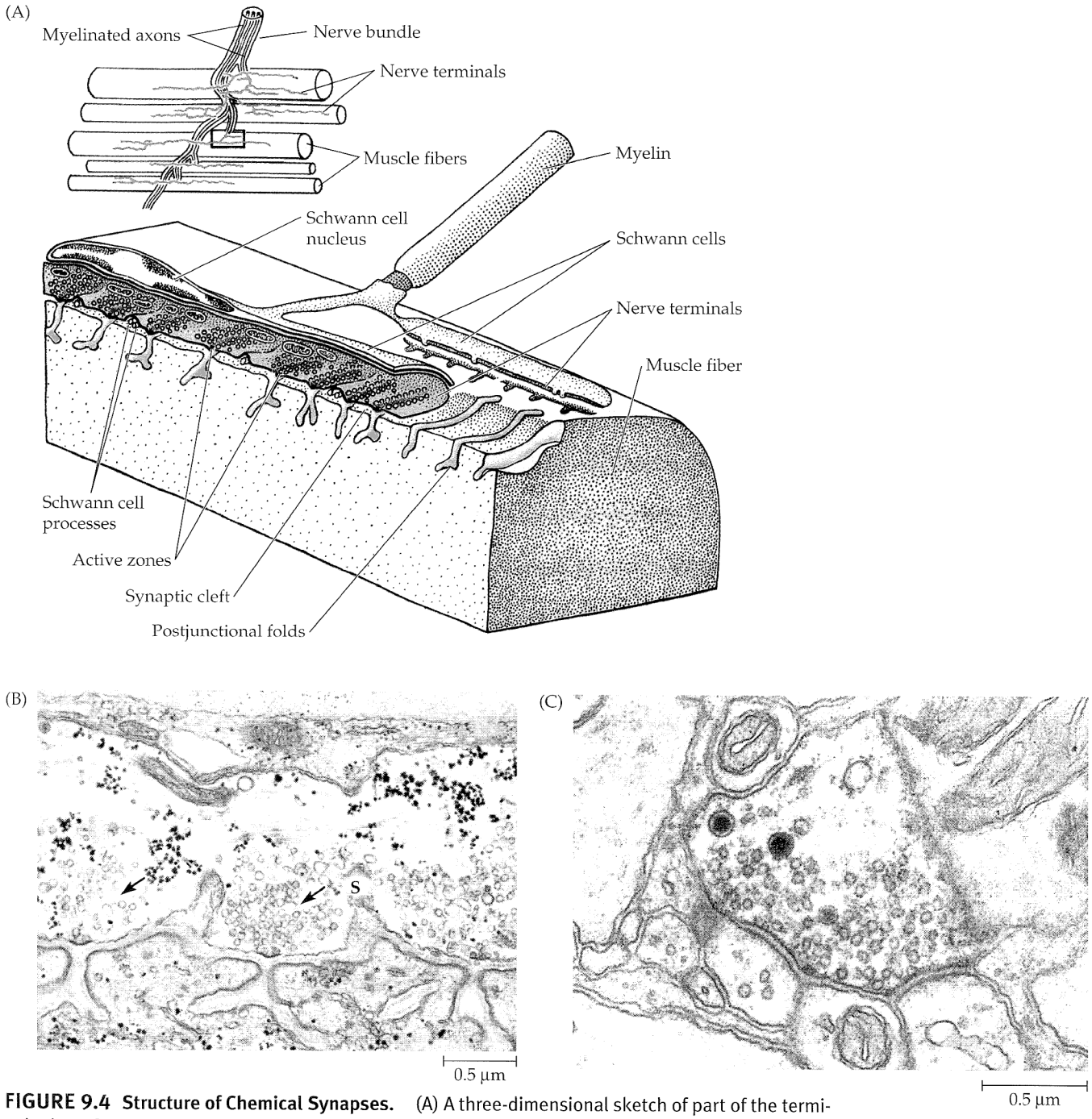


FIGURE 9.4 Structure of Chemical Synapses. (A) A three-dimensional sketch of part of the terminal arbor of a motor axon at the frog skeletal neuromuscular junction. The low-power view (inset) shows several skeletal muscle fibers and their innervation (the region depicted in more detail is indicated by the box). Synaptic vesicles are clustered in the nerve terminal in special regions opposite the openings of the postjunctional folds. These regions, called active zones, are the sites of transmitter release into the synaptic cleft. Fingerlike processes of Schwann cells extend between the terminal and the postsynaptic membrane, separating active zones. (B) Electron micrograph of a longitudinal section through a portion of the neuromuscular junction. In the nerve terminal, clusters of vesicles lie over thickenings in the presynaptic membrane—the active zones (arrows). Schwann cell processes (S) separate the clusters. In the muscle, postjunctional folds open into the synaptic cleft directly under the active zone. The band of fuzzy material in the cleft, which follows the contours of the postjunctional folds, is the synaptic basal lamina. (C) Electron micrograph of synapses in the central nervous system of the leech. As at the frog neuromuscular junction, clusters of synaptic vesicles are focused on dense regions of the presynaptic membrane, forming active zones, and are juxtaposed to postsynaptic densities. (B kindly provided by U. J. McMahan; C kindly provided by K. J. Muller.)

Stephen Kuffler, John Eccles, and Bernard Katz (left to right) in Australia, about 1941.

ing motor nerve, lose their myelin sheath, and give off terminal branches that run in shallow grooves on the surface of the muscle. The **synaptic cleft** between the terminal and the muscle membrane is about 30 nm wide. Within the cleft is the **basal lamina**, which follows the contours of the muscle fiber surface. On the muscle, **postjunctional folds** radiate into the muscle fiber from the cleft at regular intervals. The grooves and folds are peculiar to skeletal muscle and are not a general feature of chemical synapses. In muscle, the region of postsynaptic specialization is known as the **motor end plate**. Schwann cell lamellae cover the nerve terminal, sending fingerlike processes around it at regularly spaced intervals.

Within the cytoplasm of the terminal, clusters of **synaptic vesicles** are associated with electron-dense material attached to the presynaptic membrane, forming **active zones**. Synaptic vesicles are sites of ACh storage; upon excitation of the axon terminal, they fuse with the presynaptic membrane at the active zone to spill their contents into the synaptic cleft by **exocytosis** (Chapter 11).[30]

Synapses on nerve cells are usually made by nerve terminal swellings called **boutons**, which are separated from the postsynaptic membrane by a synaptic cleft. The presynaptic membrane of the bouton displays electron-dense regions with associated clusters of synaptic vesicles, forming active zones similar to, but smaller than, those seen in skeletal muscle (Figure 9.4C). Boutons can be found contacting all regions of a nerve cell—dendrites, cell body, axon. On dendrites, many synaptic inputs occur on small **spines** that project from the main dendritic shaft. At nerve–nerve synapses the postsynaptic membrane often appears thickened and has electron-dense material associated with it.

Synaptic Potentials at the Neuromuscular Junction

Early studies by Eccles, Katz, and Kuffler used extracellular recording techniques to study the **end plate potential** (**EPP**) in muscle.[31,32] The EPP is the depolarization of the end plate region of the muscle fiber following motor nerve excitation, produced by ACh released from the presynaptic nerve terminals. Synaptic potentials similar to the EPP are seen in nerve cells. A synaptic potential that excites a postsynaptic cell is usually referred to as an **excitatory postsynaptic potential** (**EPSP**), and one that inhibits as an **inhibitory postsynaptic potential** (**IPSP**).

Normally the amplitude of the EPP in a skeletal muscle fiber is much greater than that needed to initiate an action potential. When an appropriate concentration of curare (about 1 μM) is added to the bathing solution, however, the amplitude of the EPP is reduced to below threshold, so that it no longer evokes (and becomes obscured by) the action potential (Figure 9.5). The effect of curare is graded; if the concentration is increased far enough, the end plate potential disappears entirely as all the receptors become blocked.

The intracellular microelectrode[33] was used by Fatt and Katz[34] to study in detail the time course and spatial distribution of the EPP in muscle fibers treated with curare. They stimulated the motor nerve and recorded the EPP intracellularly at various distances from

[30]Heuser, J. E. 1989. *Q. J. Exp. Physiol.* 74: 1051–1069.

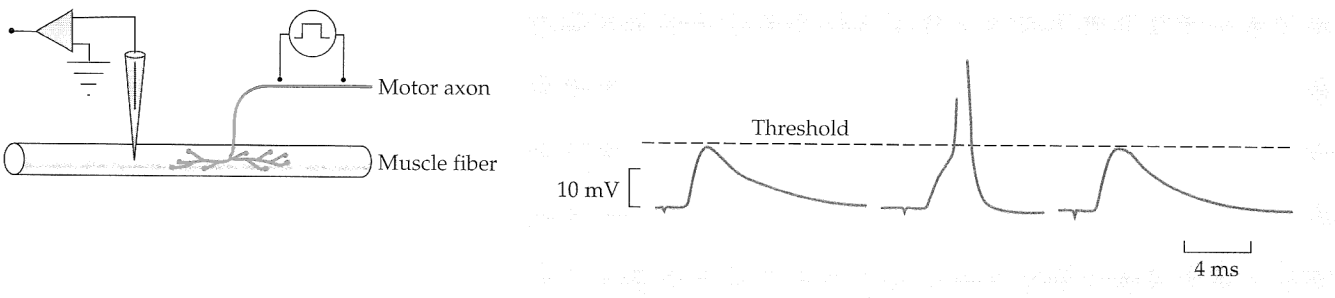
[31]Eccles, J. C., and O'Connor, W. J. 1939. *J. Physiol.* 97: 44–102.

[32]Eccles, J. C., Katz, B., and Kuffler, S. W. 1942. *J. Neurophysiol.* 5: 211–230.

[33]Ling, G., and Gerard, R. W. 1949. *J. Cell. Comp. Physiol.* 34: 383–396.

[34]Fatt, P., and Katz, B. 1951. *J. Physiol.* 115: 320–370.

FIGURE 9.5 Synaptic Potentials recorded with an intracellular microelectrode from a mammalian neuromuscular junction treated with curare. The curare concentration in the bathing solution was adjusted so that the amplitude of the synaptic potential was near threshold and so on occasion evoked an action potential in the muscle fiber. (From Boyd and Martin, 1956.)



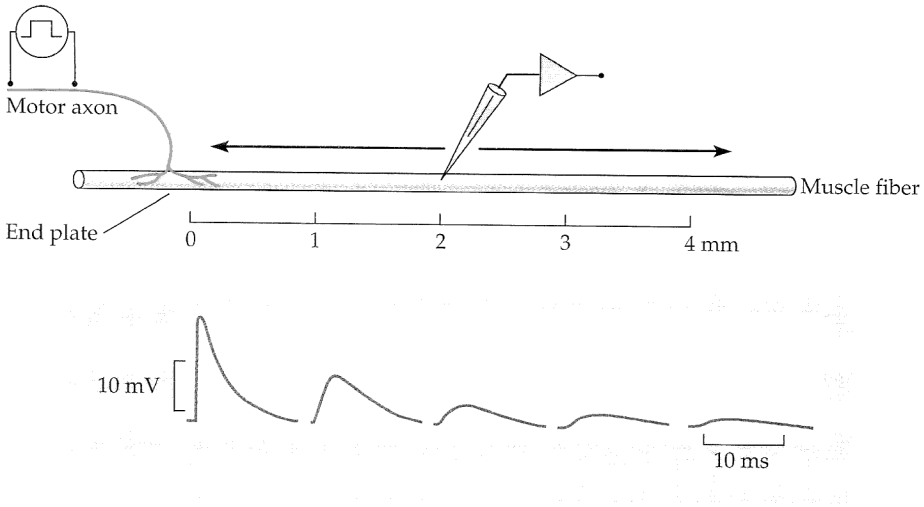


FIGURE 9.6 Decay of Synaptic Potentials with Distance from the end plate region of a muscle fiber. As the distance from the end plate increases, synaptic potentials recorded by an intracellular electrode decrease in size and rise more slowly. (After Fatt and Katz, 1951.)

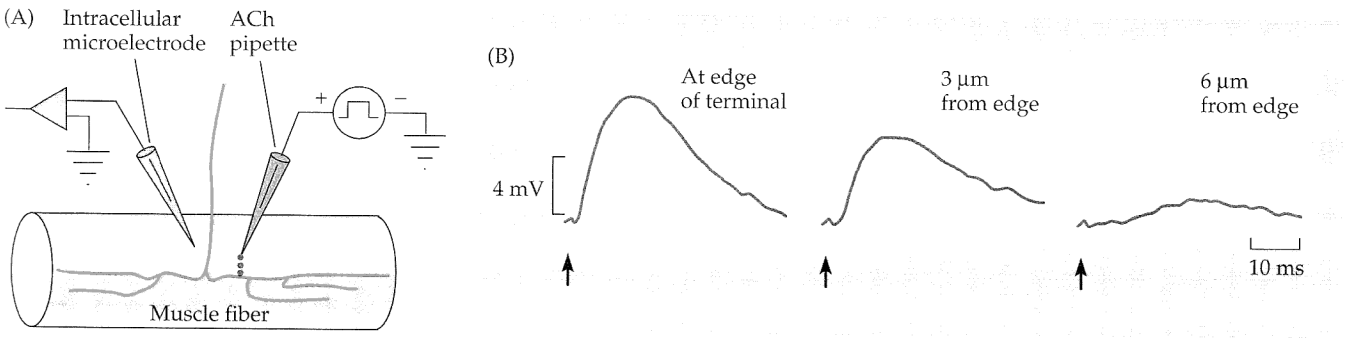
the end plate (Figure 9.6). At the end plate the depolarization rose rapidly to a peak and then declined slowly over the next 10 to 20 ms. As they moved the recording microelectrode farther and farther away from the end plate, the EPP amplitude became progressively smaller and its time to peak progressively longer. Fatt and Katz showed that after reaching its peak, the EPP decayed at a rate that was consistent with the time constant of the muscle fiber membrane, and that the decrement in EPP peak amplitude with distance from the end plate was predicted by the muscle fiber cable properties. Accordingly, they concluded that the end plate potential is generated by a brief surge of current that flows into the muscle fiber locally at the end plate and causes a rapid depolarization. The potential then decays passively, spreading beyond the end plate in both directions as it dies away.

Mapping the Region of the Muscle Fiber Receptive to ACh

The existence of special properties of skeletal muscle fibers in the region of innervation has been known since the beginning of the twentieth century. For example, Langley[35] assumed the presence of a "receptive substance" around motor nerve terminals, based on the finding that this region of the muscle fiber was particularly sensitive to various chemical agents, such as nicotine. Shortly after the introduction of the glass microelectrode for intracellular recording, microelectrodes were also used for discrete application of ACh (and later other drugs as well) to the end plate region of muscle.[36] The technique is illustrated in Figure 9.7A.

[35]Langley, J. N. 1907. *J. Physiol.* 36: 347–384.
[36]Nastuk, W. L. 1953. *Fed. Proc.* 12: 102.

FIGURE 9.7 Mapping the Distribution of ACh Sensitivity by Ionophoresis at the frog neuromuscular junction. (A) An ACh-filled pipette is placed close to the neuromuscular junction, and ACh is ejected from the tip by a brief, positive, voltage pulse (ionophoresis). An intracellular microelectrode is used to record the response from the muscle fiber. (B) Responses to small ionophoretic pulses of ACh applied at different distances from the axon terminal (indicated by the blue dots in [A]). The amplitude and rate of rise of the response decrease rapidly as ACh is applied farther from the terminal. (After Peper and McMahan, 1972.)



A microelectrode is inserted into the end plate of a muscle fiber for recording membrane potential while an ACh-filled micropipette is held just outside the fiber. To apply ACh, a brief positive voltage pulse is applied to the top of the pipette, causing a spurt of positively charged ACh ions to leave the pipette tip. This method of ejecting charged molecules from pipettes is known as ionophoresis. Using this method of application, del Castillo and Katz showed that ACh depolarized the muscle fiber only at the end plate region and only when applied to the outside of the fiber.[37] When the ACh-filled pipette is in close apposition to the end plate region, the response to ionophoresis is rapid (Figure 9.7B). Movement of the pipette by only a few micrometers results in a reduction in amplitude and slowing of the response.

The receptive substance postulated by Langley is now known to be the nicotinic acetylcholine receptor. The technique of ionophoresis made it possible to map with high accuracy the distribution of postsynaptic ACh receptors in muscle fibers[38] and nerve cells.[39] This method is particularly useful with thin preparations in which the presynaptic and postsynaptic structures can be resolved with interference contrast optics[40] and the position of the ionophoretic pipette in relation to the synapse can be determined with some precision.

One such preparation is the neuromuscular junction of the snake, shown in Figure 9.8. The end plates in snake muscle are about 50 μm in diameter, resembling in their compactness those seen in mammals. Each axon terminal consists of 50 to 70 terminal swellings, analogous to synaptic boutons, from which transmitter is released. The swellings rest in craters sunk into the surface of the muscle fiber. An electron micrograph of such a synapse is shown in Figure 9.8B, again illustrating the characteristic features observed at all chemical synapses. Figure 9.8C shows an electron micrograph of a typical ionophoretic micropipette. The opening is about 50 nm, similar in size to a synaptic vesicle.

The sharp delineation of sensitivity to ACh at the snake neuromuscular junction can be demonstrated dramatically in muscle fibers in which the motor nerve terminal has been removed by bathing the muscle in a solution of the enzyme collagenase, which frees the terminal without damaging the muscle fiber.[41,42] The process of lifting off the terminals is shown in Figure 9.9A. Each of the boutons leaves behind a circumscribed crater lined with the exposed postsynaptic membrane. This is shown in more detail in Figure 9.9B, in which an ACh-filled micropipette points at an empty crater. If the tip of the pipette is placed on the postsynaptic membrane, 1 pC (picocoulomb) of charge passed through the pipette releases enough ACh to cause, on the average, a 5 mV depolarization. The sensitivity of the membrane is then said to be 5000 mV/nC (Figure 9.9C). In contrast, at a distance of about 2 μm, just outside the crater, the same amount of ACh applied to the extrasynaptic membrane produces a response that is 50 to 100 times smaller. Along the rims of the craters the sensitivity fluctuates over a wide range.

Other Techniques for Determining the Distribution of ACh Receptors

A second way to determine the distribution of ACh receptors is to use α-bungarotoxin, a snake toxin that binds selectively and irreversibly to nicotinic ACh receptors. The distri-

[37]del Castillo, J., and Katz, B. 1955. *J. Physiol.* 128: 157–181.

[38]Miledi, R. 1960. *J. Physiol.* 151: 24–30.

[39]Dennis, M. J., Harris, A. J. and Kuffler, S. W. 1971. *Proc. R. Soc. Lond. B* 177: 509–539.

[40]McMahan, U. J., Spitzer, N. C., and Peper, K. 1972. *Proc. R. Soc. Lond. B* 181: 421–430.

[41]Betz, W. J., and Sakmann, B. 1973. *J. Physiol.* 230: 673–688.

[42]Kuffler, S. W., and Yoshikami, D. 1975. *J. Physiol.* 244: 703–730.

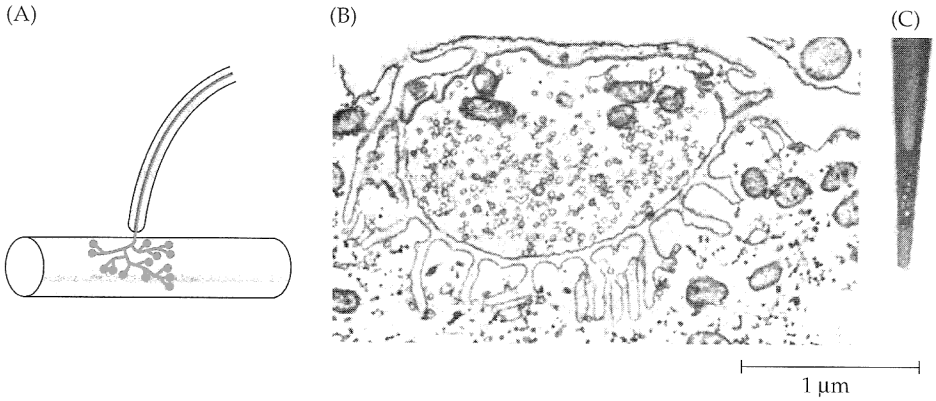


FIGURE 9.8 Skeletal Neuromuscular Junction of the Snake. (A) An end plate on a skeletal muscle of a snake. The axon terminates in a cluster of boutons. (B) Electron micrograph of a cross section through a bouton. Synaptic vesicles, which mediate ACh release from the nerve terminal, are 50 nm in diameter. (C) Electron micrograph of the tip of a micropipette used for ionophoresis of ACh, shown at the same magnification as (B). The pipette has an outer diameter of 100 nm and an opening of about 50 nm. (From Kuffler and Yoshikami, 1975.)

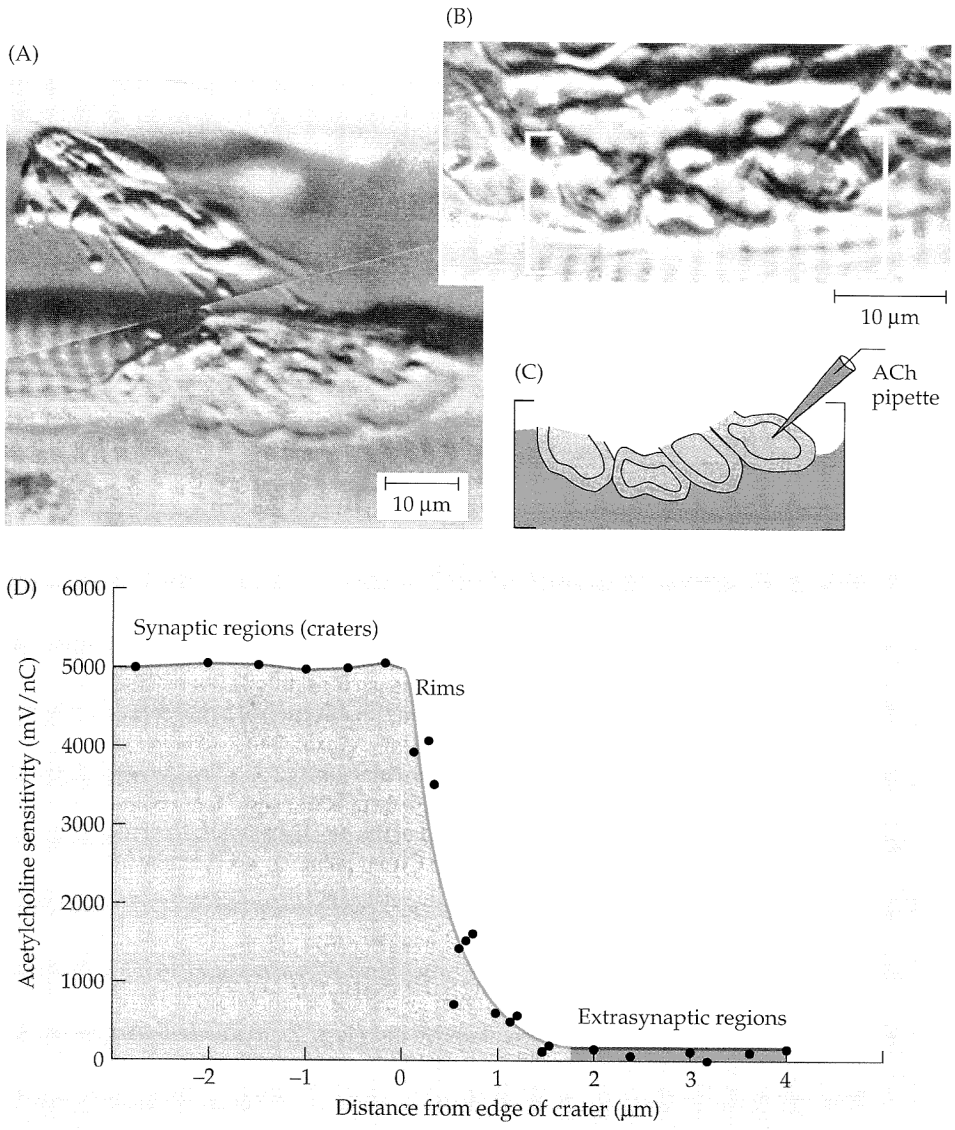


FIGURE 9.9 Acetylcholine Receptor Distribution at the skeletal neuromuscular junction of the snake. (A) Light micrograph showing the removal of the nerve terminal from the neuromuscular junction in a muscle treated with collagenase. (B) Light micrograph of the postsynaptic surface of the muscle cell exposed by removal of the nerve terminal. An ACh-filled pipette (entering from upper right; compare with part C) points to a crater that had been occupied by a terminal bouton. (C) Drawing of the area bracketed in B, showing the position of four craters formerly occupied by terminal boutons. Blue areas represent postsynaptic membrane within the craters, orange areas are the crater rims, and green areas are extrasynaptic regions. (D) Distribution of ACh sensitivity. The craters have a uniformly high sensitivity to ACh (5000 mV/nC); the sensitivity declines steeply at the rims of the craters; extrasynaptic regions have a uniformly low ACh sensitivity (100 mV/nC). (After Kuffler and Yoshikami, 1975.)

bution of bound toxin can be visualized using histochemical techniques. For example, fluorescent markers can be attached to α-bungarotoxin and the distribution of receptors visualized by fluorescence microscopy[43] (Figure 9.10A); or the enzyme horseradish peroxidase (HRP) can be linked to α-bungarotoxin and its dense reaction product visualized in the electron microscope[44] (Figure 9.10B). Such techniques confirm that receptors are highly restricted to the membrane immediately beneath the axon terminal. Even more precise, quantitative estimates of the concentration of ACh receptors can be obtained using radioactive α-bungarotoxin and autoradiography (Figure 9.10C).[45] By counting the number of silver grains exposed in the emulsion, the density of receptors can be determined. In muscle the density is highest along the crests and upper third of the junctional folds (about $10^4/\mu m^2$); the density in extrasynaptic regions is much lower (about $5/\mu m^2$).[46] Transmitter receptors are highly concentrated in the postsynaptic membrane at synapses throughout the central and peripheral nervous systems.

Another way to apply neurotransmitters and other substances to nerve and muscle fiber membranes is by **pressure ejection**. Brief pulses of pressure are applied to the top of a pipette to drive solution from the tip. The method has an advantage over ionophoresis in that the substance to be applied need not carry a net charge. Substances can also be applied to cells or membrane patches using fast flow techniques that allow extremely rapid changes between solutions of known composition.[47]

[43] Ravdin, P., and Axelrod, D. 1977. *Anal. Biochem.* 80: 585–592.

[44] Burden, S. J., Sargent, P. B., and McMahan, U. J. 1979. *J. Cell Biol.* 82: 412–425.

[45] Fertuck, H. C., and Salpeter, M. M. 1974. *Proc. Natl. Acad. Sci. USA* 71: 1376–1378.

[46] Salpeter, M. M. 1987. In *The Vertebrate Neuromuscular Junction*. Alan R. Liss, New York, pp. 1–54.

[47] Heckmann, M., and Dudel, J. 1997. *Biophys. J.* 72: 2160–2169.

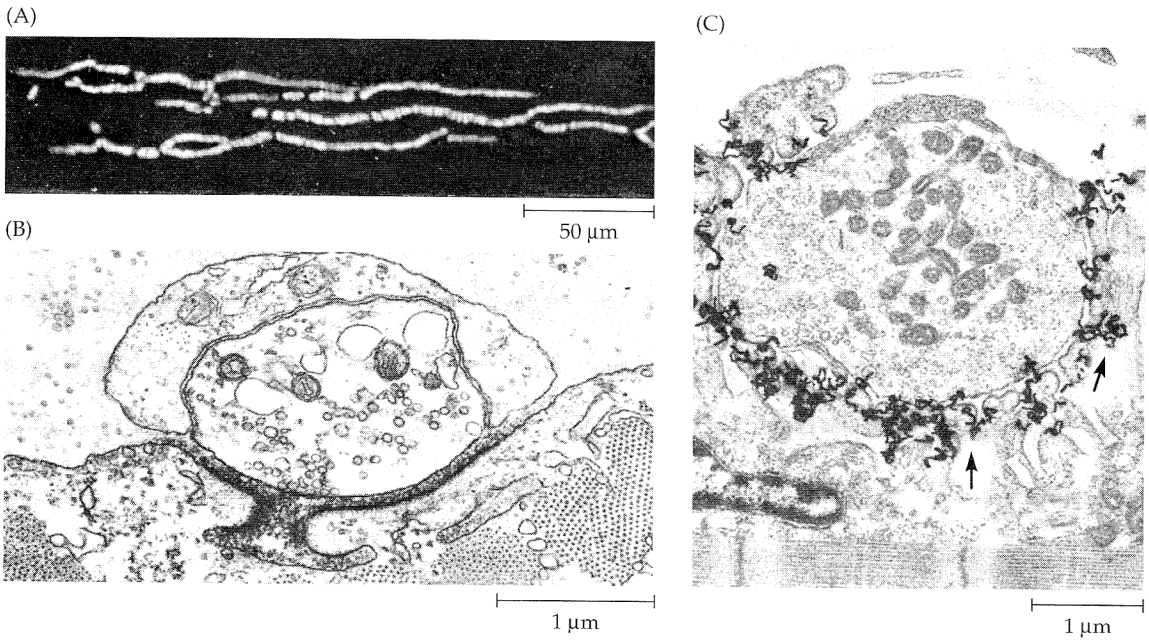


FIGURE 9.10 Visualizing the Distribution of ACh Receptors at the neuromuscular junction. (A) Fluorescence micrograph of a frog cutaneous pectoris muscle fiber stained with rhodamine α-bungarotoxin. (B) Electron micrograph of a cross section of a frog cutaneous pectoris neuromuscular junction labeled with HRP-α-bungarotoxin. Dense reaction product fills the synaptic cleft. (C) Autoradiograph of a neuromuscular junction in a lizard intercostal muscle labeled with [^{125}I]-α-bungarotoxin. Silver grains (arrows) show that receptors are concentrated at the tops and along the upper third of the junctional folds. (A kindly provided by W. J. Betz; B kindly provided by U. J. McMahan; C from Salpeter, 1987, kindly provided by M. M. Salpeter.)

Measurement of Ionic Currents Produced by ACh

How does ACh produce an inward current at the end plate? Experiments by Fatt and Katz led them to conclude that ACh produces a marked, nonspecific increase in permeability of the postsynaptic membrane to small ions.[33] Two techniques were subsequently used to assess the permeability changes produced by ACh. One involved the use of radioactive isotopes, which showed that the permeability of the postsynaptic membrane was increased to sodium, potassium, and calcium, but not to chloride.[48] This experiment provided convincing evidence concerning the ion species involved but did not reveal the details of the conductance changes, their timing, or their voltage dependence. This information was provided by voltage clamp experiments, first performed by A. and N. Takeuchi, who used two microelectrodes to voltage-clamp the end plate region of muscle fibers.[49] The experimental arrangement is shown in Figure 9.11A. Two microelectrodes were inserted into the end plate region of a frog muscle fiber—one for recording membrane potential (V_m), the other for injecting current to clamp the membrane potential at the desired level. The nerve was then stimulated to release ACh, or, in later experiments, ACh was applied directly by ionophoresis. Subsequently, similar experiments were carried out by Magleby and Stevens[50] in muscle fibers treated with hypertonic glycerol, which has the advantage of preventing muscle fibers from contracting when depolarized, but the disadvantage of leaving the muscle fibers in an artificially depolarized state.

Figure 9.11B illustrates results from such a glycerol-treated muscle fiber. With the muscle membrane potential clamped at –40 mV, nerve stimulation produced an inward current, which would have caused a depolarization if the fiber had not been voltage-clamped. At more negative holding potentials, the end plate current increased in amplitude. When the membrane was depolarized, the end plate current decreased in amplitude. With further depolarization the current reversed direction and was outward.

[48]Jenkinson, D. H., and Nicholls, J. G. 1961. *J. Physiol.* 159: 111–127.

[49]Takeuchi, A., and Takeuchi, N. 1959. *J. Neurophysiol.* 22: 395–411.

[50]Magleby, K. L., and Stevens, C. F. 1972. *J. Physiol.* 223: 151–171.

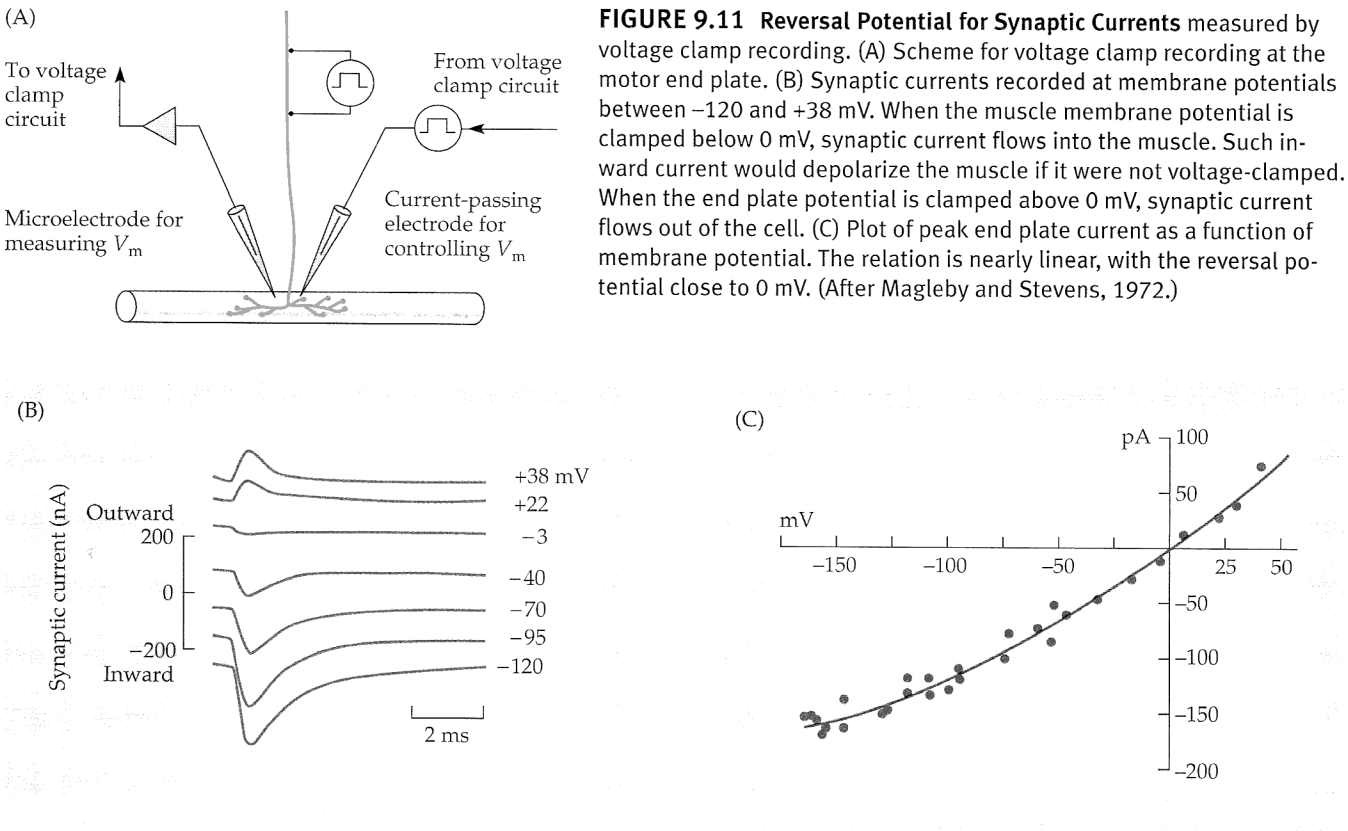


FIGURE 9.11 Reversal Potential for Synaptic Currents measured by voltage clamp recording. (A) Scheme for voltage clamp recording at the motor end plate. (B) Synaptic currents recorded at membrane potentials between −120 and +38 mV. When the muscle membrane potential is clamped below 0 mV, synaptic current flows into the muscle. Such inward current would depolarize the muscle if it were not voltage-clamped. When the end plate potential is clamped above 0 mV, synaptic current flows out of the cell. (C) Plot of peak end plate current as a function of membrane potential. The relation is nearly linear, with the reversal potential close to 0 mV. (After Magleby and Stevens, 1972.)

Figure 9.11C shows a plot of the peak amplitude of the end plate current as a function of holding potential. The current changed from inward to outward near zero membrane potential. Accordingly, zero is called the **reversal potential**, V_r. In earlier experiments on intact muscle fibers, A. and N. Takeuchi estimated the reversal potential to be about −15 mV.[51]

Significance of the Reversal Potential

The reversal potential for the end plate current gives us information about the ionic currents flowing through the channels activated by ACh in the postsynaptic membrane. For example, if the channels were permeable exclusively to sodium, then current through the channels would be zero at the sodium equilibrium potential (about +50 mV). The other major ions, potassium and chloride, have equilibrium potentials near −90 mV, the normal resting membrane potential (Chapter 5); the calcium equilibrium potential is approximately +120 mV. None of the ions has an equilibrium potential in the range of 0 to −15 mV. What ions, then, are involved in the response? Consistent with the results of radioactive tracer experiments, A. and N. Takeuchi showed that changing the concentrations of sodium, potassium, or calcium in the bathing solution resulted in changes in the reversal potential, but changes in extracellular chloride did not.[33,52] They concluded that the effect of ACh was to produce a general increase in *cation* permeability.

Relative Contributions of Sodium, Potassium, and Calcium to the End Plate Potential

ACh opens channels in the end plate membrane that, at the normal resting potential, allow sodium and calcium ions to leak inward and potassium ions outward along their electrochemical gradients. Because the calcium conductance of the channels is small, the contribution of calcium to the overall synaptic current can be ignored, as can that of other

[51]Takeuchi, A., and Takeuchi, N. 1960. *J. Physiol.* 154: 52–67.

[52]Takeuchi, N. 1963. *J. Physiol.* 167: 128–140.

cations, such as magnesium. (It should be noted that the low calcium *conductance* is due to its low extracellular and intracellular concentrations; calcium *permeability* is about 20% of the sodium permeability.) The equivalent electrical circuit is shown in Figure 9.12A. The resting membrane consists of the usual sodium, potassium, and chloride channels. It is in parallel with ACh-activated channels for sodium and potassium, Δg_{Na} and Δg_K. The Takeuchis calculated that for a reversal potential $V_r = -15$ mV, the ratio of the sodium to potassium conductance changes, $\Delta g_{Na}/\Delta g_K$, is about 1.3 (Box 9.1). The channel opened by ACh is, in fact, nearly equally permeable to sodium and potassium.[53] However, taking the extracellular and intracellular solutions together, there are more sodium than potassium ions available to move through the channels (Chapter 5). Thus, for the same permeability change, the sodium conductance change is slightly larger (Chapter 2).

Resting Membrane Conductance and Synaptic Potential Amplitude

The electrical circuit shown in Figure 9.12A can be simplified by representing the resting membrane as a single conductance, g_{rest} (equal to the sum of all the ionic conductances), and a single battery, V_{rest} (equal to the resting membrane potential). Likewise, the synaptic membrane can be represented by a single conductance Δg_s and a battery whose voltage is equal to the reversal potential V_r (Figure 9.12B). A feature of this electrical circuit is that the amplitude of a synaptic potential depends on both Δg_s and g_{rest}.

For simplicity, let us consider the steady-state membrane potential that would develop if the synaptic conductance were activated for a long period of time. If Δg_s were much larger than g_{rest}, then the membrane potential would approach V_r. However, if Δg_s were equal to g_{rest}, then the change in membrane potential produced by activating the synaptic conductance would be only one-half as great. Thus, the amplitude of a synaptic potential can be increased by either increasing the synaptic conductance (i.e., activating more synaptic channels) or decreasing the resting conductance. Indeed, a reduction in mem-

[53] Adams, D. J., Dwyer, T. M., and Hille, B. 1980. *J. Gen. Physiol.* 75: 493–510.

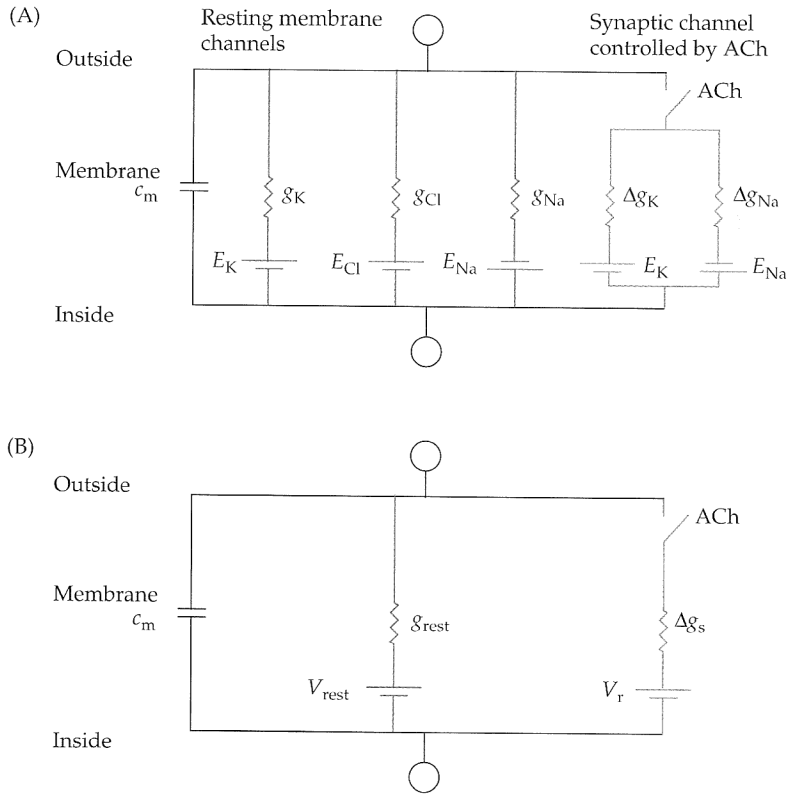


FIGURE 9.12 Electrical Model of the Postsynaptic Membrane with channels activated by ACh in parallel with the resting membrane channels and with the membrane capacitance, c_m. (A) The synaptic channel opened by ACh is electrically equivalent to two independent pathways for sodium and potassium. The resting membrane has channels for potassium, chloride, and sodium. (B) The synaptic channel can be represented as a single pathway with conductance Δg_s and a battery equal to the reversal potential V_r. The resting membrane can be represented as a single pathway with conductance g_{rest} and a battery equal to V_{rest}.

Box 9.1 Electrical Model of the Motor End Plate

How did A. and N. Takeuchi calculate the ratio of sodium to potassium conductance for the channels opened by ACh? They proposed an electrical model of the muscle cell membrane similar to that shown in Figure 9.12A. Although ACh receptors do not form separate pathways for sodium and potassium, the two ions move through the channel independently. Therefore, the synaptic conductance and reversal potential can be represented by separate conductances (Δg_{Na} and Δg_K) and driving potentials (E_{Na} and E_K) for sodium and potassium. Accordingly, separate expressions can be written for the sodium and potassium currents (ΔI_{Na} and ΔI_K):

$$\Delta I_{Na} = \Delta g_{Na}(V_m - E_{Na})$$

$$\Delta I_K = \Delta g_K(V_m - E_K)$$

These equations provide a means of determining the relative conductance changes to sodium and potassium produced by ACh, once the reversal potential (V_r) is determined. Since the Takeuchis considered only *changes* in current resulting from the action of ACh, they could ignore the resting membrane channels. The net synaptic current is zero at the reversal potential; therefore, at this potential the inward sodium current is exactly equal and opposite to the outward potassium current. So when $V_m = V_r$,

$$\Delta g_{Na}(V_r - E_{Na}) = -\Delta g_K(V_r - E_K)$$

It follows that

$$\frac{\Delta g_{Na}}{\Delta g_{Na}} = \frac{-(V_r - E_K)}{(V_r - E_{Na})}$$

We can rearrange the equations regarding synaptic sodium and potassium currents to predict the reversal potential when the relative conductances are known:

$$V_r = \frac{\Delta g_{Na}E_{Na} + \Delta g_K E_K}{(\Delta g_{Na} + \Delta g_K)}$$

Thus, the reversal potential is simply the average of the individual equilibrium potentials, weighted by the relative conductance changes. This relationship can be extended to include any number or variety of ions, so it is applicable at any synapse where transmitters produce a change in conductance of the postsynaptic membrane to one or more ions. This relationship was found to predict how changes in E_{Na} and E_K, produced by changes in extracellular concentrations of sodium and potassium, affected the reversal potential at the neuromuscular junction.[51]

Such predictions were accurate only for small changes in extracellular sodium and potassium, however, because channel conductance is determined in part by ion concentration (Chapters 2 and 5). Therefore, the effect of a large change in sodium, potassium, or calcium concentration on reversal potential is predicted accurately only if the resulting change in conductance is taken into account. Alternatively, the analysis can be made in terms of permeabilities, using the constant field equation developed by Goldman, Hodgkin, and Katz (Chapter 5).[54,55]

[54]Ritchie, A., and Fambrough, D. M. 1975. *J. Gen. Physiol.* 65: 751–767.

[55]Lassignal, N. L., and Martin, A. R. 1977. *J. Gen. Physiol.* 70: 23–36.

brane conductance is an important mechanism for modulating synaptic strength. For example, certain inputs to autonomic ganglion cells in the bullfrog close potassium channels, thereby increasing the amplitude of excitatory synaptic potentials produced by other inputs to the cell (Chapter 16).

Kinetics of Currents through Single ACh Receptor Channels

To what extent does the time course of the end plate current reflect the behavior of individual ACh channels? For example, do individual channels open and close repetitively during the end plate current, with the probability of channel opening declining with time? Or do individual channels open only once, so that the time course of the current is determined by how long channels remain open?

Definitive answers to such questions came only with the advent of patch clamp techniques, by which the behavior of individual channels could be observed directly (Chapter 2).[56] When ACh was applied continuously, ACh channels were shown to open instantaneously in an all-or-nothing fashion, and then close at a rate that matched exactly the rate of decay of the end plate current.[57] This idea can be summarized by the following

[56]Neher, E., and Sakmann, B. 1976. *Nature* 260: 799–802.

[57]Dionne, V. E., and Leibowitz, M. D. 1982. *Biophys. J.* 39: 253–261.

scheme, indicating the interaction between the transmitter molecule A (for "agonist") and the postsynaptic receptor molecule R:

$$2A + R \rightleftharpoons A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^*$$

Two ACh molecules combine with the channel (one on each α subunit; Chapter 3), which then undergoes a change in conformation from the closed (A_2R) to the open (A_2R^*) state. The transitions between the open and closed states are characterized by the rate constants α and β, as indicated. Now consider the time course of the end plate current, as illustrated in Figure 9.13. ACh arriving at the postsynaptic membrane opens a large number of channels almost simultaneously. Because ACh is lost rapidly from the synaptic cleft (because of hydrolysis by cholinesterase and diffusion), each channel opens only once. As the channels close, the synaptic current declines. Thus, the time course of decay of the end plate current reflects the rate at which individual ACh channels close. Channels close at the rate $\alpha \times [A_2R^*]$; that is, many channels close very quickly, and fewer and fewer channels close at longer and longer times (see Figure 9.13). As with all independent or random events, the open times are distributed exponentially, and the **mean open time** (τ) is equal to the time constant of the decay of the end plate current, $1/\alpha$.

Patch clamp experiments have revealed a number of details of channel activation that were previously undetectable. For example, local anesthetics such as procaine prolong the falling phase of the end plate current (in addition to blocking sodium channels and therefore action potentials). Patch clamp experiments showed that this prolongation occurs because procaine causes the current through open ACh channels to "flicker" on and off, apparently as a consequence of the anesthetic molecule itself moving rapidly into and out of the pore of the open channel and thereby intermittently obstructing current flow.[58–60] Subsequent experiments revealed that procaine also inhibits channel opening by binding to sites on the receptor *outside* the pore.[61] Indeed, channel properties can be modified by a wide variety of drugs, including cocaine,[62] barbiturates,[63] steroids,[64] and general anesthetics,[65] which bind to sites both within and outside the pore.

The properties of acetylcholine receptors change during development. There is a fetal form of the acetylcholine receptor, which has a low conductance and a long and variable open time, and an adult form, which has a higher conductance and shorter open time.[66,67] The switch from embryonic to adult receptors is caused by a change in subunit composition (Chapters 3 and 23), and splice variants of one of the embryonic subunits may account for variation in mean channel open time early in development.[68]

[58]Neher, E., and Steinbach, J. H. 1978. *J. Physiol.* 277: 153–176.

[59]Galzi, J-L., et al. 1991. *Annu. Rev. Pharmacol.* 31: 37–72.

[60]Lester, H. A. 1992. *Annu. Rev. Biophys. Biomol. Struct.* 21: 267–292.

[61]Niu, L., and Hess, G. P. 1993. *Biochemistry* 32: 3831–3835.

[62]Niu, L., Abood, L. G., and Hess, G. P. 1995. *Proc. Natl. Acad. Sci. USA* 92: 12008–12012.

[63]Dilger, J. P., et al. 1997. *J. Gen. Physiol.* 109: 401–414.

[64]Bouzat, C., and Barrantes, F. J. 1996. *J. Biol. Chem.* 271: 25835–25841.

[65]Dilger, J. P., Liu, Y., and Vidal, A. M. 1995. *Eur. J. Anaesthesiol.* 12: 31–39.

[66]Mishina, M., et al. 1986. *Nature* 321: 406–411.

[67]Grassi, F., et al. 1998. *J. Physiol.* 508: 393–400.

[68]Herlitze, S., et al. 1996. *J. Physiol.* 492: 775–787.

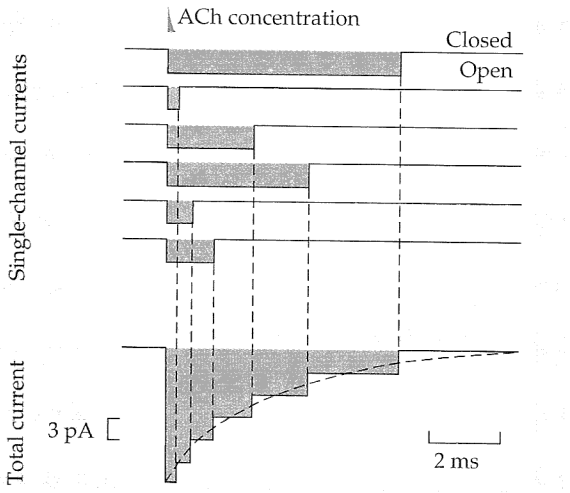


FIGURE 9.13 Total End Plate Current Is the Sum of Individual Channel Currents. Current flow through six individual channels is depicted in the top panel. Channels open instantaneously in response to ACh. ACh is rapidly hydrolyzed, preventing any further channel openings. Channel open times are distributed exponentially. The individual channel currents sum to give the total end plate current (lower panel). The time constant of the decay of the total current is equal to the mean open time of the individual channels.

DIRECT SYNAPTIC INHIBITION

The principles that underlie direct chemical synaptic excitation at the neuromuscular junction also apply to **direct chemical inhibitory synapses**. Whereas excitation occurs by opening channels in the postsynaptic membrane whose reversal potential is *positive* to threshold, direct chemical synaptic inhibition is achieved by opening channels whose reversal potential is *negative* to threshold. Direct chemical synaptic inhibition occurs by activating channels permeable to chloride, an anion that typically has an equilibrium potential at or near the resting potential. Pioneering studies of direct chemical synaptic inhibition were made on the crustacean neuromuscular junction,[69,70] the crayfish stretch receptor,[71] and spinal motoneurons of the cat.[72]

Reversal of Inhibitory Potentials

Spinal motoneurons are inhibited by sensory inputs from antagonistic muscles, by way of inhibitory interneurons in the spinal cord. The effect of activation of inhibitory inputs can be studied by an experiment similar to that illustrated in Figure 9.14A. The motoneuron is impaled with two micropipettes—one to record potential changes, the other to pass current through the cell membrane. At the normal resting potential (about –75 mV), stimulation of the inhibitory inputs causes a slight hyperpolarization of the cell—the **inhibitory postsynaptic potential** (**IPSP**) (Figure 9.14B). When the membrane is depolarized by passing positive current into the cell, the amplitude of the IPSP is increased. When the cell is hyperpolarized to –82 mV, the inhibitory potential is very small and reversed in sign, and at –100 mV the reversed inhibitory potential is increased in amplitude. The reversal potential in this experiment is thus about –80 mV.

[69]Dudel, J., and Kuffler, S. W. 1961. *J. Physiol.* 155: 543–562.

[70]Takeuchi, A., and Takeuchi, N. 1967. *J. Physiol.* 191: 575–590.

[71]Kuffler, S. W., and Eyzaguirre, C. 1955. *J. Gen. Physiol.* 39: 155–184.

[72]Coombs, J. S., Eccles, J. C. and Fatt, P. 1955. *J. Physiol.* 130: 326–373.

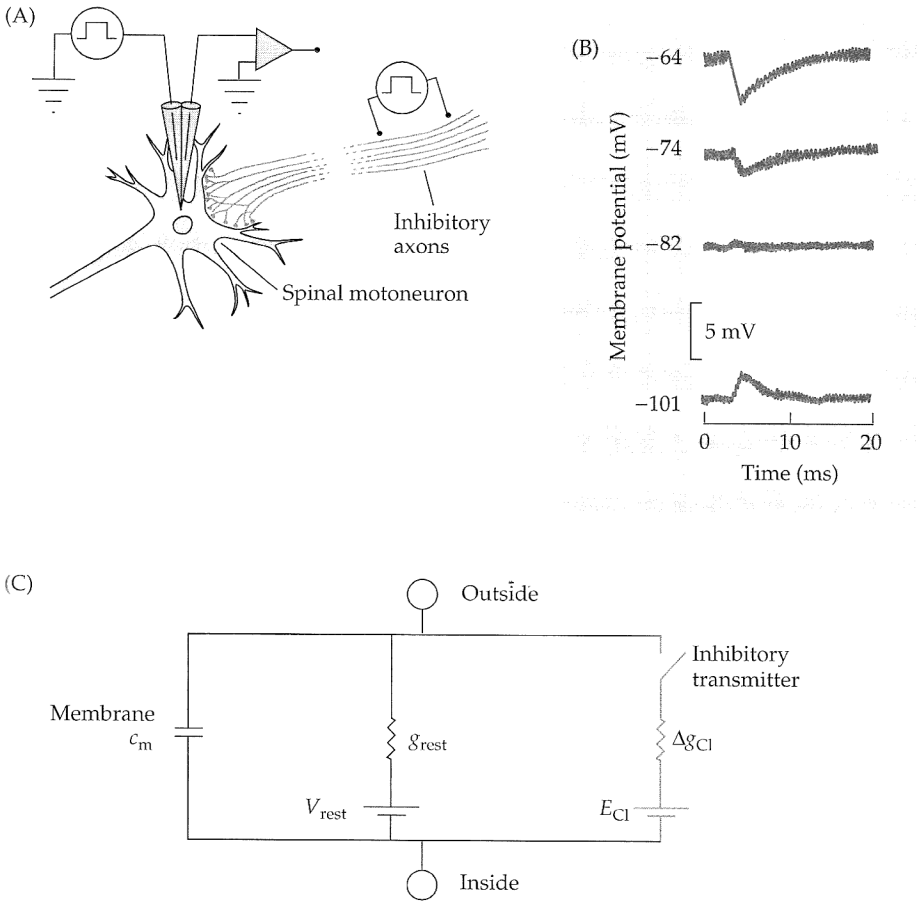


FIGURE 9.14 Direct Inhibitory Chemical Synaptic Transmission. (A) Scheme for intracellular recording from a cat spinal motoneuron and stimulation of inhibitory synaptic inputs. The membrane potential of the motoneuron is set to different levels by passing current through a second intracellular microelectrode. (B) Intracellular records of synaptic potentials evoked at membrane potentials between –64 and –101 mV. The reversal potential is between –74 and –82 mV. (C) Electrical model of the motoneuron membrane with chloride channels activated by the inhibitory transmitter, Δg_{Cl}, in parallel with the resting membrane channels, g_{rest}, and the membrane capacitance, c_m. (A and B after Coombs, Eccles, and Fatt, 1955.)

Inhibitory channels are permeable to anions, with permeabilities roughly correlated with the hydrated radius of the penetrating ion.[72,73] In physiological circumstances, the only small anion present in any quantity is chloride. Thus, in spinal motoneurons injection of chloride into the cell from a micropipette shifts the chloride equilibrium potential, and hence the reversal potential for the IPSP, in the positive direction. In other preparations, changes in extracellular chloride have been shown to produce corresponding changes in the chloride equilibrium potential and the IPSP reversal potential, but such experiments often give ambiguous results. This is because changes in extracellular chloride concentration lead eventually to proportionate changes in intracellular concentration as well (Chapter 5), so any change in chloride equilibrium potential is only transient.

One way around this difficulty is to remove chloride entirely, as shown in Figure 9.15. The records are from a reticulospinal cell in the brainstem of the lamprey, in which inhibitory synaptic transmission is mediated by glycine.[74] Membrane potential was recorded with an intracellular microelectrode. A second electrode was used to pass brief hyperpolarizing current pulses into the cell; the resulting changes in potential provided a measure of the cell's input resistance. Finally, a third micropipette was used to apply glycine to the cell close to an inhibitory synapse, using brief pressure pulses. Glycine application resulted in a slight hyperpolarization, with a marked reduction in input resistance (Figure 9.15A), as would be expected if glycine activated a large number of chloride channels. To test this idea, chloride was removed from the bathing solution and replaced by the impermeant ion isethionate. As a result, intracellular chloride was also removed, by efflux through chloride channels open at rest. After 20 min, glycine application produced no detectable change in membrane potential or input resistance (Figure 9.15B), indicating that no ions other than chloride pass through the inhibitory channels. The restoration of normal extracellular chloride concentration (Figure 9.15C) resulted in restoration of the response.

Given that the inhibitory response involves an increase in chloride permeability, the reversal potential for the inhibitory current will be equal to the chloride equilibrium potential and the magnitude of the current will be given by

$$\Delta i_{\text{inhibitory}} = \Delta i_{\text{Cl}} = \Delta g_{\text{Cl}}(V_{\text{m}} - E_{\text{Cl}})$$

At membrane potentials positive to E_{Cl} the current is outward, resulting in membrane hyperpolarization. In this case outward current is carried by an influx of negatively charged

[73]Hille, B. 1992. *Ionic Channels of Excitable Membranes*, 2nd Ed. Sinauer, Sunderland, MA.

[74]Gold, M. R., and Martin, A. R. 1983. *J. Physiol.* 342: 99–117.

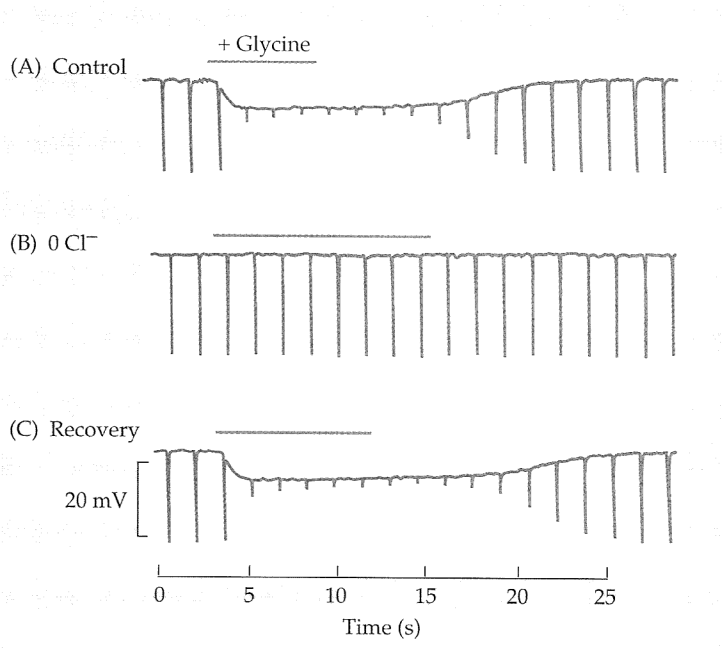


FIGURE 9.15 Inhibitory Response to Glycine Depends on Chloride. Intracellular microelectrode recordings from a neuron in the brainstem of the lamprey. (A) Resting membrane potential is –63 mV. Brief downward voltage deflections are produced by 10 nA current pulses from a second intracellular microelectrode; their amplitude indicates membrane resistance. On application of glycine (bar), the cell is hyperpolarized by about 7 mV and membrane resistance is reduced drastically. (B) After 20 min in chloride-free bathing solution, the response to glycine is abolished. (C) Five minutes after return to normal chloride solution the response has recovered. (From Gold and Martin, 1983.)

chloride ions. At membrane potentials negative to E_{Cl}, inhibition causes an efflux of chloride ions, resulting in depolarization. The equivalent circuit is shown in Figure 9.14C.

Early in postnatal development of the mammalian central nervous system, GABA and glycine paradoxically depolarize and thereby excite neurons in the hippocampus.[75] This effect is due not to differences in the properties of the channels opened by GABA and glycine, but to a difference in the regulation of intracellular chloride that results in a change in the chloride equilibrium potential.

Stephen W. Kuffler in 1975

Presynaptic Inhibition

So far we have defined excitatory and inhibitory synapses on the basis of the effect of the transmitter on the postsynaptic membrane—that is, on whether the postsynaptic permeability change is to cations or to anions. However, a number of early experiments indicated that in some instances it was difficult to account for inhibition in terms of postsynaptic permeability changes alone.[76,77] The paradox was resolved by the discovery of an additional inhibitory mechanism, **presynaptic inhibition**, described in the mammalian spinal cord by Eccles and his colleagues[78] and at the crustacean neuromuscular junction by Dudel and Kuffler.[69] Presynaptic inhibition results in a reduction in the amount of transmitter released from excitatory nerve terminals.[69,79,80]

As shown in Figure 9.16, the action of the inhibitory nerve at the crustacean neuromuscular junction is exerted not only on the muscle fibers, but also on the excitatory terminals. The presynaptic effect is brief, reaching a peak in a few milliseconds and declining to zero after a total of 6 to 7 ms. For the maximum inhibitory effect to occur, the impulse must arrive in the inhibitory presynaptic terminal several milliseconds before the action potential arrives in the excitatory terminal. The importance of accurate timing is shown in Figure 9.16, where parts A and B show the excitatory and inhibitory potentials following separate stimulation of the corresponding nerves. In Figure 9.16C both nerves are stimulated, but the action potential in the inhibitory nerve follows that in the excitatory nerve by 1.5 ms, arriving too late to exert any effect. In Figure 9.16D, on the other hand, the action potential in the inhibitory nerve precedes that in the excitatory nerve and causes a marked reduction in the size of the excitatory postsynaptic potential.

The presynaptic effect, like that on the postsynaptic membrane, is mediated by γ-aminobutyric acid (GABA), and is associated with a marked increase in chloride per-

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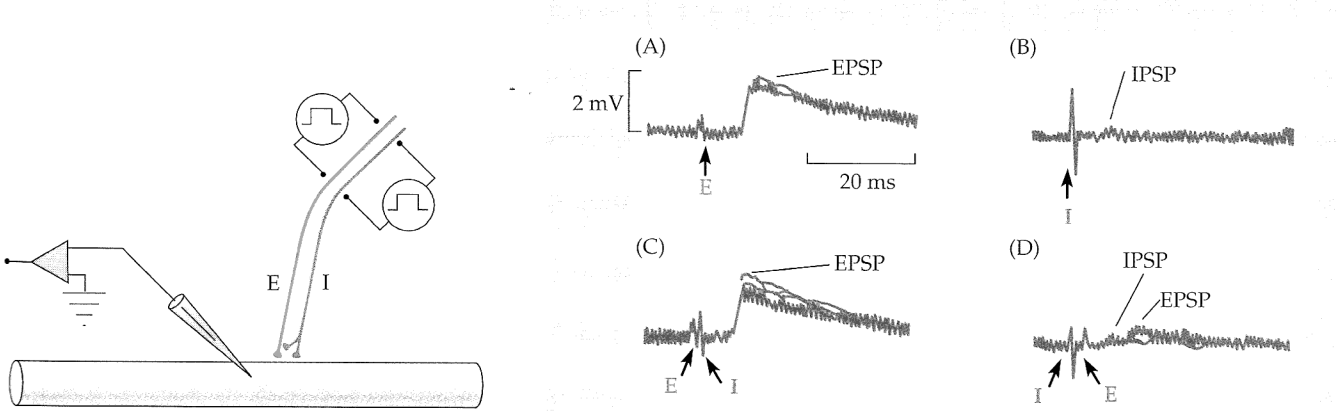
FIGURE 9.16 Presynaptic Inhibition in a crustacean muscle fiber innervated by one excitatory and one inhibitory axon. (A) Stimulation of the excitatory axon (E) produces a 2 mV EPSP. (B) Stimulation of the inhibitory axon (I) produces a depolarizing IPSP of about 0.2 mV. (C) If the inhibitory stimulus follows the excitatory one by a short interval, there is no effect on the EPSP. (D) If the inhibitory stimulus precedes the excitatory one by a few milliseconds, the EPSP is almost abolished. The importance of precise timing indicates that the inhibitory nerve is having a presynaptic effect, reducing the amount of excitatory neurotransmitter that is released. (After Dudel and Kuffler, 1961.)



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meability in the presynaptic terminals.[81,82] An explanation that has been suggested is that when chloride permeability is high, the depolarizing effect of sodium influx during the rising phase of the action potential is canceled in part by an accompanying influx of chloride. As a result, the presynaptic action potential is smaller in amplitude, and its effectiveness in releasing transmitter is reduced. At many mammalian synapses, presynaptic inhibition has been shown to be due to inhibition of voltage-dependent calcium channels in the axon terminal.[83]

In the nervous system in general, presynaptic and postsynaptic inhibition serve quite different functions. Postsynaptic inhibition reduces the excitability of the cell itself, rendering it relatively less responsive to all excitatory inputs. Presynaptic inhibition is much more specific, aimed at a particular input and leaving the postsynaptic cell free to go about its business of integrating information from other sources.[84] Presynaptic inhibition implies that inhibitory axons make synaptic contact with axon terminals. Such axo-axonic synapses have been demonstrated directly by electron microscopy at the crustacean neuromuscular junction[85] and at numerous locations in the mammalian central nervous system.[86] Moreover, inhibitory nerve terminals themselves can be influenced presynaptically;[87] the requisite ultrastructural arrangement has been reported at inhibitory synapses on crayfish stretch receptors.[88] There is also evidence for **presynaptic excitation**, synaptic inputs that enhance the release of transmitter from presynaptic nerve terminals.[89]

Desensitization

The response to a neurotransmitter often decreases during repeated or prolonged application, a phenomenon termed **desensitization**. It was described in detail at the neuromuscular junction by Katz and Thesleff, who showed that with prolonged application of ACh the depolarizing response of the muscle fiber steadily declines (Figure 9.17).[90] Desensitization is an intrinsic molecular property of the ACh receptor;[91] however, the rate of receptor desensitization and recovery is modulated by phosphorylation.[92–94] Under normal physiological conditions desensitization does not play a significant role in the response of skeletal muscle to ACh released from axon terminals.[95] However, in muscles that have been poisoned with inhibitors of cholinesterase, such as the organophosphorus compounds used as insecticides and nerve gases, the persistence of ACh in the synaptic cleft is sufficient to cause desensitization and block synaptic transmission.[96]

Receptors for glutamate and GABA also desensitize.[97] At synapses in the central nervous system where glutamate and GABA are released as direct chemical transmitters, desensitization of the postsynaptic receptors occurs even under normal physiological conditions and appears to play an important role in determining the amplitude and time course of postsynaptic potentials.[95,98]

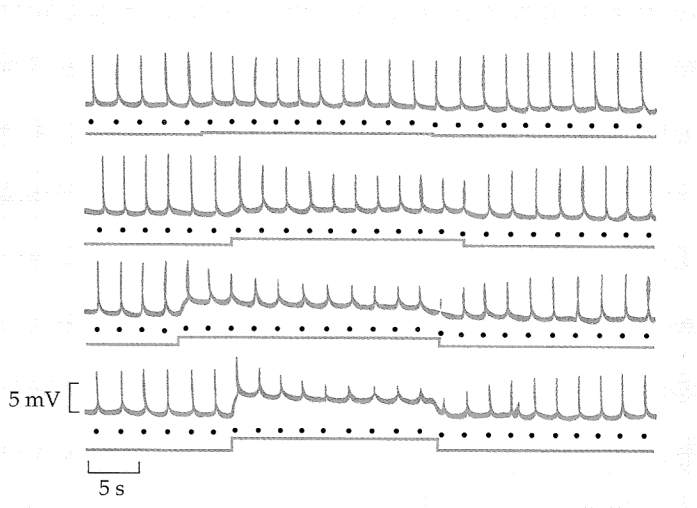

FIGURE 9.17 Prolonged Application of ACh Causes Receptor Desensitization at the frog neuromuscular junction. Intracellular recordings of potential changes produced by brief ionophoretic pulses of ACh from a micropipette (dots). Steady conditioning doses of ACh were delivered from a second pipette (upward deflections of lower traces). During the conditioning pulse the response to the test pulse decreases in amplitude as the receptors desensitize. With increasing doses the rate and extent of desensitization increase. (After Katz and Thesleff, 1957.)

Receptors Mediating Direct and Indirect Chemical Transmission

Direct chemical synaptic transmission is mediated by ion channels in the postsynaptic membrane that are activated by binding the neurotransmitter released by the presynaptic cell. Such ligand-activated ion channels are also referred to as **ionotropic neurotransmitter receptors.** In the mammalian central nervous system three major transmitters act at direct chemical synapses. Two of these, GABA and glycine, activate receptors that are anion channels and so are inhibitory, at least in the adult (Chapter 13). Glutamate, the most prevalent excitatory transmitter in the mammalian central nervous system, acts on several different types of cation-selective ionotropic receptors. Other important transmitters in the mammalian CNS that activate cation-selective ionotropic receptors include acetylcholine, serotonin, and purines.

All the directly acting neurotransmitters mentioned here, as well as transmitters such as dopamine, histamine, norepinephrine, and neuropeptides, also influence postsynaptic cells by a quite different mechanism, **indirect chemical transmission.** Typically, indirect chemical synaptic transmission is mediated by **metabotropic receptors,** postsynaptic receptors that produce an intracellular second messenger. The second messenger, in turn, influences the activity of ion channels, causing excitation or inhibition, and often affects other intracellular targets as well. Indirect chemical synaptic transmission is discussed in detail in Chapter 10.

Summary

- Signaling between nerve cells and their targets can occur by chemical or electrical synaptic transmission.
- Electrical synaptic transmission is mediated by the direct flow of current from cell to cell.
- At chemical synapses a neurotransmitter released from the presynaptic terminal activates receptors in the postsynaptic membrane; the time required for transmitter release imposes a minimum synaptic delay of approximately 1 ms.
- Direct chemical synaptic transmission occurs when the postsynaptic receptor activated by a neurotransmitter is itself an ion channel. Such ligand-activated ion channels are called ionotropic transmitter receptors.
- At direct excitatory synapses, such as the vertebrate skeletal neuromuscular junction, the neurotransmitter (in this case ACh) opens cation-selective channels, allowing sodium, potassium, and calcium ions to flow down their electrochemical gradients.
- The relative permeability of a channel for various ions determines the reversal potential; at excitatory synapses the reversal potential is more depolarized than the threshold for action potential initiation.
- Direct chemical synaptic inhibition occurs when a neurotransmitter opens anion-selective channels, which allow chloride ions to flow down their electrochemical gradient. The reversal potential for such currents is the chloride equilibrium potential (E_{Cl}); inhibition occurs if E_{Cl} is hyperpolarized to threshold.
- Many transmitter receptors desensitize; that is, their response decreases during repeated or prolonged transmitter application.

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