10
Synaptic Integration in the Central Nervous System

LIKE SYNAPTIC TRANSMISSION at the neuromuscular junction, most rapid signaling between neurons in the central nervous system involves ionotropic receptors in the postsynaptic membrane. Thus, many principles that apply to the synaptic connection between the motor neuron and skeletal muscle fiber at the neuromuscular junction also apply in the central nervous system. Synaptic transmission between central neurons is more complex, however, for several reasons. First, although most muscle fibers are innervated by only one motor neuron, a central nerve cell (such as the motor neuron in the spinal cord) receives connections from hundreds or even thousands of neurons. Second, muscle fibers receive only excitatory inputs, whereas central neurons receive both excitatory and inhibitory inputs. Third, all synaptic actions on muscle fibers are mediated by one neurotransmitter, acetylcholine (ACh), which activates only one type of receptor (the ionotropic nicotinic ACh receptor); however, a single central neuron can respond to different types of inputs, each mediated by a distinct transmitter that alters the activity of specific types of receptor. These receptors include both ionotropic receptors, where binding of transmitter directly opens an ion channel, and metabotropic receptors, where transmitter binding indirectly regulates a channel by activating second messengers. As a result, unlike muscle fibers, central neurons must integrate diverse inputs into a single coordinated action. Finally, the nerve–muscle synapse is a model of efficiency—every action potential in the motor neuron produces an action potential in the muscle fiber. In comparison, connections made by a presynaptic neuron onto the motor neuron are only modestly effective—often 50 to 100 excitatory neurons must fire together to produce a synaptic potential large enough to trigger an action potential in a motor cell.

The first insights into synaptic transmission in the central nervous system came from experiments by John Eccles and his colleagues in the 1950s on the synaptic inputs onto spinal motor neurons that control the

stretch reflex, the simple behavior we considered in Chapter 2. The spinal motor neurons remain particularly useful for examining central synaptic mechanisms because they have large, accessible cell bodies and, most important, they receive both excitatory and inhibitory connections and therefore allow us to study the integrative action of the nervous system on the cellular level.

Central Neurons Receive Excitatory and Inhibitory Inputs

To analyze the synapses that mediate the stretch reflex, Eccles activated a large population of axons of the sensory cells that innervate the stretch receptor organs in the quadriceps (extensor) muscle (Figure 10–1A,B). Nowadays the same experiments can be done by stimulating a single sensory neuron. For example, passing sufficient current through a microelectrode into the cell body of a stretch-receptor neuron that innervates the extensor muscle generates an action potential in the sensory cell. This in turn produces a small excitatory postsynaptic potential (EPSP) in the motor neuron that innervates precisely the same muscle (in this case the quadriceps) monitored by the sensory neuron (Figure 10–1B upper panel). The EPSP produced by the one sensory cell, the unitary EPSP, depolarizes the extensor motor neuron by less than 1 mV, often only 0.2 to 0.4 mV, far below the threshold for generating an action potential (typically, a depolarization of 10 mV or more is required to reach threshold).

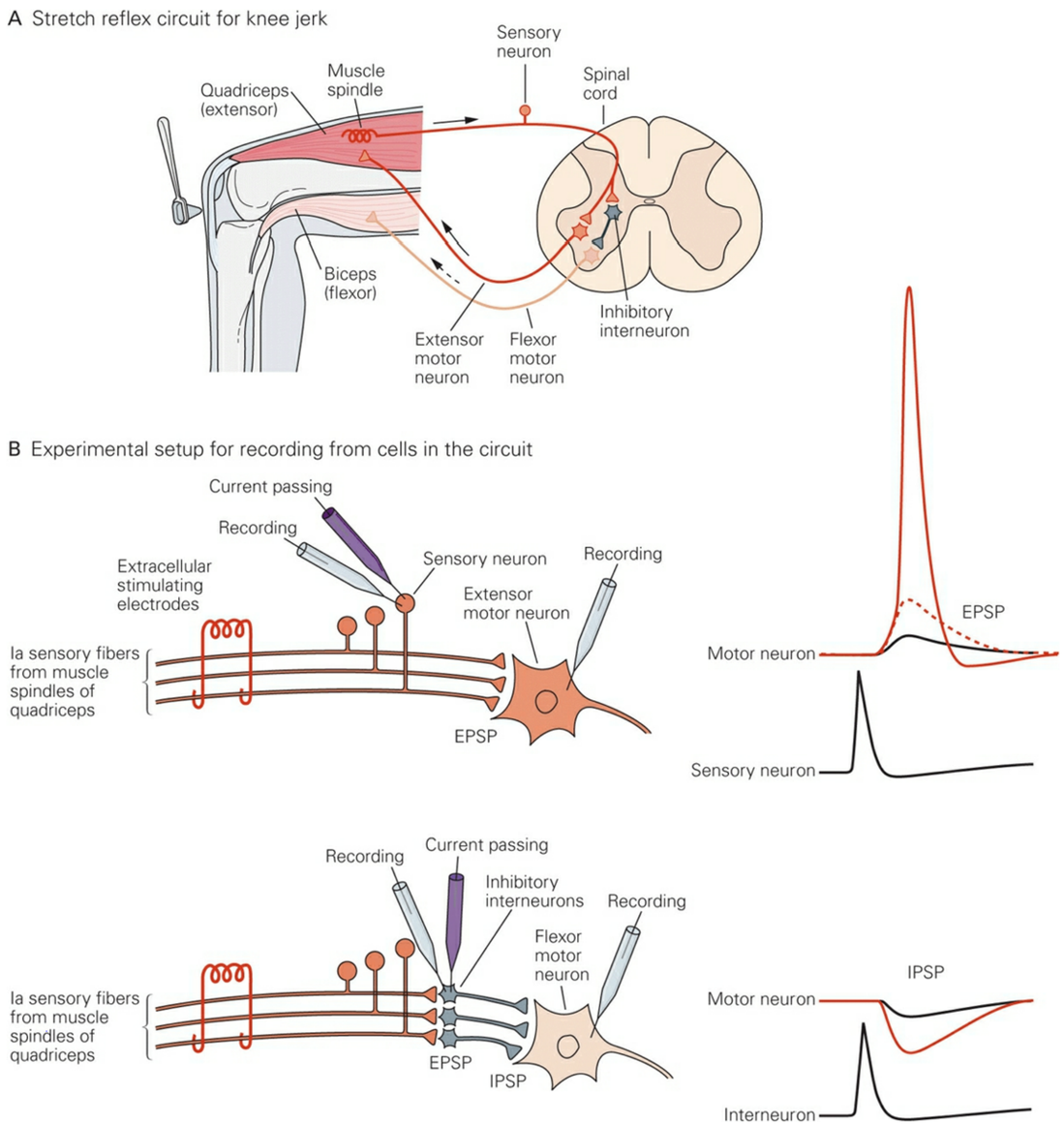


Figure 10-1 The combination of excitatory and inhibitory synaptic connections mediating the stretch reflex of the quadriceps muscle is typical of circuits in the central nervous system.

A. The stretch-receptor sensory neuron at the extensor (quadriceps) muscle makes an excitatory connection with an extensor motor neuron that innervates this same muscle group. It also makes an excitatory connection with an interneuron, which in turn makes an inhibitory connection with a flexor motor neuron that innervates the antagonist biceps femoris muscle group. Conversely, an afferent fiber from the bi-

ceps (not shown) excites an interneuron that makes an inhibitory synapse on the extensor motor neuron.

B. This idealized experimental setup shows the approaches to studying the inhibition and excitation of motor neurons in the pathway illustrated in part A. **Above**: Two alternatives for eliciting excitatory postsynaptic potentials (EPSPs) in the extensor motor neuron. A single presynaptic axon can be stimulated by inserting a current-passing electrode into the sensory neuron cell body. An action potential in the sensory neuron stimulated in this way triggers a small EPSP in the extensor motor neuron (**black trace**). Alternatively, the whole afferent nerve from the quadriceps can be stimulated electrically with extracellular electrodes. The excitation of many afferent neurons through the extracellular electrode generates a synaptic potential (**dashed trace**) large enough to initiate an action potential (**red trace**). **Below**: The setup for eliciting and measuring inhibitory potentials in the flexor motor neuron. Intracellular stimulation of a single inhibitory interneuron receiving input from the quadriceps pathway produces a small inhibitory (hyperpolarizing) postsynaptic potential (IPSP) in the flexor motor neuron (**black trace**). Extracellular stimulation recruits numerous inhibitory neurons and generates a larger postsynaptic IPSP (**red trace**). (Action potentials from the sensory neuron and interneuron appear smaller because they were recorded at lower amplification than the motor neuron action potentials).

The generation of an action potential requires the near-synchronous firing of a number of sensory neurons. This can be observed in an experiment in which a population of sensory neurons is stimulated by passing current through an extracellular electrode. As the strength of the extracellular stimulus is increased, more sensory afferent fibers are excited, and the depolarization produced by the EPSP becomes larger. The depolarization eventually becomes large enough to bring the membrane potential of the axon initial segment (the integrative component of the motor neuron) to the threshold for an action potential.

In contrast to the EPSP produced in the extensor motor neuron, stimulation of the extensor stretch-receptor neuron produces a small inhibitory postsynaptic potential (IPSP) in the motor neuron that innervates the flexor muscle, which is antagonistic to the extensor muscle (Figure 10–1B lower panels). This hyperpolarizing action is mediated by an inhibitory interneuron, which receives excitatory input from the sensory neurons of the extensor muscle and in turn makes synapses with the motor neurons that innervate the flexor muscle. In the laboratory a single interneuron can be stimulated intracellularly to directly elicit a small unitary IPSP in the motor neuron. Extracellular activation of an entire population of interneurons elicits a larger IPSP.

Although a single EPSP in the extensor motor neuron is not nearly large enough to elicit an action potential, the neuron integrates many EPSPs from a large number of afferent sensory fibers to initiate an action potential. At the same time, IPSPs, if strong enough, can counteract the sum of the excitatory actions and prevent the membrane potential from reaching threshold. In addition to counteracting synaptic excitation, synaptic inhibition can exert powerful control over action potential firing in neurons that are spontaneously active because of the presence of intrinsic pacemaker channels. This function, called the *sculpting* role of inhibition, shapes the pattern of firing in such cells (Figure 10–2).

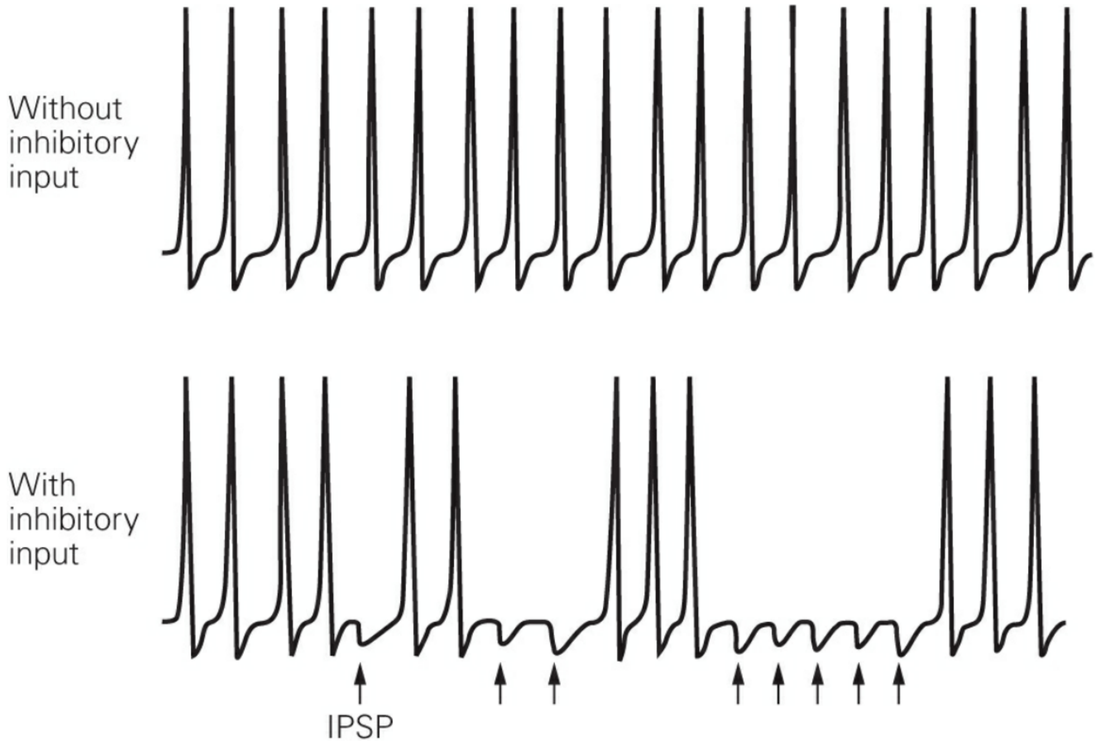


Figure 10-2 Inhibition can shape the firing pattern of a spontaneously

active neuron. Without inhibitory input the neuron fires continuously at a fixed interval. With inhibitory input (arrows) some action potentials are inhibited, resulting in a distinctive pattern of impulses.

Excitatory and Inhibitory Synapses Have Distinctive Ultrastructures

As we learned in Chapter 8, the effect of a synaptic potential—whether it is excitatory or inhibitory—is determined not by the type of transmitter released from the presynaptic neuron but by the type of ion channels in the postsynaptic cell activated by the transmitter. Although some transmitters can produce both excitatory and inhibitory postsynaptic potentials, by acting on distinct classes of ionotropic receptors at different synapses, most transmitters produce a single predominant type of synaptic response; that is, a transmitter is usually inhibitory or excitatory. For example, in the vertebrate brain neurons that release glutamate typically act on receptors that produce excitation; neurons that release γ-aminobutyric acid (GABA) or glycine act on receptors that produce inhibition.

The synaptic terminals of excitatory and inhibitory neurons can be distinguished by their morphology. Two morphological types are common in the brain: Gray type I and type II (named after E. G. Gray, who described them). Most type I (asymmetric) synapses are glutamatergic and therefore excitatory, whereas most type II synapses (symmetric) are GABAergic and therefore inhibitory. Type I synapses have round synaptic vesicles, an electron-dense region at the active zone of the presynaptic membrane, and an even larger dense region in the postsynaptic membrane apposed to the active zone, known as the *postsynaptic density* (PSD). Type II synapses have oval or flattened synaptic vesicles with less obvious presynaptic membrane specializations and PSD (Figure 10–3). Although type I synapses are mostly excitatory and type II inhibitory, the two morphological types have proved to be only a first approximation to transmitter biochemistry. As we shall learn in Chapter 13, immunocytochemistry affords much more reliable distinctions between transmitter types based on the biochemical nature of the transmitters or the enzymes involved in their synthesis.

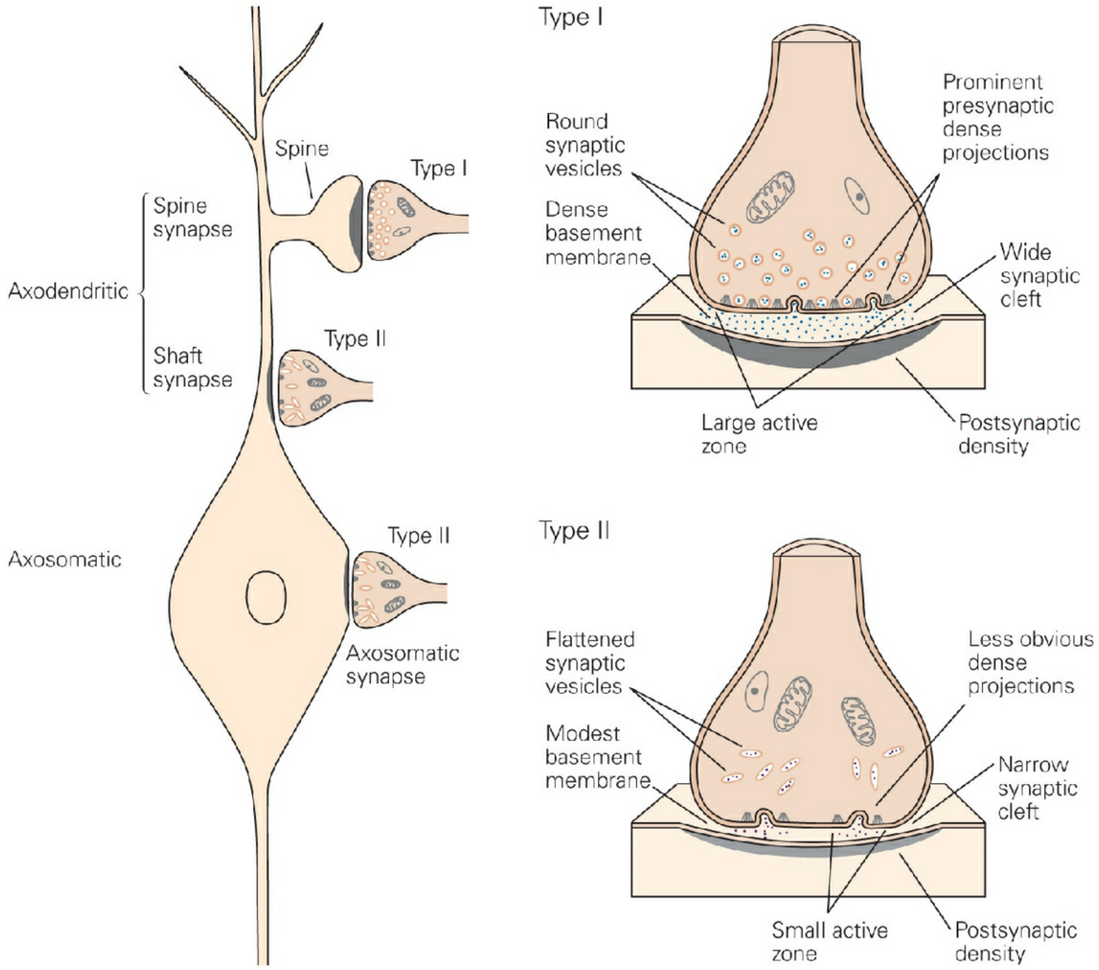


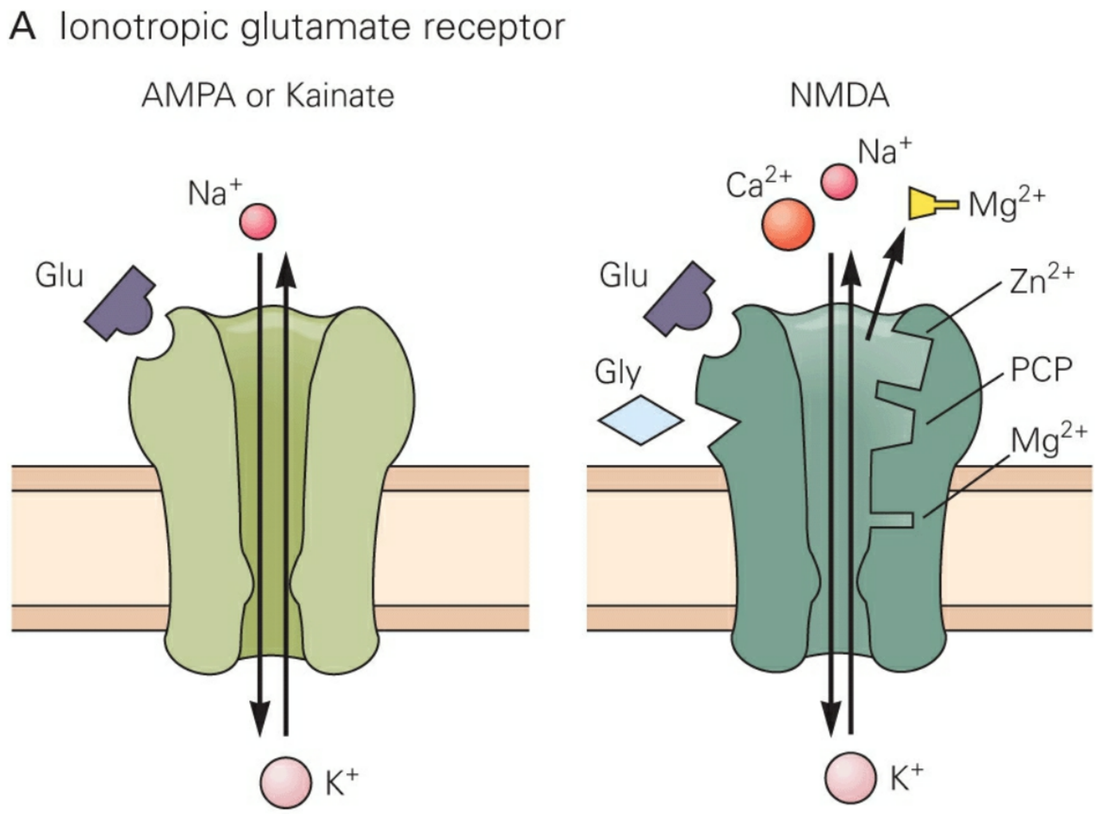
Figure 10-3 The two most common morphological types of synapses in the central nervous system are Gray type I and type II. Type I is usually excitatory, exemplified by glutamatergic synapses; type II is usually inhibitory, exemplified by GABAergic synapses. Differences include the shape of vesicles, prominence of presynaptic densities, total area of the active zone, width of the synaptic cleft, and presence of a dense basement membrane. Type I synapses typically contact specialized projections on the dendrites, called spines, and less commonly contact the shafts of dendrites. Type II synapses often contact the cell body and dendritic shaft.

Excitatory Synaptic Transmission Is Mediated by Ionotropic Glutamate Receptor-Channels That Are

Permeable to Sodium and Potassium

The excitatory transmitter released from the presynaptic terminals of stretch-receptor neurons is the amino acid L-glutamate, the major excitatory transmitter in the brain and spinal cord. Eccles and his colleagues discovered that the EPSP in spinal motor cells results from the opening of glutamate-gated channels permeable to both Na^+, and K^+. This ionic mechanism is similar to that produced by ACh at the neuromuscular junction described in Chapter 9. Like the ACh-gated channels, the glutamate-gated channels conduct both Na^+, and K^+ with nearly equal permeability. As a result, the reversal potential for current flow through these channels is 0 mV (see Figure 9-7).

Glutamate receptors can be divided into two broad categories: the ionotropic receptors, which are ligand-gated channels where glutamate binding directly opens the channel, and metabotropic receptors, which are G protein-coupled receptors that indirectly gate channels through the production of second messengers (Figure 10–4). There are three major subtypes of ionotropic glutamate receptors: AMPA, kainate, and NMDA, named according to the types of synthetic agonists that activate them (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, kainate, and *N*-methyl-D-aspartate, respectively). The NMDA receptor is selectively blocked by the drug APV (2-amino-5-phosphonovaleric acid). The AMPA and kainate receptors are not affected by APV but both are blocked by the drug CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), and thus they are sometimes called the *non-NMDA receptors*. The metabotropic glutamate receptors can be selectively activated by *trans*-(1S,3R)-1-amino-1, 3-cyclopentanedicarboxylic acid (ACPD). The action of ionotropic glutamate receptors is always excitatory or depolarizing, as the reversal potential of their ionic current is near zero, whereas the metabotropic receptors can produce either excitation or inhibition, depending on the reversal potential of the ionic currents that they regulate.



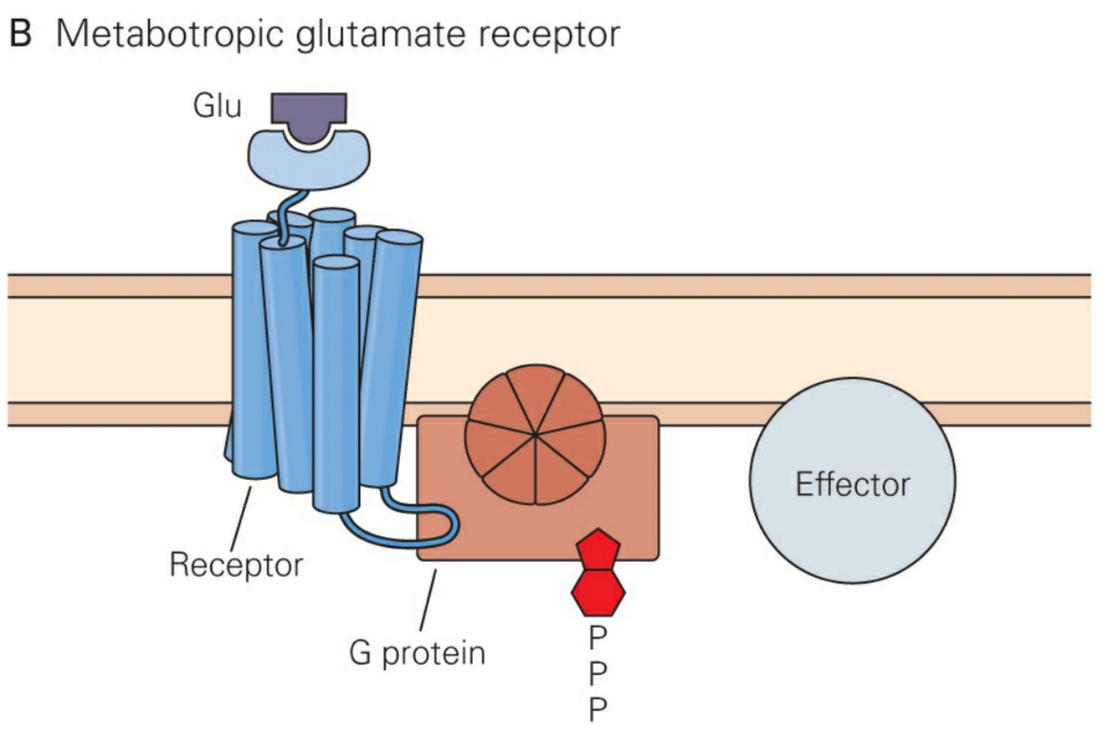


Figure 10-4 Different classes of glutamate receptors regulate excitatory synaptic actions in neurons in the spinal cord and brain.

A. Three classes of ionotropic glutamate receptors directly gate ion channels permeable to cations. The AMPA and kainate type of receptors bind the glutamate agonists AMPA or kainate, respectively. These receptors contain a channel that is permeable to Na^+, and K^+. The NMDA receptor, which binds the glutamate agonist NMDA, contains a channel permeable to Ca^{2+}, K^+, and Na^+. It has binding sites for glutamate, glycine, Zn^{2+}, phencyclidine (PCP, or *angel dust*), MK801 (an experimental drug), and Mg^{2+}, each of which regulates the functioning of the channel differently.

B. The metabotropic glutamate receptors indirectly gate ion channels by activating a GTP-binding protein, which in turn interacts with effector molecules that alter metabolic and ion channel activity (see Chapter 11).

The NMDA receptor has several interesting properties. First, this ligand-gated channel is permeable to Ca^{2+} as well as to Na^+, and K^+ (Figure 10-4A). Second, opening the channel requires extracellular glycine as a cofactor. Under normal conditions the concentration of extracellular glycine is sufficient to allow the NMDA receptor-channel to be activated efficiently by glutamate. Third, the NMDA receptor is unique among ligand-gated channels thus far characterized because its opening depends on membrane voltage as well as transmitter. The voltage-dependence is caused by a mechanism that is quite different from that employed by the voltage-gated channels that generate the action potential. In the latter, changes in membrane potential are translated into conformational changes in the channel by an intrinsic voltage-sensor. In the NMDA receptors, however, depolarization removes an extrinsic plug from the channel. At the resting membrane potential (-65 mV) extracellular Mg^{2+} binds tightly to a site in the pore of the channel, blocking ionic current. But when the membrane is depolarized (for example, by the opening of AMPA receptor-channels), Mg^{2+} is expelled from the channel by electrostatic repulsion, allowing Na^+, and Ca^2 to enter (Figure 10–5).

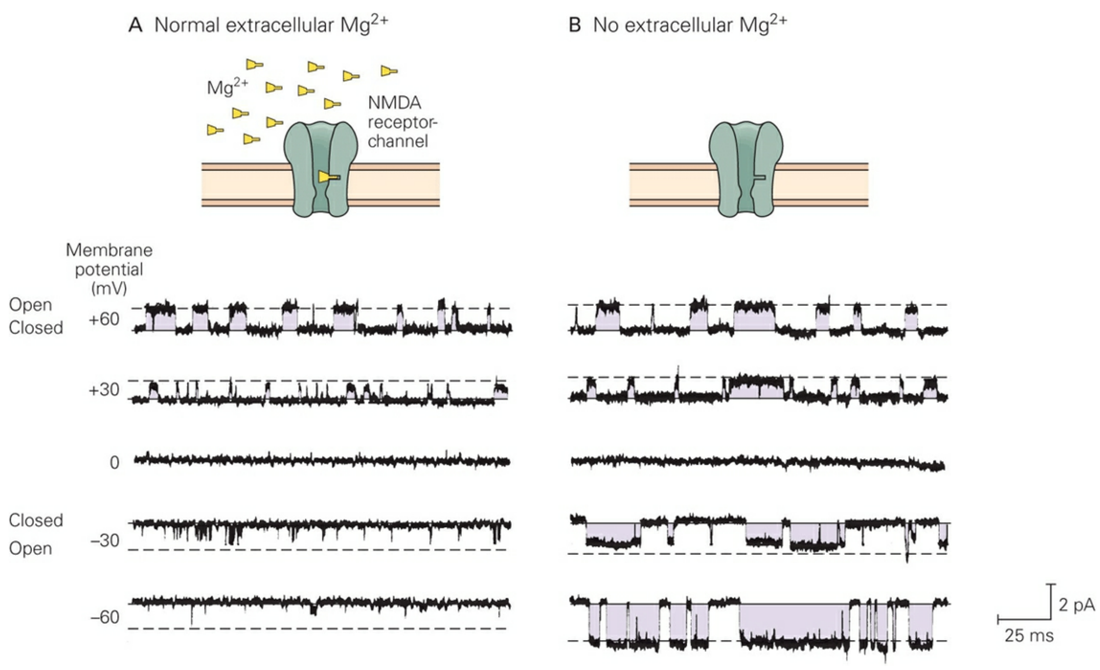


Figure 10-5 Opening of single NMDA receptor-channels depends on voltage in addition to glutamate. These recordings are from individual NMDA receptor-channels (from rat hippocampal cells in culture). Downward deflections indicate pulses of inward (negative) current;

upward deflections indicate outward (positive) current. (Reproduced, with permission, from J. Jen and C. F. Stevens.)

A. When Mg^{2+} is present in normal concentration in the extracellular solution (1.2 mM), the channel is largely blocked at the resting potential (-60 mV). At negative membrane potentials only brief, flickery, inward currents are seen upon channel opening because of the Mg^{2+} block. Substantial depolarization (to +30 mV or +60 mV) relieves the Mg^{2+} block, permitting longer-lasting pulses of outward current through the channel.

B. When Mg^{2+} is removed from the extracellular solution, the opening and closing of the channel do not depend on voltage. The channel is open at the resting potential of -60 mV, and the synaptic current reverses near 0 mV, like the total synaptic current (see Figure 10-6B).

The NMDA receptor has the further interesting property that it is inhibited by the hallucinogenic drug phencyclidine (PCP, also known as angel dust) and by MK801, both of which bind to a site in the pore of the channel that is distinct from the Mg^{2+} binding site (Figure 10-4A). Indeed, blockade of NMDA receptors produces symptoms that resemble the hallucinations associated with schizophrenia, whereas certain antipsychotic drugs enhance current flow through the NMDA receptor-channels. This has led to the hypothesis that schizophrenia may involve a defect in NMDA receptor function.

At most central synapses that use glutamate as the transmitter, the postsynaptic membrane contains both NMDA and AMPA receptors. The contributions of current through NMDA and AMPA receptors to the total excitatory postsynaptic current (EPSC) can be dissected using pharmacological antagonists in a voltage-clamp experiment (Figure 10–6). At the normal resting potential of most neurons, the NMDA receptor-channels are largely inhibited by Mg^{2+}. As a result, the EPSC is predominantly determined by charge flow through the AMPA receptors, which generate a current with a very rapid rising phase and very rapid decay phase. However, as a neuron becomes depolarized, Mg^{2+} is driven out of the mouth of the NMDA receptors and more charge flows through these channels. Thus, the NMDA receptor conducts current maximally when two conditions are met: Glutamate is present, and the cell is depolarized (Figure 10–6). That is, the NMDA receptor acts as a "coincidence detector," detecting a timing relationship between activation of the presynaptic and postsynaptic cells. In addition, because of the intrinsic kinetics of ligand gating, the current through the NMDA receptor rises and decays with a much slower time course than the AMPA receptor current. As a result, the NMDA receptors contribute to a late, slow phase of the EPSC and EPSP.

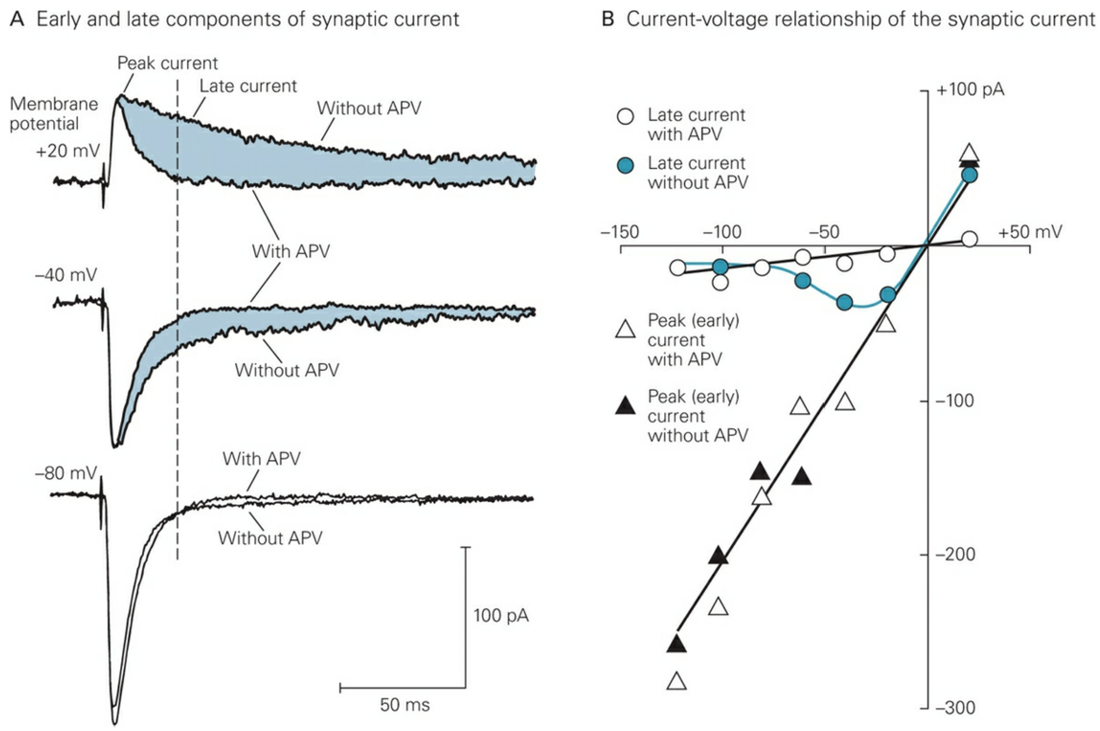


Figure 10-6 The contributions of the AMPA and NMDA glutamate receptor-channels to the excitatory postsynaptic current. These voltage-clamp current records are from a cell in the hippocampus. Similar receptor-channels are present in motor neurons and throughout the brain. (Adapted, with permission, from Hestrin et al. 1990.)

A. The drug APV selectively binds to and blocks the NMDA receptor. Shown here is the excitatory postsynaptic current (EPSC) before and during application of 50 μM APV at three different membrane potentials. The difference between the traces (**blue region**) represents the contribution of the NMDA receptor-channel to the EPSC. The current that remains in the presence of APV is the contribution of the AMPA receptor-channels. At -80 mV there is no current through the NMDA receptor-channels because of pronounced Mg^{2+} block (see Figure 10-5).

At -40 mV a small late inward current through NMDA receptor-channels is evident. At +20 mV the late component is more prominent and has reversed to become an outward current. The vertical dotted line indicates the time 25 ms after the peak of the synaptic current, which is used for the calculations of late current in part B.

B. The postsynaptic currents through the NMDA and AMPA receptor-channels differ in their dependence on the membrane potential. The current through the AMPA receptor-channels contributes to the early phase of the synaptic current (**filled triangles**). The early phase is measured at the peak of the synaptic current and plotted here as a function of membrane potential. The current through the NMDA receptor-channels contributes to the late phase of the synaptic current (**filled circles**). The late phase is measured 25 ms after the peak of the synaptic current (**dotted line** in part A), a time at which the AMPA receptor component has decayed almost to zero. Note that the AMPA receptor-channels behave as simple resistors; current and voltage have a linear relationship. In contrast, current through the NMDA receptor-channels is nonlinear and increases as the membrane is depolarized from -80 to -40 mV, owing to progressive relief of Mg^{2+} block. The reversal potential of both receptor-channel types is at 0 mV. The components of the synaptic current in the presence of 50 μm APV are indicated by the unfilled circles and triangles. Note how APV blocks the late (NMDA receptor) component but not the early (AMPA receptor) component of the EPSC.

As most glutamatergic synapses contain AMPA receptors that are capable of triggering an action potential, what is the function of the NMDA receptor? At first glance the function of these receptors is even more puzzling because they are normally blocked by Mg^{2+} at the resting potential. However, when glutamate is paired with depolarization, the NMDA receptors uniquely conduct Ca^{2+} into the postsynaptic cell. This leads to a rise in intracellular [Ca^{2+}] that can activate various calcium-dependent signaling cascades, including calcium-calmodulin-dependent protein kinase II (CaMKII) (see Chapter 11). Thus NMDA receptor activation can translate electrical signals into biochemical ones. Some of these biochemical reactions lead to long-lasting changes in synaptic strength, a set of processes called long-term synaptic plasticity that are thought to be important during synapse development and for regulating neural circuits in the adult brain. In particular, an NMDA receptor-dependent long-term potentiation (LTP) of excitatory synaptic transmission has been implicated in certain forms of memory storage (see Chapters 66 and 67).

However, there is also a potential downside to the entry of Ca^{2+} through the NMDA receptors. Excessively high concentrations of glutamate are thought to result in an overload of Ca^{2+} in the postsynaptic neurons. Such high levels of Ca^{2+} can be toxic to neurons. In tissue culture even a brief exposure to high concentrations of glutamate can kill many neurons, an action called *glutamate excitotoxicity*. The high concentrations of intracellular Ca^{2+} are thought to activate calcium-dependent proteases and phospholipases and lead to the production of free radicals that are toxic to the cell. Glutamate toxicity may contribute to cell damage after stroke, to the cell death that occurs with episodes of rapidly repeated seizures experienced by patients who have status epilepticus, and to degenerative diseases such as Huntington disease. Agents that selectively block the NMDA receptor may protect against the toxic effects of glutamate and have been tested clinically. Unfortunately, the hallucinations that accompany NMDA receptor blockade have so far limited the usefulness of such compounds. A further complication of attempts to control excitotoxicity by blocking NMDA receptor function is that physiological levels of NMDA receptor activation can actually protect neurons from damage and cell death.

The Excitatory Ionotropic Glutamate Receptors Are Encoded by a Distinct Gene Family

What are the molecular bases for the biophysical function of glutamate receptors and how are these receptors related to other ligand-gated ion channels? Over the past 20 years the genes coding for the subunits of all the major neurotransmitter receptors have been identified. This molecular analysis demonstrates evolutionary linkages among the structure of receptors that enable us to classify them into three distinct families (Figure 10–7). One family includes the genes encoding the kainate, AMPA, and NMDA receptors; the genes encoding the AMPA and kainate receptors are more closely related to one another than are the genes encoding the NMDA receptors. Surprisingly this gene family bears little resemblance to the two other gene families that encode ionotropic receptors (one that

encodes the ACh, GABA, and glycine receptors, and one that encodes ATP receptors, as described below).

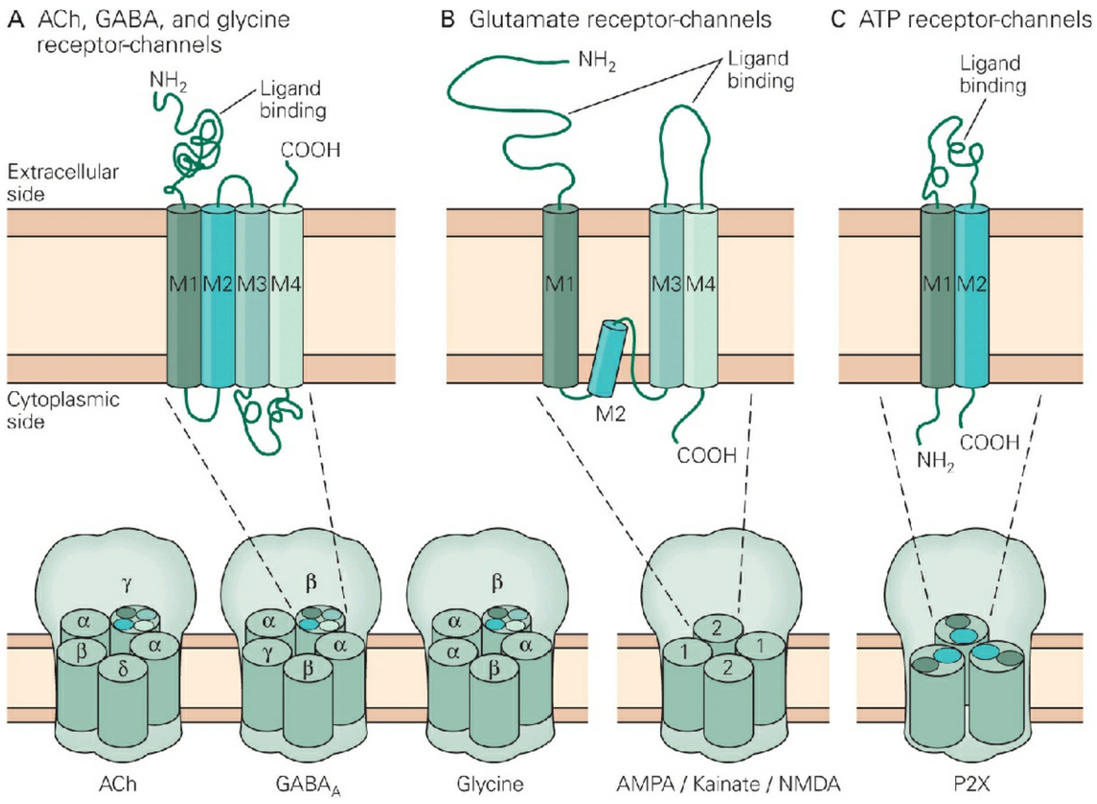


Figure 10-7 The three families of ligand-gated channels.

A. The nicotinic ACh, $GABA_A$, and glycine receptor-channels are all pentamers composed of several types of related subunits. As shown here, the ligand-binding domain is formed by the extracellular amino-terminal region of the protein. Each subunit has a membrane domain with four membrane-spanning α-helixes (M1–M4) and a short extracellular carboxyl terminus. The M2 helix lines the channel pore.

B. The glutamate receptor-channels are tetramers, often composed of two different types of closely related subunits (here denoted 1 and 2). The subunits have a large extracellular amino terminus, a membrane domain with three membrane-spanning α-helixes (M1, M3, and M4), a large extracellular loop connecting the M3 and M4 helixes, and an intracellular carboxyl terminus. The M2 segment forms a loop that dips into and out of the cytoplasmic side of the membrane, contributing to the selectivity filter of the channel. The glutamate binding site is formed by residues in the extracellular amino terminus and in the M3-M4 extracellular loop.

C. The ATP receptor-channels (or purinergic P2X receptors) are trimers. Each subunit possesses two membrane-spanning α-helixes (M1 and M2) and a large extracellular loop that binds ATP. The M2 helix lines the pore.

Unlike the pentameric nicotinic ACh receptor family, the AMPA, kainate, and NMDA receptors are tetrameric proteins with four subunits arranged around a central pore. The AMPA receptor subunits are encoded by four separate genes (*GluA1-GluA4*), and there are five different kainate receptor subunit genes (*GluK1-GluK5*). Most of the AMPA and kainate receptors are heteromers composed of two different types of *GluA* and *GluK* subunits, respectively. The NMDA receptors are encoded by a family consisting of five genes that fall into two groups, the single *GluN1* gene and the four *GluN2A-D* genes. Each NMDA receptor contains two GluN1 subunits and two of the different types of GluN2 subunits.[1] In addition, many of these subunit genes are alternatively spliced, generating further diversity. Autoantibodies to the AMPA receptor GluA3 subunit are thought to play an important role in some forms of epilepsy. These antibodies actually mimic glutamate by activating GluA3-containing receptors, resulting in excessive excitation and seizures.

The amino acid sequence of the ionotropic glutamate receptor subunits and subsequent functional and biochemical studies provided the initial compelling evidence that the transmembrane topology of these subunits is very different from that of the nicotinic ACh receptor (Figure 10–7). Our understanding of the ionotropic glutamate receptors was then greatly expanded by Eric Gouaux and colleagues' determination of the high-resolution X-ray crystal structures of the isolated AMPA receptor ligand binding domain and of an intact AMPA receptor-channel formed by GluA2 subunits (Figure 10–8).

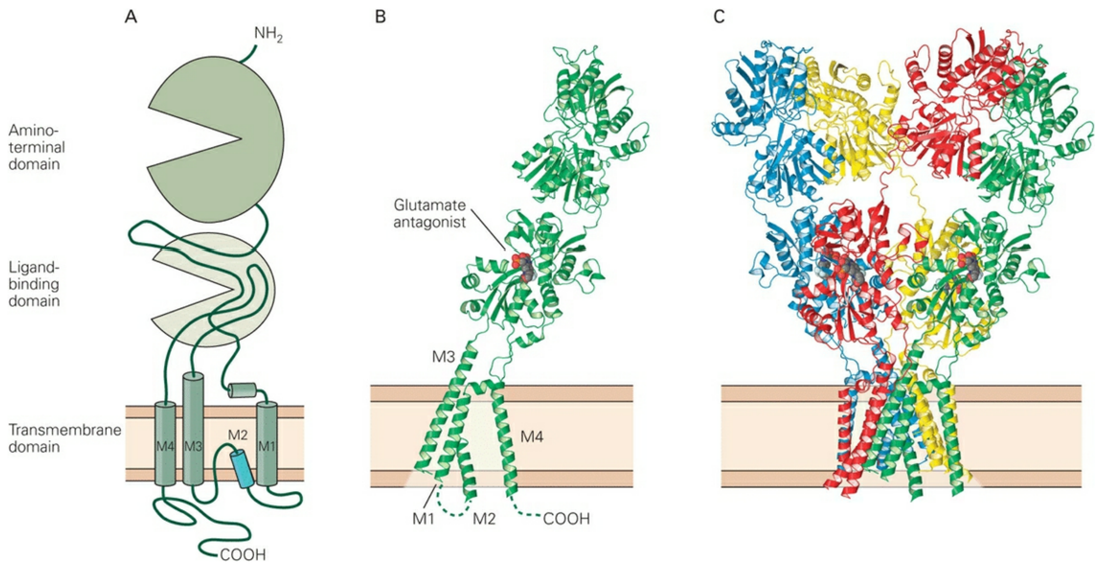


Figure 10-8 Structure of an ionotropic glutamate receptor.

A. Schematic organization of the ionotropic glutamate receptors. The receptors contain a large extracellular amino terminus, followed by a transmembrane domain containing three membrane-spanning α-helixes (M1, M3, and M4) and a loop that dips into the cytoplasmic side of the membrane (M2). The ligand-binding domain is formed by the extracellular region of the receptor on the amino-terminal side of the M1 segment and by the extracellular loop connecting M3 and M4. These two regions intertwine to form a clamshell structure that binds glutamate and various pharmacological agonists and competitive antagonists. A second clamshell structure is formed at the extreme amino terminus of the receptor. This amino-terminal domain is thought to modulate receptor function and synapse development. It does not bind glutamate in the ionotropic receptors. (Reproduced, with permission, from Armstrong et al. 1998.)

B. Three-dimensional X-ray crystal structure of an AMPA receptor composed solely of GluA2 subunits. A side view of the structure of a single GluA2 subunit showing the amino-terminal domain, ligand-binding domain, and transmembrane domain. The M1, M3, and M4 transmembrane α-helixes are indicated, as is the short α-helix in the M2 loop. A molecule of a competitive antagonist of glutamate bound to the ligand-binding domain is shown in a space-filling representation. The cytoplasmic loops connecting the membrane α-helixes were not resolved in the structure and have been drawn as dashed lines. (Reproduced, with permission, from Sobolevsky, Rosconi and Gouaux, 2009.)

C. A side view of the structure of the tetrameric receptor. The four GluA2 subunits associate through the extracellular domains as a pair of dimers (two-fold symmetry). In the amino-terminal domain, one dimer is formed by the blue and yellow subunits, and the other dimer is formed by the red and green subunits. In the ligand-binding domain, the subunits change partners. In one dimer the blue subunit associates with the red subunit, whereas in the other dimer the yellow subunit associates with the green subunit. In the transmembrane region the subunits associate as a four-fold symmetric tetramer. This is a highly unusual subunit arrangement whose significance is not fully understood. (Reproduced, with permission, from Sobolevsky, Rosconi and Gouaux, 2009.)

Glutamate Receptors Are Constructed from a Set of Modules

AMPA receptors are composed of three distinct modules: an extracellular amino-terminal domain, an extracellular ligand-binding domain, and a transmembrane domain (Figure 10–8A,B). The transmembrane domain contains three transmembrane α-helixes (M1, M3, and M4) and a loop (M2) between the M1 and M3 helixes that dips into and out of the cytoplasmic side of the membrane. This M2 loop is thought to form the selectivity filter of the channel. It adopts a structure similar to the pore-lining P loop of K^+ channels, except that in K^+ channels the P loop dips into and out of the extracellular surface of the membrane (see Figure 5-15).

Both the extracellular amino-terminal domain and the extracellular ligand binding domain are homologous to bacterial amino acid binding proteins. Each domain forms a bi-lobed clamshell-like structure similar to the structure of the bacterial proteins, in which the amino acid is bound within the clamshell. The amino-terminal domain does not bind glutamate but is homologous to the glutamate binding domain of metabotropic glutamate receptors. In the ionotropic glutamate receptors this domain is involved in subunit assembly, the modulation of receptor

function by ligands other than glutamate, and the interaction with other synaptic proteins to regulate synapse development.

The ligand-binding domain is formed by two distinct regions in the linear sequence of the protein. One region is located in the extracellular amino terminus of the protein from the end of the amino-terminal domain up to the M1 transmembrane helix; the second region is formed by the large extracellular loop connecting the M3 and M4 helixes. In the ionotropic receptors, the binding of a molecule of glutamate within the clamshell triggers the closure of the lobes of the clamshell; competitive antagonists also bind to the clamshell but fail to trigger clamshell closure. Thus the conformational change associated with clamshell closure is thought to be coupled to the opening of the ion channel.

Given the homology among the various subtypes of glutamate receptors, it is likely that the kainate and NMDA receptors adopt an overall structure similar to that of the homomeric GluA2 receptor. However, there are also likely to be some important differences that give rise to the distinct physiological functions of the different receptors. As we saw previously, the NMDA receptor-channels are permeable to Ca^{2+}, whereas most AMPA receptors are not. These differences have been localized to a single amino acid residue in the pore-forming M2 loop (Figure 10–9A). All NMDA receptor subunits contain the neutral residue asparagine at this position in the pore. In most types of AMPA receptor subunits this residue is the uncharged amino acid glutamine. However, in the GluA2 subunit the corresponding M2 residue is arginine, a positively charged basic amino acid. Inclusion of even a single GluA2 subunit causes the AMPA receptor-channels to have a very low permeability to Ca^{2+}, most likely as a result of strong electrostatic repulsion by the arginine. Some cells form AMPA receptors that lack the GluA2 subunit. Such AMPA receptor-channels generate a significant Ca^{2+} influx, because their pores lack the positively charged arginine residue.

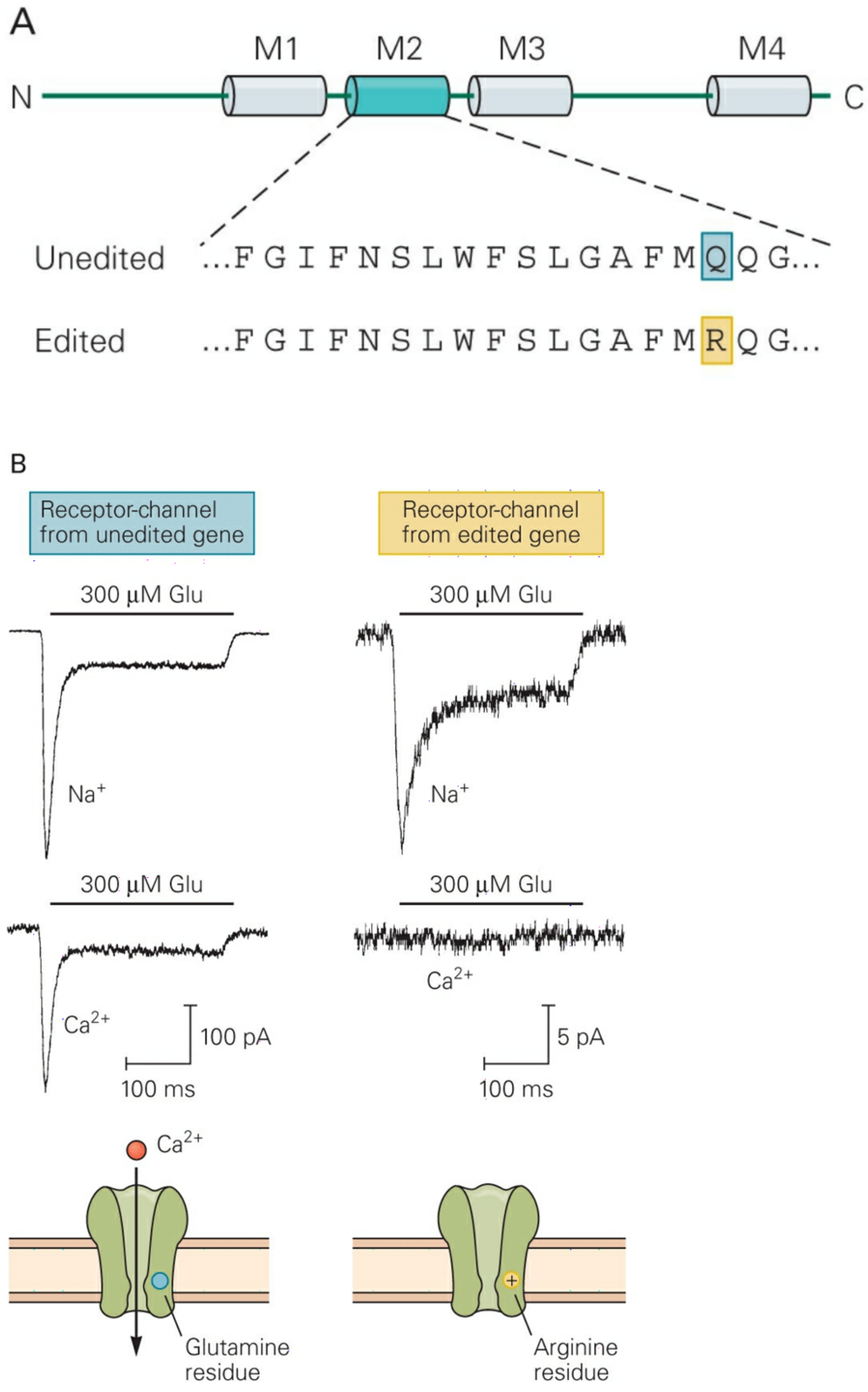


Figure 10-9 Determinants of Ca^{2+} permeability of the AMPA receptor.

A. Comparison of amino acid sequences in the M2 region of the AMPA receptor-channel coded by unedited and edited transcripts of the *GluA2* gene. The unedited transcript codes for the polar residue glutamine (**Q**, using the single-letter amino acid notation), whereas the edited transcript codes for the positively charged residue arginine (R). In the adult the GluA2 protein exists almost exclusively in the edited form.

B. AMPA receptor-channels expressed from unedited transcripts conduct Ca^{2+} (left traces), whereas those expressed from edited transcripts do not (right traces). The top and bottom traces show currents elicited by glutamate with either extracellular Na^{+} (top traces) or Ca^{2+} (bottom traces) as the predominant permeant cation. (Reproduced, with permission, from Sakmann 1992.)

Peter Seeburg and his colleagues made the remarkable discovery that the DNA of the *GluA2* gene does not actually encode an arginine residue at this position in the M2 loop but rather codes for a glutamine residue. After transcription the codon for glutamine in the *GluA2* mRNA is replaced with one for arginine because of a chemical modification of a single nucleotide base through an enzymatic process termed RNA editing (Figure 10-9A). The importance of this RNA editing is underscored by a genetically engineered mouse that Seeburg and colleagues designed to express a *GluA2* gene in which the glutamine residue could no longer be edited to an arginine. Such mice develope seizures and die within a few weeks after birth, presumably caused by excess intracellular Ca^{2+} as all the AMPA receptors in these mice have a high Ca^{2+} permeability.

NMDA and AMPA Receptors Are Organized by a Network of Proteins at the Postsynaptic Density

How are the different glutamate receptors localized and arranged at excitatory synapses? Like most ionotropic receptors, glutamate receptors are normally clustered at postsynaptic sites in the membrane, opposed to glutamatergic presynaptic terminals. The vast majority of excitatory synapses in the mature nervous system contain both NMDA and AMPA, whereas in early development synapses containing only NMDA receptors are common. How are synaptic receptors clustered and targeted to appropriate sites? We are now beginning to appreciate that a large number of regulatory proteins that constitute the postsynaptic density help organize the three-dimensional structure of the postsynaptic cell membrane, including the localization of postsynaptic receptors (Figure 10–10).

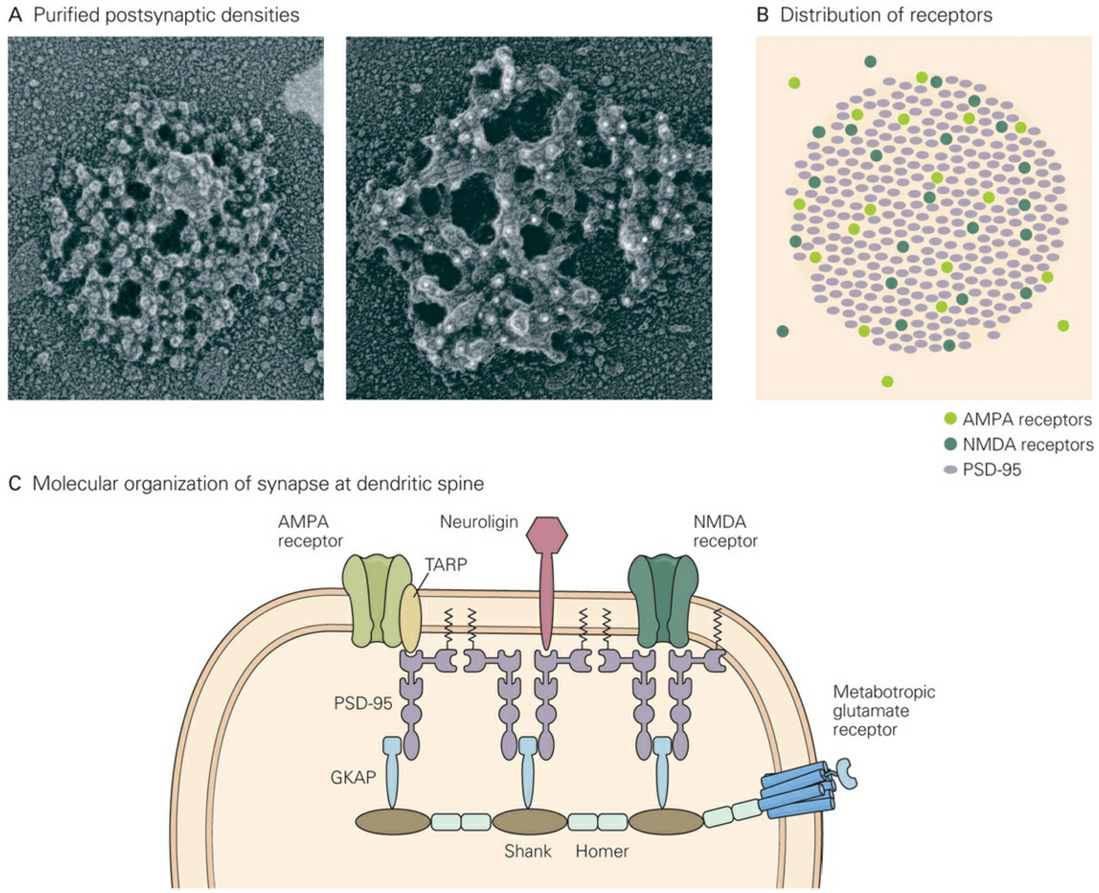


Figure 10-10 The postsynaptic cell membrane is organized into a macromolecular complex at excitatory synapses.

Proteins containing PDZ domains help organize the distribution of AMPA and NDMA receptors of the postsynaptic membrane at the postsynaptic density. (Reproduced, with permission, from Sheng and Hoogenrad 2007. Micrographs originally provided by Thomas S. Reese and Xiaobing Chen, National Institutes of Health, USA.)

A. Electron microscope images of biochemically purified postsynaptic densities, showing organization of protein network. The mem-

brane lipid bilayer is no longer present. **Left**: View of postsynaptic density from what would normally be the outside of the cell. This image consists of the extracellular domains of various receptors and membrane proteins. **Right**: View of a postsynaptic density from what would normally be the cytoplasmic side of the membrane. White dots show immunolabeled guanylate kinase anchoring protein, an important component of the PSD.

B. Schematic view of localization and typical number of NMDA receptors, AMPA receptors, and PSD-95, a prominent postsynaptic density protein, at a synapse.

C. Schematic view of the network of receptors and their interacting proteins in the postsynaptic density. PSD-95 contains three PDZ domains at its amino terminus and two other protein interacting motifs at its carboxyl terminus, an SH3 domain and guanylate kinase (GK) domain. Certain PDZ domains of PSD-95 bind to the carboxyl terminus of the GluN2 subunit of the NMDA receptor. PSD-95 does not directly interact with AMPA receptors but binds to the carboxyl terminus of the TARP family of membrane proteins, which interact with the AMPA receptors as auxiliary subunits. PSD-95 also acts as a scaffold for various cytoplasmic proteins by binding to the guanylate-kinase-associated protein (GKAP), which interacts with Shank, a large protein that associates into a meshwork linking the various components of the postsynaptic density. PSD-95 also interacts with the cytoplasmic region of neuroligin. The metabotropic glutamate receptor is localized on the periphery of the synapse. It interacts with the protein Homer, which in turn binds to Shank.

The postsynaptic density is a remarkably stable structure, permitting its biochemical isolation, purification and characterization. Electron microscopic studies of intact and isolated postsynaptic densities provide a strikingly detailed view of their structure. By using gold-labeled antibodies it is possible to identify specific protein components of the postsynaptic membrane, including the location and number of glutamate receptors. A typical PSD of around 350 nm in diameter contains about 20 NMDA receptors, which tend to be localized near the center of the PSD, and 10 to 50 AMPA receptors, which are less centrally localized. The metabotropic glutamate receptors are located on the periphery, outside the main area of the PSD. All three receptor types interact with a wide array of cytoplasmic and membrane proteins to ensure their proper localization.

One of the most prominent proteins in the postsynaptic density important for the clustering of glutamate receptors is PSD-95 (postsynaptic density protein of 95 kD molecular weight). PSD-95 is a membrane-associated protein that contains three repeated regions—the so-called PDZ domains—important for protein-protein interactions. The PDZ domains bind to specific sequences at the extreme carboxy terminus of a number of cellular proteins. They are named PDZ after the three proteins in which they were first identified: PSD-95, the DLG tumor suppressor protein in *Drosophila*, and a protein termed ZO-1. The PDZ domains of PSD-95 bind the NMDA receptor and the Shaker-type voltage-gated K^+ channel, thereby localizing and concentrating these channels at postsynaptic sites. PSD-95 also interacts with the postsynaptic membrane protein neuroligin, which forms an extracellular contact in the synaptic cleft with the presynaptic membrane protein neurexin, an interaction important for synapse development. Mutations in neuroligin are thought to contribute to some cases of autism.

Although PSD-95 does not directly bind to AMPA receptors, it does interact with an auxiliary subunit of these receptors termed the *transmembrane AMPA receptor regulatory protein* (TARP). The TARP proteins contain four transmembrane segments with a cytoplasmic C-terminus. These proteins strongly regulate the trafficking, synaptic localization, and gating of the AMPA receptors. The first TARP family member to be identified was stargazin, which was isolated through a genetic screen in the *stargazer* mutant mouse, so named because these animals have a tendency to tip their heads backward and stare upward. Loss of stargazin leads to a complete loss of AMPA receptors from cerebellar granule cells, which results in cerebellar ataxia and frequent seizures. Other members of the TARP family are similarly required for AMPA receptor trafficking to the surface membrane of other types of neurons.

The proper localization of AMPA receptors by stargazin depends on the interaction between its C-terminus and PSD-95. AMPA receptors also bind to a distinct PDZ domain protein called GRIP, and metabo-tropic glutamate receptors interact with yet another PDZ domain protein called Homer. In addition to interacting with receptors, proteins with PDZ domains interact with many other cellular proteins, including proteins

that bind to the actin cytoskeleton, providing a scaffold around which a complex of postsynaptic proteins is constructed. Indeed, a biochemical analysis of the postsynaptic density has identified dozens of proteins that participate in NMDA or AMPA receptor complexes.

Inhibitory Synaptic Action Is Usually Mediated by Ionotropic GABA and Glycine Receptor-Channels That Are Permeable to Chloride

Although glutamatergic excitatory synapses account for the vast majority of synapses in the brain, inhibitory synapses play an essential role in the nervous system both by preventing too much excitation and by helping coordinate activity among networks of neurons. Inhibitory postsynaptic potentials in spinal motor neurons and most central neurons are generated by the amino acid neurotransmitters GABA and glycine. GABA is a major inhibitory transmitter in the brain and spinal cord. It acts on two receptors, $GABA_A$ and $GABA_B$. The $GABA_A$ receptor is an ionotropic receptor that directly opens a Cl^- channel. The $GABA_B$ receptor is a metabo-tropic receptor that activates a second-messenger cascade, which often indirectly activates a K^+ channel (see Chapter 11). Glycine, a less common inhibitory transmitter in the brain, also activates ionotropic receptors that directly open Cl^- channels. Glycine is the major transmitter released in the spinal cord by the inter-neurons that inhibit antagonist muscles.

Eccles and his colleagues determined the ionic mechanism of the IPSP in spinal motor neurons by systematically changing the level of the resting membrane potential in a motor neuron and stimulating a presynaptic inhibitory interneuron (Figure 10–11). When the motor neuron membrane is held at the normal resting potential (-65 mV), a small hyperpolarizing potential is generated when the interneuron is stimulated. When the motor neuron membrane is held at -70 mV, no change in potential is recorded when the interneuron is stimulated. But at potentials more negative than -70 mV the motor neuron generates a *depolarizing* response following stimulation of the inhibitory interneuron. This reversal potential of -70 mV corresponds to the Cl^- equilibrium potential in spinal motor neurons (the extracellular concentration of Cl^- is much greater than the intracellular concentration). Thus, at -70 mV the tendency of Cl^- to diffuse into the cell down its chemical concentration gradient is balanced by the electrical force (the negative membrane potential) that opposes Cl^- influx. Replacement of extracellular Cl^- with an impermeant anion reduces the size of the IPSP and shifts the reversal potential to more positive values in accord with the predictions of the Nernst equation. Thus, the IPSP results from an increase in Cl^- conductance.

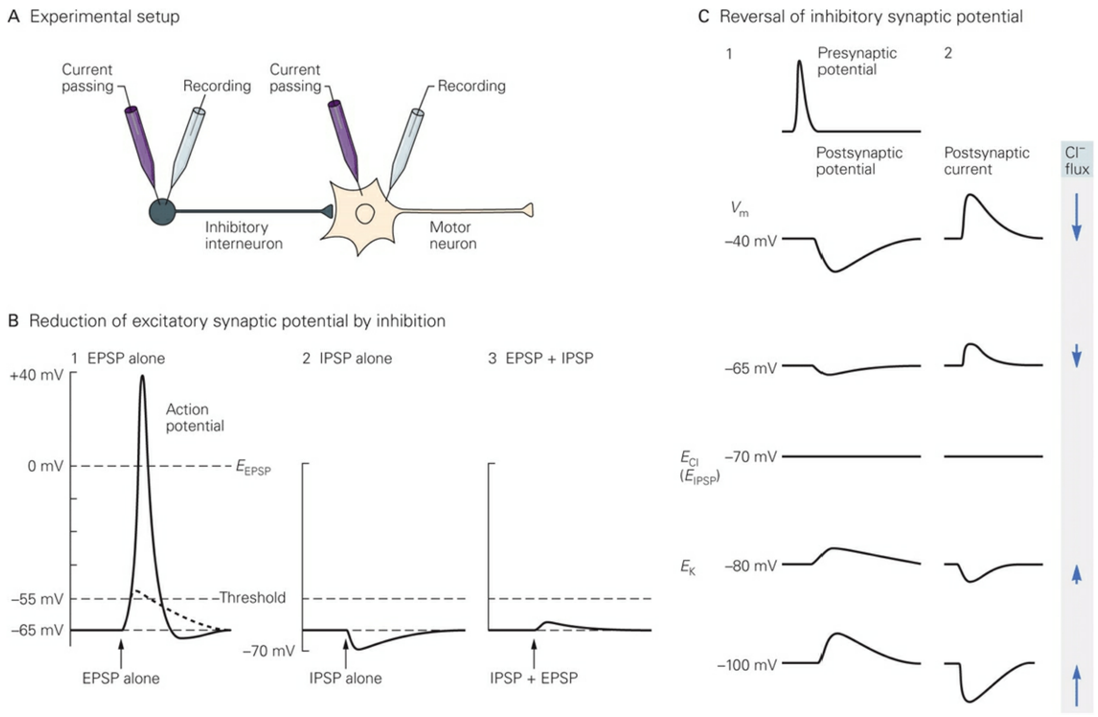


Figure 10-11 Inhibitory actions at chemical synapses result from the opening of ion channels selective for Cl^-.

A. In this hypothetical experiment two electrodes are placed in the presynaptic interneuron and two in the postsynaptic motor neuron. The current-passing electrode in the presynaptic cell is used to produce an action potential; in the postsynaptic cell it is used to alter the membrane potential systematically prior to the presynaptic input.

B. Inhibitory actions counteract excitatory actions. **1**. A large EPSP occurring alone depolarizes the membrane toward E_{EPSP} and exceeds the threshold for generating an action potential.

2. An IPSP alone moves the membrane potential away from the thresh-

old toward E_{Cl}, the Nernst potential for Cl^- (-70 mV).

3. When inhibitory and excitatory synaptic potentials occur together, the effectiveness of the EPSP is reduced, preventing it from reaching the threshold for an action potential.

C. The IPSP and inhibitory synaptic current reverse at the equilibrium potential for Cl^-. **1**. A presynaptic spike produces a hyperpolarizing IPSP at the resting membrane potential (-65 mV). The IPSP is larger when the membrane potential is set at -40 mV due to the increased driving force on Cl^-. When the membrane potential is set at -70 mV the IPSP is nullified. This reversal potential for the IPSP occurs at E_{Cl}. With further hyperpolarization of the membrane the IPSP is inverted to a depolarizing postsynaptic potential (at -80 and -100 mV) because the membrane potential is negative to E_{Cl}. **2**. The reversal potential of the inhibitory postsynaptic current measured under voltage clamp. An inward (negative) current flows at membrane potentials negative to the reversal potential (corresponding to an efflux of Cl^-) and an outward (positive) current flows at membrane potentials positive to the reversal potential (corresponding to an influx of Cl^-). (Up arrows = efflux, down arrows = influx.)

Currents Through Single GABA and Glycine Receptor-Channels Can Be Recorded

The currents through single GABA and glycine receptor-channels, the unitary currents, have been measured using the patch-clamp technique. Both transmitters activate Cl^- channels that open in an all-or-none manner, similar to the opening of ACh and glutamate-gated channels. The conductance of a glycine receptor-channel (46 pS) is larger than that of a $GABA_A$ receptor-channel (30 pS). As a result, the unitary current through glycine-gated channels is somewhat larger than that of $GABA_A$ receptor-channels (Figure 10–12). This difference results because the diameter of the glycine receptor-channel pore is slightly larger than that of the $GABA_A$ receptor-channel pore.

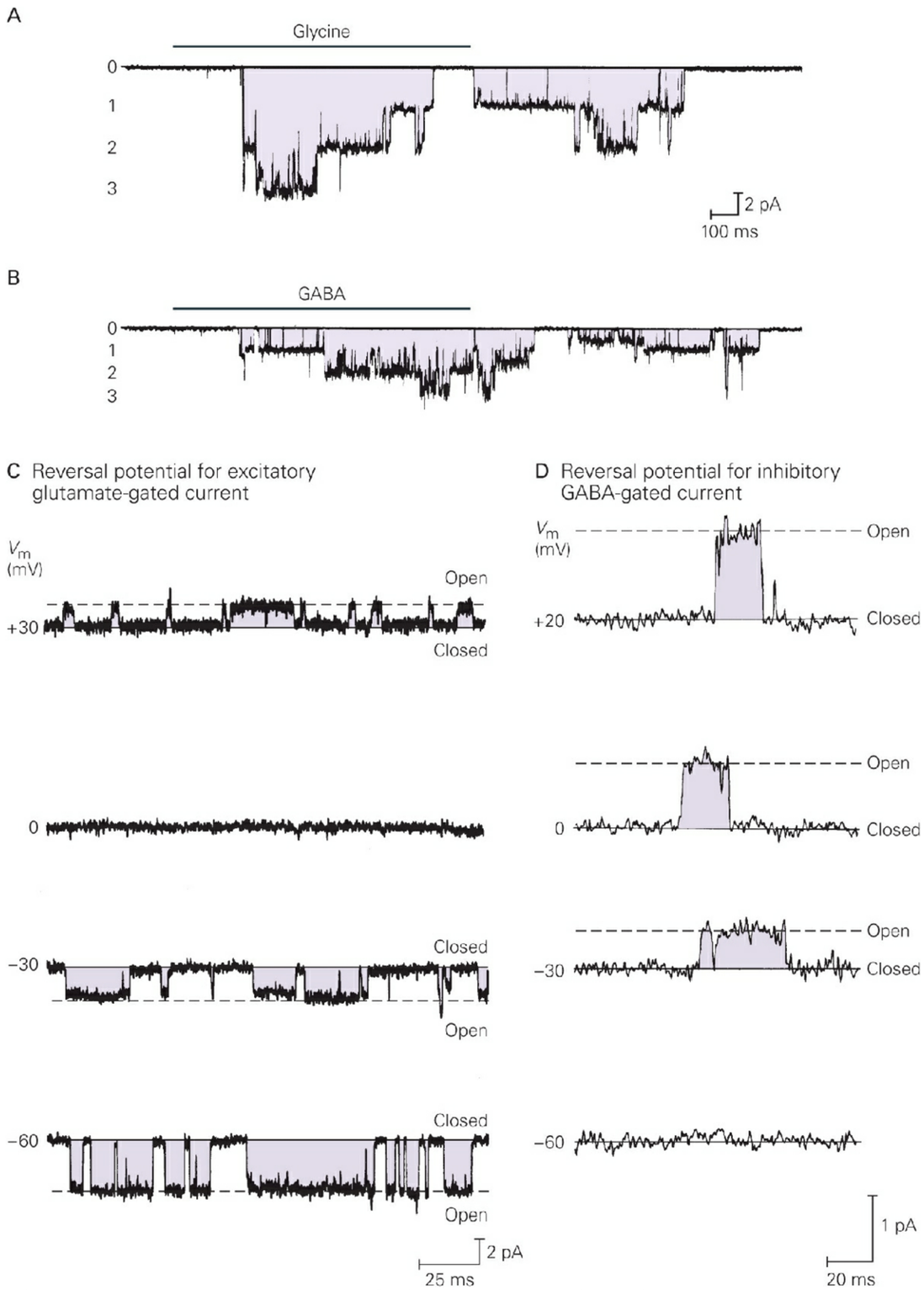


Figure 10-12 Comparison of single-channel excitatory currents acti-

vated by glutamate and inhibitory currents activated by GABA and glycine.

A. Single-channel currents through three glycine receptor-channels in a patch from a mouse spinal neuron. (Reproduced, with permission, from Bormann et al. 2007.)

B. Single-channel currents through GABA receptor-channels in the same patch. In A and B the membrane was held at a voltage negative to the Cl^- equilibrium potential, so channel openings generate an inward current owing to an efflux of Cl^-. (Reproduced, with permission, from Bormann et al. 2007.)

C. Excitatory current through a single NMDA receptor-channel in a rat hippocampal neuron. As the membrane potential is moved in a depolarizing direction (from -60 to -30 mV), the current pulses become smaller. At 0 mV (the reversal potential for the EPSP) the current pulses are nullified, and at +30 mV they invert and are outward. The reversal potential at 0 mV is the weighted average of equilibrium potentials for Na^+, Ca^{2+}, and K^+, the three ions responsible for generating this current. Note: In this recording there was no Mg^{2+} in the external solution so the NMDA receptor-channel openings are voltage-independent. (Reproduced, with permission, from J. Jen and C. F. Stevens.)

D. Inhibitory current through a single GABA-activated channel in a rat hippocampal neuron. The current is nullified at approximately -60 mV (the reversal potential for the IPSP in this cell). At more depolarized levels the current pulses are outward (corresponding to the influx of Cl^-). This reversal potential lies near the equilibrium potential for Cl^-, the only ion contributing to this current. (Reproduced, with permission, from B. Sakmann.)

The inhibitory action of these Cl^- channels can be demonstrated by comparing the reversal potentials of current through a $GABA_A$ receptor-channel and a glutamate receptor-channel. The excitatory current reverses at 0 mV (Figure 10-12C). Therefore, opening of glutamate receptor-channels at the normal resting potential generates an inward current (influx of positive charge), driving the membrane past threshold. In contrast, the inhibitory current becomes nullified and begins to reverse at values more negative than -60 mV (Figure 10-12D). Thus, the opening of $GABA_A$ receptor-channels at typical resting potentials normally generates an outward current (influx of negative charge), preventing the membrane from reaching threshold.

Chloride Currents Through Inhibitory $GABA_A$ and Glycine Receptor-Channels Normally Inhibit the Postsynaptic Cell

In a typical neuron the resting potential of -65 mV is slightly more positive than E_{Cl} (-70 mV). At this resting potential the chemical force driving Cl^- into the cell is slightly greater than the electrical force opposing Cl^- influx—that is, the electrochemical driving force on Cl^- $(V_m - E_{Cl})$ is positive. As a result, the opening of Cl^- channels leads to a positive current, based on the relation $I_{Cl} = g_{Cl}(V_m - E_{Cl})$. Because the charge carrier is the negatively charged Cl^- ion, the positive current corresponds to an influx of Cl^- into the neuron, down its electrochemical gradient. This causes a net increase in the negative charge on the inside of the membrane—the membrane becomes hyperpolarized.

Some central neurons have a resting potential that is approximately equal to E_{Cl}. In such cells an increase in Cl^- conductance does not change the membrane potential—the cell does not become hyperpolarized—because the electrochemical driving force on Cl^- is nearly zero. However, the opening of Cl^- channels in such a cell still inhibits the cell from firing an action potential in response to a near-simultaneous EPSP. This is because the depolarization produced by an excitatory input depends on a weighted average of the batteries for all types of open channels—that is, the batteries for the excitatory and inhibitory synaptic conductances and the resting conductances—with the weighting factor equal to the total conductance for a particular type of channel (see Chapter 9, Postscript). Because the battery for Cl^- channels lies near the resting potential, opening these channels helps hold the membrane near its resting potential during the EPSP by increasing the weighting factor for the Cl^- battery.

The effect that the opening of Cl^- channels has on the magnitude of an EPSP can also be described in terms of Ohm's law. Accordingly, the amplitude of the depolarization during an EPSP, ΔV_{EPSP}, is given by:

$$\Delta V_{EPSP} = I_{EPSP}/g_1$$

where I_{EPSP} is the excitatory synaptic current and g_l is the total conductance of all other channels open in the membrane, including resting channels and any contributions from the transmitter-gated Cl^- channels. Because the opening of the Cl^- channels increases the resting conductance, the depolarization during the EPSP decreases. This consequence of synaptic inhibition is called the *short-circuiting* or *shunting* effect.

The different biophysical properties of synaptic conductances can be also understood as distinct mathematical operations carried out by the postsynaptic neuron. Thus, inhibitory inputs that hyperpolarize the cell perform a *subtraction* on the excitatory inputs, whereas those that shunt the excitation perform a *division*. Adding excitatory inputs (or removing nonshunting inhibitory inputs) results in a *summation*. Finally, the combination of an excitatory input with the removal of an inhibitory shunt produces a *multiplication*.

In some cells, such as those with metabotropic $GABA_B$ receptors, inhibition is caused by the opening of K^+ channels. Because the K^+ equilibrium potential of neurons ($E_K = -80$ mV) is always negative to the resting potential, opening K^+ channels inhibits the cell even more profoundly than opening Cl^- channels (assuming a similar-size synaptic conductance). $GABA_B$ responses turn on more slowly and persist for a longer time compared with $GABA_A$ responses.

Paradoxically, under some conditions the activation of $GABA_A$ receptors in brain cells can cause excitation. This is because the influx of Cl^- after intense periods of stimulation can be so great that the intracellular Cl^- concentration increases substantially. It may even double. As a result, the Cl^- equilibrium potential may become more positive than the resting potential. Under these conditions the opening of Cl^- channels leads to Cl^- efflux and depolarization of the neuron. Such depolarizing Cl^- responses occur normally in some neurons in newborn animals, where the intracellular Cl^- concentration tends to be high even at rest. This is because the K^+-Cl^- cotransporter is expressed at low levels during early development, as discussed in Chapter 6. Depolarizing Cl^- responses may also occur in the distal dendrites of more mature neurons and perhaps also at their axon initial segment. Such excitatory $GABA_A$ receptor actions in adults may contribute to epileptic discharges in which large, synchronized, and depolarizing GABA responses are observed.

Ionotropic Glutamate, GABA, and Glycine Receptors Are Transmembrane Proteins Encoded by Two Distinct Gene Families

The genes coding for the $GABA_A$ and glycine receptors are closely related to each other. More surprisingly, the $GABA_A$ and glycine receptors are structurally related to the nicotinic ACh receptors, even though the latter select for cations. Thus, these receptors are members of one large gene family (Figure 10-7A). In contrast, the glutamate receptors have evolved from a different class of proteins and thus represent a second gene family of ligand-gated channels (Figure 10-7B).

Ionotropic $GABA_A$ and Glycine Receptors Are Homologous to Nicotinic ACh Receptors

Like nicotinic ACh receptor-channels, the $GABA_A$ and glycine receptor-channels are each composed of five subunits that are encoded by related gene families (Figure 10-7A). The $GABA_A$ receptors are usually composed of two α-, two β-, and one γ- or Δ-subunit. The receptors are activated by the binding of two molecules of GABA in clefts formed between the two α- and α-subunits. The glycine receptors are composed of three α- and two α-subunits and require the binding of up to three molecules of ligand to open. The transmembrane topology of each $GABA_A$ and glycine receptor subunit is similar to that of a nicotinic ACh receptor subunit, consisting of a large extracellular ligand-binding domain followed by four hydrophobic transmembrane α-helices (labeled M1, M2, M3, and M4), with the M2 helix forming the lining of the channel pore. However, the amino acids flanking the M2 domain are strikingly different from those of the nicotinic ACh receptor. As discussed in Chapter 9, the pore of the ACh receptor contains rings of negatively charged acidic residues that help the channel select for cations over anions. In contrast, the GABA and glycine receptor-channels contain either neutral or positively charged basic residues at the homologous positions, which contributes to the selectivity of these channels for anions.

Most of the classes of receptor subunits are encoded by multiple related

genes. Thus, there are six subtypes of $GABA_A$ α-subunits (α1-α6), three α-subunits (β1-β3), three γ-subunits (γ1-γ3) and one Δ-subunit. The genes for these different subtypes are often differentially expressed in different types of neurons, endowing their inhibitory synapses with distinct properties. The possible combinatorial arrangements of these subunits in a fully assembled pentameric receptor provides an enormous potential diversity of receptor subtypes.

The $GABA_A$ and glycine receptors play important roles in disease and in the actions of drugs. $GABA_A$ receptors are the target for several types of drugs that are clinically important and socially abused, including general anesthetics, benzodiazepines, barbiturates, and alcohol. General anesthetics can be either gases or injectable compounds that induce a loss of consciousness and are therefore widely used during surgery. Benzodiazepines are antianxiety agents and muscle relaxants that include diazepam (Valium), lorazepam (Ativan), and clonazepam (Klonopin). Zolpidem (Ambien) is a non-benzodiazepine compound that promotes sleep. The barbiturates comprise a distinct group of hypnotics that includes phenobarbital and secobarbital. The different classes of compounds—GABA, general anesthetics, benzodiazepines, barbiturates, and alcohol—bind to different sites on the receptor but act similarly to increase the opening of the GABA receptor-channel. For example, whereas GABA binds to a cleft between the α- and α-subunits, benzodiazepines bind to a cleft between the α- and γ-subunits. In addition, the binding of any one of these classes of drug influences the binding of the others. For example, a benzodiazepine (or a barbiturate) binds more strongly to the receptor-channel when GABA also is bound, and this tight binding helps stabilize the channel in the open state. In this manner, the various compounds all enhance inhibitory synaptic transmission.

How do these various compounds that all act on $GABA_A$ receptors to promote channel opening produce such diverse behavioral and psychological effects, for example, reducing anxiety versus promoting sleep? It turns out that many of these compounds interact selectively with specific subunit subtypes, which can be localized to different regions of the brain. For example, zolpidem binds selectively to $GABA_A$ receptors containing the α1-subtype of subunit. In contrast, the anxiolytic effect of benzodiazepines requires binding to the α2-and γ-subunits.

In addition to being important pharmacological targets, the $GABA_A$ and glycine receptors are targets of disease and poisons. Missense mutations in the α subunit of the glycine receptor underlie an inherited neurological disorder called *familial startle disease* (or *hyperekplexia*), characterized by abnormally high muscle tone and exaggerated responses to noise. These mutations decrease the opening of the glycine receptor and so reduce the normal levels of inhibitory transmission in the spinal cord. The poison strychnine, a plant alkaloid compound, causes convulsions by blocking the glycine receptor and decreasing inhibition. Nonsense mutations that result in truncations of $GABA_A$ receptor α- and γ-subunits have been implicated in congenital forms of epilepsy.

Some Synaptic Actions Depend on Other Types of Ionotropic Receptors in the Central Nervous System

Certain fast excitatory synaptic actions are mediated by the neurotransmitter serotonin (5-HT) acting at the $5\text{-}HT_3$ class of ionotropic receptor-channels. These ionotropic receptors have four transmembrane segments and are structurally similar to the nicotinic ACh receptors. Like the ACh receptor-channels, $5\text{-}HT_3$ receptor-channels are permeable to monovalent cations and have a reversal potential near 0 mV.

Finally, ionotropic receptors for adenosine triphosphate (ATP), which serves as an excitatory transmitter at selected synapses, constitute a third major family of transmitter-gated ion channels. These so-called purinergic receptors (named for the purine ring in adenosine) occur on smooth muscle cells innervated by sympathetic neurons of the autonomic ganglia as well as on certain central and peripheral neurons. At these synapses ATP activates an ion channel that is permeable to both monovalent cations and Ca^{2+}, with a reversal potential near 0 mV. Several genes coding for this family of ionotropic ATP receptors (termed the P2X receptors) have been identified. The amino acid sequence and subunit structure of these ATP receptors is different from the other two ligand-gated channel families. An X-ray crystal structure of the P2X receptor reveals that it has an exceedingly simple organization in which three subunits, each containing only two transmembrane segments, surround a central pore (Figure 10-7C).

Excitatory and Inhibitory Synaptic Actions Are Integrated by the Cell into a Single Output

Up to now we have mostly focused on the physiological and molecular properties of excitatory or inhibitory synapses in isolation. However, each neuron in the central nervous system is constantly bombarded by an array of synaptic inputs from many other neurons. A single motor neuron, for example, may be innervated by as many as 10,000 different presynaptic endings. Some are excitatory, others inhibitory; some are strong, others weak. Some inputs contact the motor cell on the tips of its apical dendrites, others on proximal dendrites, some on the dendritic shaft, others on the soma. The different inputs can reinforce or cancel one another. How does a given neuron integrate these signals into a coherent output?

As we saw earlier, the synaptic potentials produced by a single presynaptic neuron typically are not large enough to depolarize a postsynaptic cell to the threshold for an action potential. The EPSPs produced in a motor neuron by most stretch-sensitive afferent neurons are only 0.2 to 0.4 mV in amplitude. If the EPSPs generated in a single motor neuron were to sum linearly, at least 25 afferent neurons would have to fire together and release transmitter to depolarize the trigger zone by the 10 mV required to reach threshold. But at the same time the postsynaptic cell is receiving excitatory inputs, it may also be receiving inhibitory inputs that prevent the firing of action potentials by either a subtractive or shunting effect. The net effect of the inputs at any individual excitatory or inhibitory synapse will therefore depend on several factors: the location, size, and shape of the synapse, the proximity and relative strength of other synergistic or antagonistic synapses, and the resting potential of the cell.

Inputs are coordinated in the postsynaptic neuron by a process called *neuronal integration*. This cellular process reflects the task that confronts the nervous system as a whole: decision making. A cell at any given moment has two options: to fire or not to fire an action potential. Charles Sherrington described the brain's ability to choose between competing alternatives as the *integrative action of the nervous system*. He regarded this decision making as the brain's most fundamental operation.

Synaptic Inputs Are Integrated to Fire an Action Potential at the Axon Initial Segment

In most neurons the decision to initiate an action potential is made at one site: the initial segment of the axon (see Chapter 2). Here the cell membrane has a lower threshold for action potential generation than at the cell body or dendrites because it has a higher density of voltage-dependent Na^+ channels (Figure 10–13). With each increment of membrane depolarization, more Na^+ channels open, thus generating a higher density of inward current (per unit area of membrane) at the axon initial segment than elsewhere in the cell. At the initial segment the depolarization increment required to reach the threshold for an action potential (-55 mV) is only 10 mV from the resting level of -65 mV. In contrast, the membrane of the cell body must be depolarized by 30 mV before reaching its threshold (-35 mV). Therefore, synaptic excitation first discharges the region of membrane at the initial segment, also called the *trigger zone*. The action potential generated at this site then depolarizes the membrane of the cell body to threshold and at the same time is propagated along the axon.

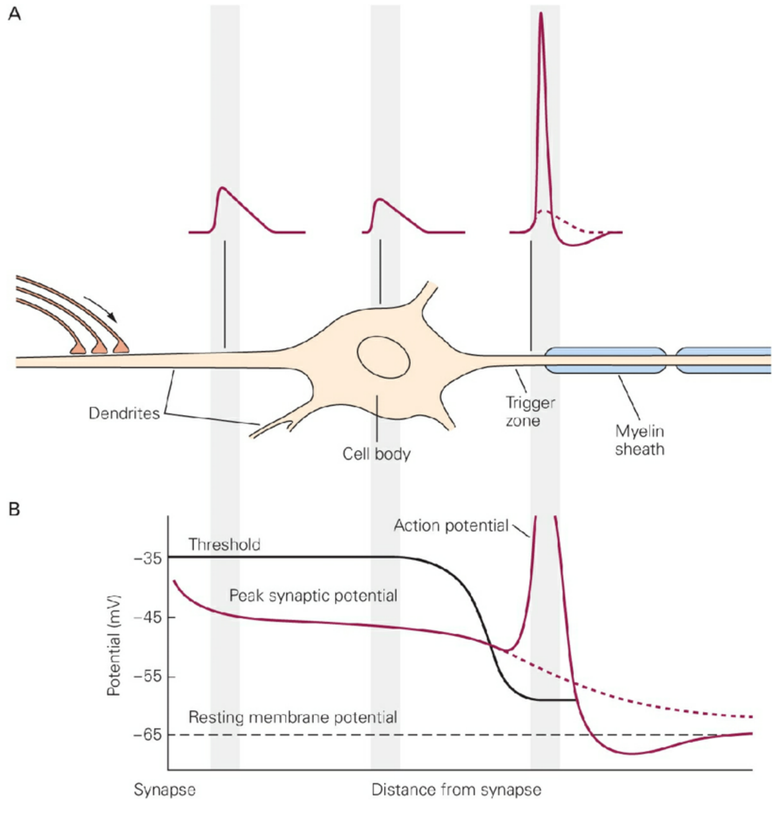


Figure 10-13 A synaptic potential arising in a dendrite can generate an action potential at the axon initial segment. (Adapted, with permission, from Eckert et al. 1988.)

A. An excitatory synaptic potential originating in the dendrites decreases with distance as it propagates passively to the soma. Nevertheless, an action potential can be initiated at the trigger zone (the axon initial segment) because the density of the Na^+ channels in this region is high, and thus the threshold is low.

B. Comparison of the threshold for initiation of the action potential at different sites in the neuron (corresponding to drawing A). An action potential is generated when the amplitude of the synaptic potential exceeds the threshold. The dashed line shows the decay of the synaptic potential if no action potential is generated at the axon initial segment.

Because neuronal integration involves the summation of synaptic potentials that spread to the trigger zone, it is critically affected by two passive membrane properties of the neuron (see Chapter 6). First, the membrane time constant helps determine the time course of the synaptic potential and thereby controls *temporal summation*, the process by which consecutive synaptic potentials at the same site are added together in the postsynaptic cell. Neurons with a large membrane time constant have a greater capacity for temporal summation than do neurons with a shorter time constant (Figure 10–14A). As a result, the longer the time constant, the greater the likelihood that two consecutive inputs from an excitatory presynaptic neuron will summate to bring the cell membrane to its threshold for an action potential.

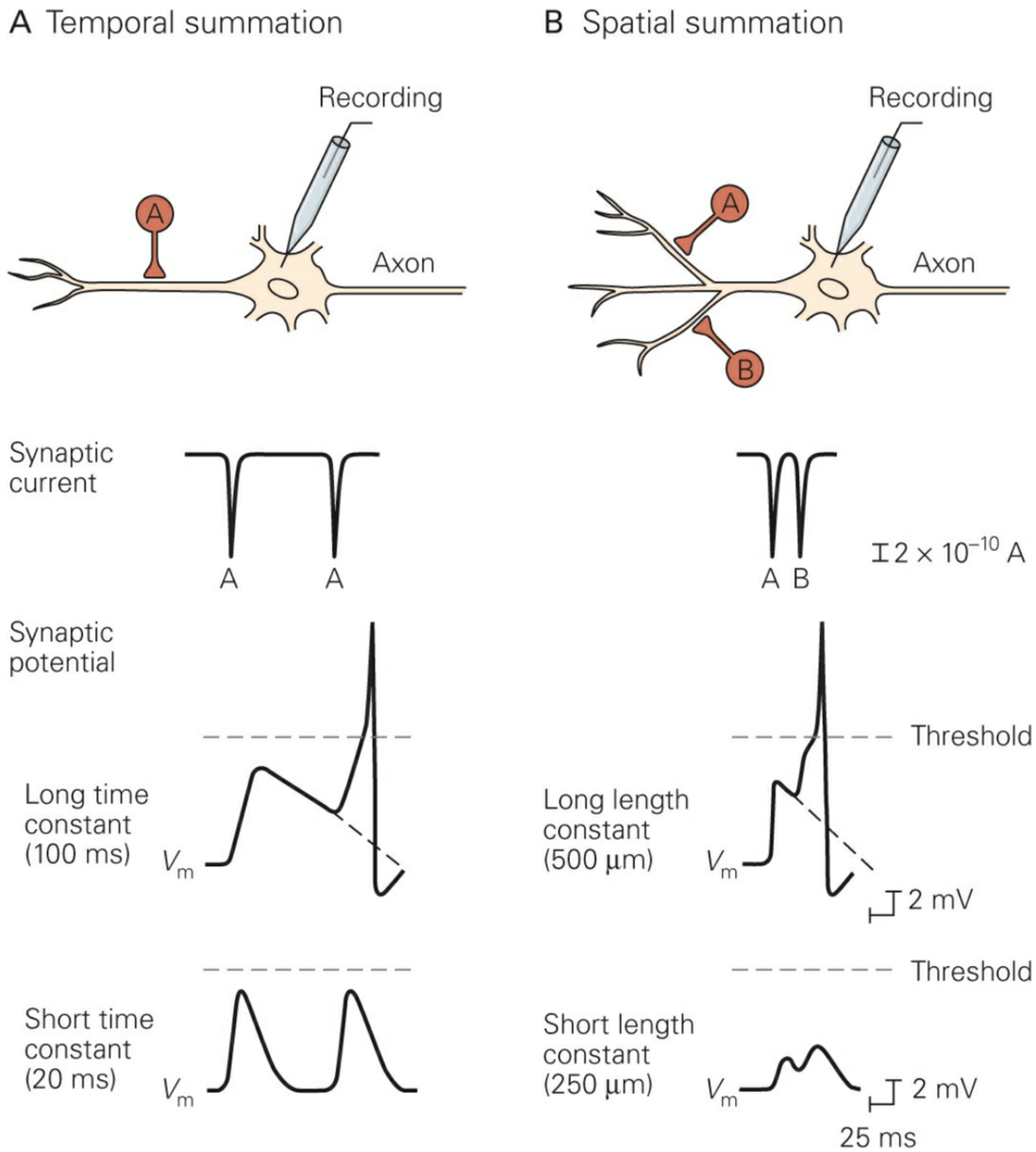


Figure 10-14 Central neurons are able to integrate a variety of synaptic inputs through temporal and spatial summation of synaptic potentials.

A. The time constant of a postsynaptic cell (see Figure 6-15) affects the amplitude of the depolarization caused by consecutive EPSPs produced by a single presynaptic neuron (A). Here the synaptic current generated by the presynaptic neuron is nearly the same for both EPSPs. In a cell with a *long* time constant the first EPSP does not fully decay by the

time the second EPSP is triggered. Therefore, the depolarizing effects of both potentials are additive, bringing the membrane potential above the threshold and triggering an action potential. In a cell with a *short* time constant the first EPSP decays to the resting potential before the second EPSP is triggered. The second EPSP alone does not cause enough depolarization to trigger an action potential.

B. The length constant of a postsynaptic cell (see Figure 6-16) affects the amplitudes of two excitatory postsynaptic potentials produced by two presynaptic neurons (A and B). For illustrative purposes, both synapses are the same (500 μm) distance from the postsynaptic cell's trigger zone at the axon initial segment, and the current produced by each synaptic contact is the same. If the distance between the site of synaptic input and the trigger zone in the postsynaptic cell is only one length constant (that is, the postsynaptic cell has a length constant of 500 μm), the synaptic potentials produced by each of the two presynaptic neurons will decrease to 37% of their original amplitude by the time they reach the trigger zone. Summation of the two potentials results in enough depolarization to exceed threshold, triggering an action potential. If the distance between the synapse and the trigger zone is equal to two length constants (ie, the postsynaptic cell has a length constant of 250 μm), each synaptic potential will be less than 15% of its initial amplitude, and summation will not be sufficient to trigger an action potential.

Second, the *length* constant of the cell determines the degree to which a local depolarization decreases as it spreads passively from a synapse along the length of the dendrite. In cells with a longer length constant, signals spread to the trigger zone with minimal decrement; in cells with a short length constant, the signals decay rapidly with distance. Because the depolarization produced at one synapse is almost never sufficient to trigger an action potential at the trigger zone, the inputs from many presynaptic neurons acting at different sites on the postsynaptic neuron must be added together. This process is called *spatial summation*. Neurons with a large length constant are more likely to be brought to threshold by inputs arising from different sites than are neurons with a short length constant (Figure 10-14B).

Dendrites Are Electrically Excitable Structures That Can Fire Action Potentials

Propagation of signals in dendrites was originally thought to be purely passive. However, intracellular recordings from the cell body of neurons in the 1950s and from dendrites beginning in the 1970s demonstrated that dendrites could produce action potentials. Indeed, we now know that the dendrites of most neurons contain voltage-gated Na^+, K^+, and Ca^{2+} channels in addition to ligand-gated channels and leakage channels. In fact, the rich diversity of dendritic conductances suggests that central neurons rely on a sophisticated repertory of electrophysiological properties to integrate synaptic inputs.

One function of the voltage-gated Na^+, and Ca^{2+} channels in dendrites may be to amplify the EPSP. In some neurons there are sufficient concentrations of voltage-gated channels in the dendrites to serve as a local trigger zone. This can further amplify weak excitatory input that arrives at remote parts of the dendrite. When a cell has several dendritic trigger zones, each one sums the local excitation and inhibition produced by nearby synaptic inputs; if the net input is above threshold, a dendritic action potential may be generated, usually by voltage-dependent Na^+ or Ca^{2+} channels (Figure 10–15). Nevertheless, the number of voltage-gated Na^+ or Ca^{2+} channels in the dendrites is usually not sufficient to support the all-or-none regenerative propagation of these action potentials to the cell body. Rather, action potentials generated in the dendrites are local events that propagate electrotonically to the cell body and axon initial segment, where they are integrated with all other input signals in the cell.

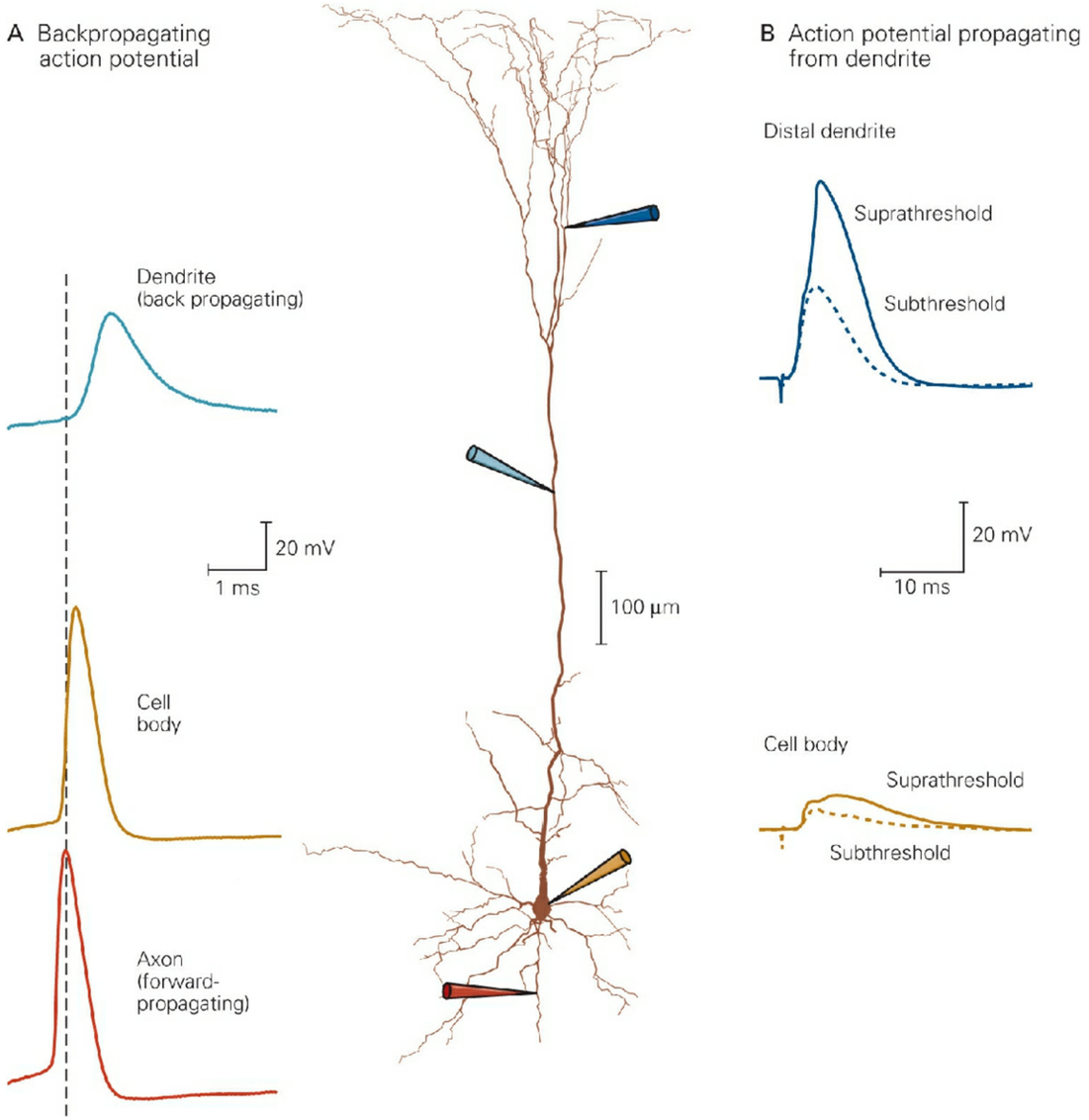


Figure 10-15 Dendrites support propagation of action potentials to and from the axon initial segment. The figure illustrates an experiment in which several electrodes are used to record membrane voltage and pass stimulating currents in the axon, the soma, and at several locations along the dendritic tree. The recording electrode and corresponding voltage trace are matched according to color. (Adapted, with permission, from Stuart et al. 2000.)

A. An action potential can be propagated from the axon initial segment to the dendrites. Such backpropagation depends on activation of voltage-gated Na^+ channels in the dendrites. Unlike the action potential that is continually regenerated along an axon, the amplitude of a backpropagating action potential decreases as it travels along a dendrite due to the relatively low density of voltage-gated Na^+ channels in dendrites.

B. A strong depolarizing EPSP at a dendrite can generate an action potential that travels to the cell body. Such forward-propagating action potentials are often generated by dendritic voltage-gated Ca^{2+} channels and have a high threshold. They propagate relatively slowly and decrement with distance, often failing to reach the cell body. The solid line shows a suprathreshold response generated in the dendrite, and the dotted line shows a subthreshold response.

Do active conductances influence dendritic integration? There is currently a vigorous debate as to what arithmetic rules dendrites use to summate inputs. While some results indicate that dendrites are highly nonlinear devices that work by firing local spikes in individual dendritic branches, others argue that dendrites behave essentially as linear integrators (ie, they sum inputs arithmetically). In this linear scenario, dendritic conductances would balance each other to achieve a stable integration regime.

The dendritic voltage-gated channels also permit action potentials generated at the axon initial segment to propagate backward into the dendritic tree. These *back-propagating* action potentials are found in most neurons and are largely generated by dendritic voltage-gated Na^+ channels. Although the precise role of backpropagating action potentials is unclear, they may provide a temporally precise mechanism for regulating current through the NMDA receptor by providing the depolarization necessary to remove the Mg^{2+} block (see Figure 10-5).

Synapses on a Central Neuron Are Grouped According to Physiological Function

All four regions of the nerve cell—axon, terminals, cell body, and dendrites—can in principle be presynaptic or postsynaptic sites. The most common types of contact, illustrated in Figure 10–16, are axodendritic, axosomatic, and axo-axonic (by convention, the presynaptic element is identified first). Axodendritic synapses can occur on the dendritic shaft or

on spines. Dendrodendritic and somasomatic contacts are also found, but they are rare.

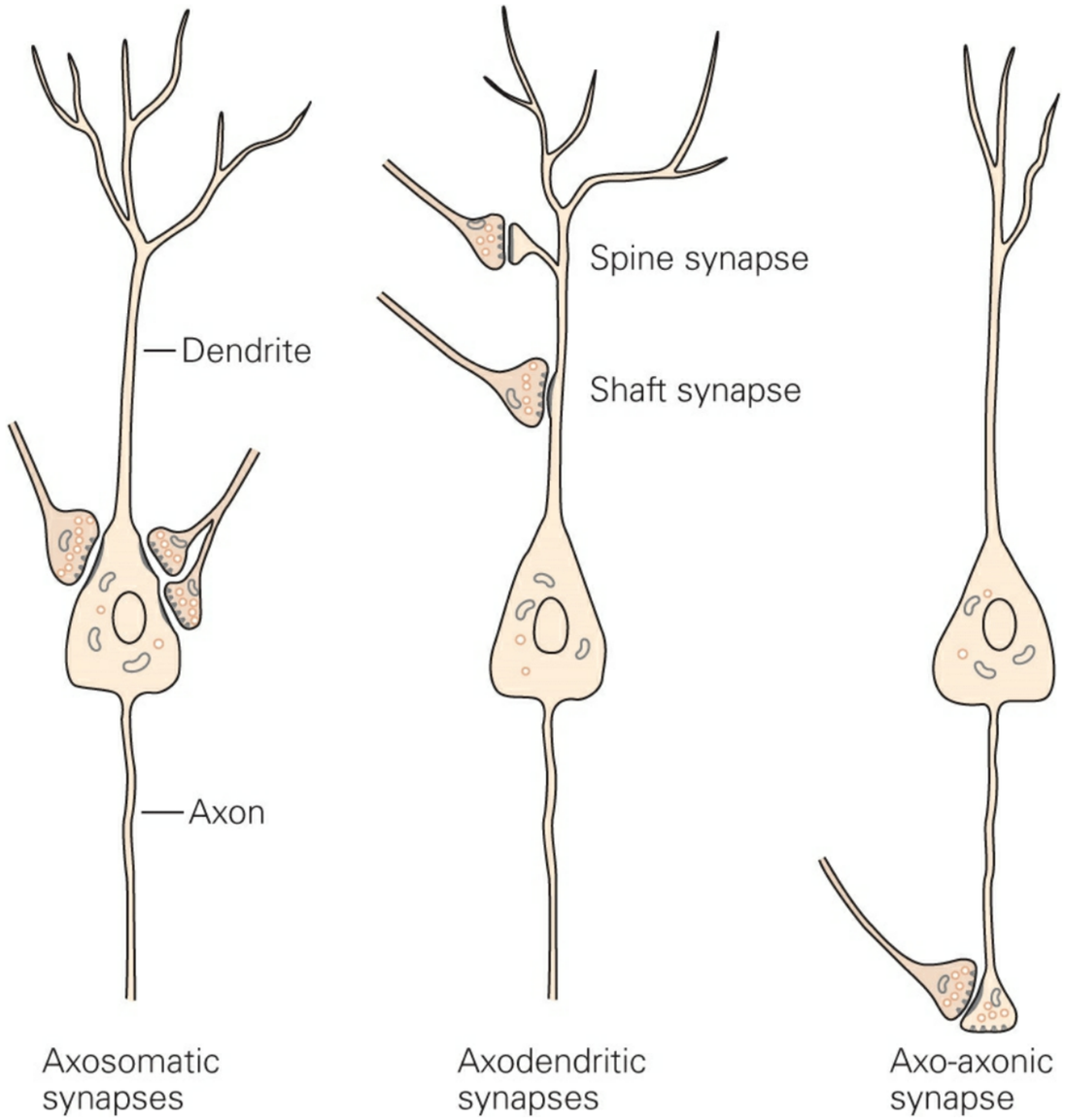


Figure 10-16 Synaptic contact can occur on the cell body, the dendrites, or the axon of the postsynaptic cell. The names of various kinds of synapses—axosomatic, axodendritic, and axo-axonic—identify the contacting regions of both the presynaptic and postsynaptic neurons (the presynaptic element is identified first). Axodendritic synapses can occur on either the shaft of a dendrite or on a specialized input zone, the spine.

The proximity of a synapse to the trigger zone is traditionally thought to be important to its effectiveness. A postsynaptic current generated at an axosomatic site should produce a greater change in membrane potential at the trigger zone, and therefore a greater influence on action potential output, than does an equal current at more remote axodendritic contacts, because of the passive cable properties of a dendrite (Figure 10–17). Nevertheless, it seems that some neurons compensate for this effect by placing more glutamate receptors at distal synapses than at proximal synapses, ensuring that inputs along the dendritic tree have a more equivalent strength at the initial segment, thereby minimizing spatial effects in dendritic integration.

In contrast to axodendritic and axosomatic input, most axo-axonic synapses have no direct effect on the trigger zone of the postsynaptic cell. Instead, they affect the activity of the postsynaptic neuron by controlling the amount of transmitter released from the presynaptic terminals (see Chapter 12).

The location of inhibitory inputs in relation to excitatory ones is critical for the effectiveness of the inhibitory stimuli. Inhibitory shunting is more significant when initiated at the cell body near the axon hillock. The depolarization produced by an excitatory current from a dendrite must pass through the cell body as it moves toward the axon initial segment. Inhibitory actions at the cell body open Cl^- channels, thus increasing Cl^- conductance and reducing by shunting much of the depolarization produced by the spreading excitatory current. As a result, the influence of the excitatory current on the membrane potential at the trigger zone is strongly curtailed (Figure 10–17). In contrast, inhibitory actions at a remote part of a dendrite are much less effective in shunting excitatory actions or in affecting the more distant trigger zone. Thus, it is not surprising that in the brain significant inhibitory input frequently occurs on the cell body of neurons.

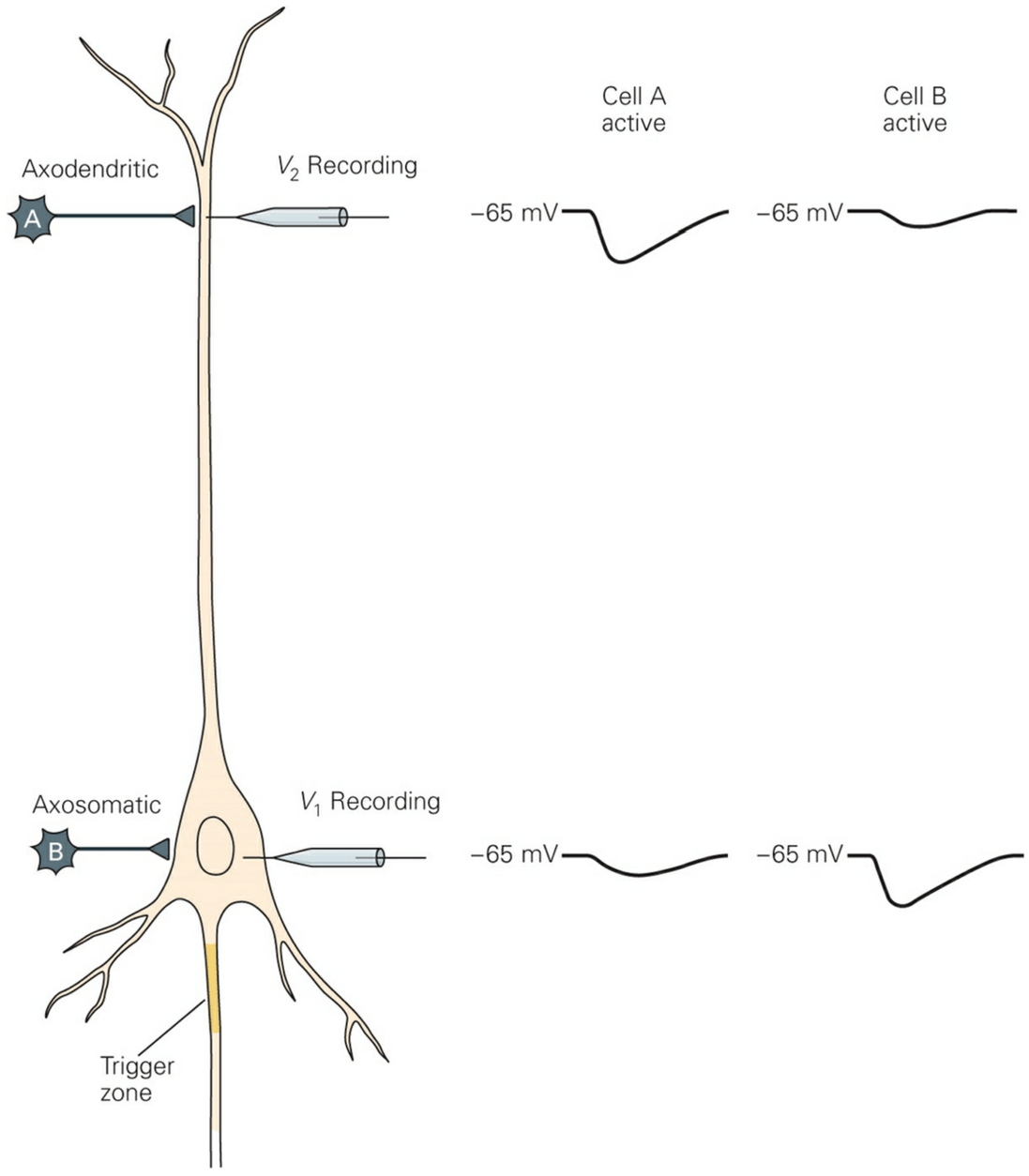


Figure 10-17 The effect of an inhibitory current in the postsynaptic neuron depends on the distance the current travels from the synapse to the cell's trigger zone. In this hypothetical experiment the inputs from inhibitory axosomatic and axodendritic synapses are compared by recording from both the cell body (V_1) and the dendrite (V_2) of the postsynaptic cell. Stimulating the axosomatic synapse from presynaptic cell B produces a large IPSP in the cell body. Because the synaptic potential is initiated in the cell body it will undergo little decrement before arriving at the trigger zone in the initial segment of the axon. The IPSP decays as it propagates up the dendrite, producing only a small hyperpolarization at the site of dendritic recording. Stimulating the axodendritic synapse from presynaptic cell **A** produces a large local IPSP in the dendrite but only a small IPSP in the cell body because the synaptic potential decays as it propagates down the dendrite to the cell body. Thus the axosomatic IPSP is more effective than the axodendritic IPSP in inhibiting action potential firing in the postsynaptic cell.

Even though some excitatory inputs occur on dendritic shafts, close to 95% of all excitatory inputs in the brain terminate on dendritic spines, surprisingly avoiding dendritic shafts (see Figure 10-3). Although the function of spines is not completely understood, their thin necks provide a barrier to diffusion of various signaling molecules from the spine head to the dendritic shaft. As a result a relatively small Ca^{2+} current through the NMDA receptors can lead to a relatively large increase in $[Ca^{2+}]$ that is localized to the head of the individual spine that is synaptically activated (Figure 10–18A). Moreover, because action potentials can backpropagate from the cell body to the dendrites (see below), spines also serve as sites at which information about presynaptic and postsynaptic activity is integrated. Indeed, when a backpropagating action potential is paired with presynaptic stimulation, the spine Ca^{2+} signal is greater than the linear sum of the individual Ca^{2+} signals from synaptic stimulation alone or action potential stimulation alone. This "supralinearity" is specific to the activated spine and occurs because depolarization during the action potential causes Mg^{2+} to be expelled from the NMDA receptor, allowing it to conduct Ca^{2+} into the spine (see Figure 10-5). The resultant Ca^{2+} accumulation thus provides, at an individual synapse, a biochemical detector of the near simultaneity of the input (EPSP) and output (backpropagating action potential), which is thought to be a key requirement of memory storage.

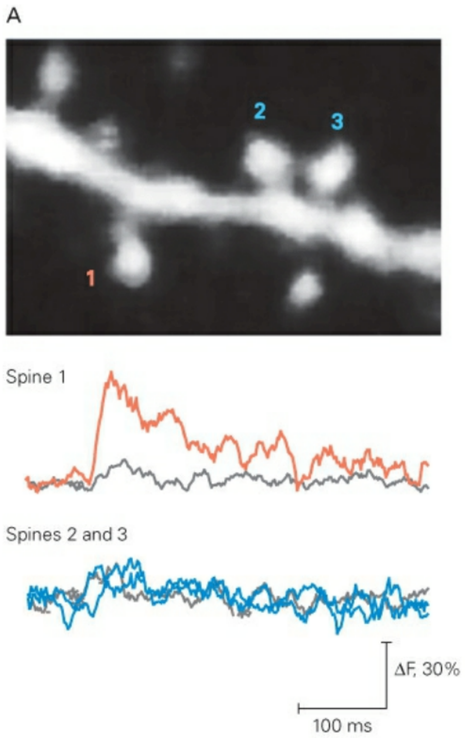


Figure 10-18 Dendritic spines compartmentalize calcium influx through NMDA receptors.

A. This fluorescence image of a hippocampal CA1 pyramidal neuron filled with a calcium-sensitive dye shows the outline of a dendritic shaft with several spines. When the dye binds Ca^{2+} its fluorescence intensity increases. The traces plot fluorescence intensity as a function of time following the extracellular stimulation of the presynaptic axon. Spine 1 shows a large, rapid fluorescence increase (ΔF) in response to synaptic stimulation (**red trace**), reflecting Ca^{2+} influx through the NMDA receptors. In contrast, there is little change in the fluorescence intensity in the neighboring dendrite shaft (**grey trace**), showing that Ca^{2+} accumulation is restricted to the head of the spine. Spines 2 and 3 show little increase in fluorescence in response to synaptic stimulation because their presynaptic axons were not activated by the external stimulus. (Reproduced, with permission, from Lang et al. 2004.)

B. Calcium accumulation is greatest in spines when synaptic stimulation is paired with postsynaptic action potentials. The vertical axis is proportional to the intracellular Ca^{2+} concentration in a dendritic spine. The Ca^{2+} signal generated when an EPSP and a backpropagating action potential are evoked at the same time (EPSP + AP [measured]) is greater than the expected sum of the individual Ca^{2+} signals (EPSP + AP [calculated]) when either an EPSP or a backpropagating action potential (AP) is evoked alone. (Adapted, with permission, from Yuste and Denk, 1995.)

As the thin spine neck, at least partly, restricts the rise in Ca^{2+}, and thus long-term plasticity, to the spine that receives the synaptic input, spines also ensure that activity-dependent changes in synaptic function, and thus memory storage, are restricted to the synapses that are activated. The ability of spines to implement such synapse-specific local learning rules may be of fundamental importance for the ability of neural networks to store meaningful information (see Appendix E). Finally, the local synaptic potentials in some spines are filtered as they propagate through the spine neck and enter the dendrite, reducing the size of the EPSP at the soma. The regulation of this electrical filtering could control the efficacy with which local EPSPs are conducted to the soma.

An Overall View

The past several years have seen a remarkable growth in our understanding of the diversity of molecules and mechanisms underlying the postsynaptic responses of neurons in the central nervous system to transmitter released from their presynaptic inputs. Nonetheless, nearly all fast synaptic actions in the brain are mediated by only three main amino acid

neurotransmitters: the excitatory transmitter glutamate and the inhibitory transmitters GABA and glycine. These produce rapid changes in the postsynaptic membrane potential by acting on specific classes of ligand-gated channels, known as ionotropic receptors. Glutamate depolarizes a postsynaptic cell by acting on three types of ionotropic receptors: the kainate, AMPA, and NMDA receptors. In addition, glutamate acts on metabotropic receptors to produce slower modulatory synaptic actions that can be either excitatory or inhibitory. The fast inhibitory transmitters hyperpolarize cells by acting on $GABA_A$ receptors or glycine receptors. GABA also binds to inhibitory metabotropic $GABA_B$ receptors,

The inhibitory $GABA_A$ and glycine receptors belong to the same superfamily of receptors as do the excitatory nicotinic ACh receptors present at the neuromuscular junction. They are composed of five homologous subunits, with each subunit containing four membrane-spanning α-helixes. The three types of glutamate receptors belong to a separate receptor gene family. They are composed of four homologous subunits, with each subunit containing two extracellular ligand binding regions and a transmembrane domain containing three membrane-spanning α-helixes and a reentrant P-loop that forms the selectivity filter of the channel.

Despite the fact that fast synaptic transmission in the brain depends on only three major neurotransmitters acting on five main classes of ionotropic receptors, there is an enormous diversity in the postsynaptic properties of synapses in the brain. This is due, in part, to the presence of a large number of subtypes of the different receptors, resulting from the presence of multiple isoforms of receptor subunits encoded by distinct but related genes. Further diversity is generated through posttranscriptional processing, including differential RNA splicing and RNA editing. For $GABA_A$ receptors alone it has been estimated that there is a potential for the existence of up to 800 different receptor subtypes due to combinatorial arrangements of the different subunit isoforms, although it is likely that far fewer are actually generated in the brain.

Clearly, a major challenge is the identification of which receptor subunit combinations actually exist in the brain and where those subunits are located. Although this is a daunting task, the potential payoff is great, offering the possibility of subtype-specific drugs that produce highly specific actions. For example, whereas compounds that produce a nonspecific blockade of all types of $GABA_A$ receptors lead to excess excitation and seizures, a drug that selectively blocks the subtype of $GABA_A$ receptor containing the α5-subunit has a beneficial effect on memory storage. A different drug that activates the $GABA_A$ α2/α3-subtype of receptors alleviates neuropathic pain.

As we have seen in this chapter and will return to throughout the book, one of the key properties of neuronal synapses is that their function can be modulated by experience. Some forms of synaptic plasticity involve long-term changes in receptor expression. One of the best characterized examples of activity-dependent synaptic plasticity depends on the properties of the NMDA receptors, which conduct Ca^{2+} into a postsynaptic cell when two criteria are met: Glutamate must be released from the presynaptic terminal and the postsynaptic neuron must be sufficiently depolarized to expel Mg^{2+} from the pore of the receptor through electrostatic repulsion. In normal amounts, Ca^{2+} triggers signaling pathways that enhance excitatory synaptic transmission, a process essential for certain types of memory (see Chapters 66 and 67). In excess, as occurs during stroke and certain neurodegenerative diseases, Ca^{2+} leads to neuronal death and brain damage.

Another key aspect of synaptic transmission in the CNS is the fact that individual excitatory or inhibitory inputs normally produce relatively small changes in a neuron's membrane potential. A neuron must therefore integrate information from thousands of excitatory and inhibitory inputs before deciding whether the threshold for an action potential (-55 mV) has been reached. The summation of these inputs within a single cell depends critically on the cell's passive properties, namely on its time and length constants. Moreover, a synapse's location is critically important to its efficacy. Excitatory synapses tend to be located on spines on neuronal dendrites, whereas inhibitory synapses predominate on the cell body, where they can effectively interrupt and override the excitatory inputs traveling down the cell's dendrites to the soma. The final summing of inputs to the cell is made at the axon initial segment, which contains the highest density of Na^+ channels in the cell and thus has the lowest threshold for spike initiation. The consequences of mixed excitatory and inhibitory inputs in neural networks is discussed in Appendix E.

Much of the discussion in this chapter has been based on the schematic model of the neuron outlined in Chapter 2. According to this model, the dendritic tree is specialized as the receptive pole of the neuron, the axon is

the signal-conducting portion, and the axon terminal is the transmitting pole. This model implies that the neuron, the signaling unit of the nervous system, merely sends and receives information. In reality, neurons in most brain regions are more complex. As we have seen in this chapter, active conductances allow dendrites to propagate action potentials, which interact with synaptic events to produce long-lasting changes in synaptic transmission. Moreover, dendritic spines appear ideally suited to implement input-specific learning rules. Thus, our current view is that dendrites are complex, integrative compartments in neurons that can powerfully affect the propagation of synaptic potentials to the cell body and the relay of activity-dependent information from the cell body and initial segment back to synapses on the dendrites. Although crucial to neuronal integration, the electrical properties of dendrites and spines remain rather poorly understood and are an area of active investigation. In fact, as we shall see when considering the sensory and motor systems, the integrative properties of neurons in many brain regions allow the neurons to perform essential transformations on their inputs, rather than serve as simple relay stations.

Steven A. Siegelbaum
Eric R. Kandel
Rafael Yuste

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