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MEMBRANE PROPERTIES AND NEUROTRANSMITTER ACTIONS

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Information processing depends not only on the anatomical substrates of synaptic circuits but also on the electrophysiological properties of neurons and neuronal elements, and how these properties are altered and tuned by the plethora of neuroactive substances impinging upon them. Even if two neurons in different regions of the nervous system possess identical morphological features, they may respond to the same synaptic input in very different manners because of each cell's intrinsic properties. Understanding synaptic organization and function in different regions of the nervous system therefore requires an understanding of the electrophysiological and pharmacological properties of each of the constituent neuronal elements.

The electrophysiological behavior of a neuron is determined by the presence and distribution of different ionic currents in that cell, and by the ability of various neurotransmitters either to increase or decrease the amplitude or to modify the properties of these currents. This chapter will give a general overview of neuronal currents known to exist in brain cells, how they may be modulated by neurotransmitters, and how the interplay between the two can result in complicated patterns of activity in synaptic circuits. For a more detailed introduction to the biophysical mechanisms of ionic currents in neurons, the reader is referred to Hille, 1992; Nicholls et al., 1992; Huguenard and McCormick, 1994; and Johnston and Wu, 1995.

MEMBRANES AND IONIC CURRENTS

Neurons, like cells elsewhere in the body, are bounded by a lipid bilayer membrane that contains a large number of protein macromolecules. The lipid bilayer allows the composition of the medium on each side to be very different. Of particular importance for electrical signaling is the fact that certain key ions have different concentrations on the inside and outside of the neuron (Fig. 2.1). On the outside, Na^+, Ca^{2+}, and Cl^- exist in much higher concentrations; by contrast, K^+ ions and membrane-impermeant anions (denoted as A^-) are concentrated on the inside.

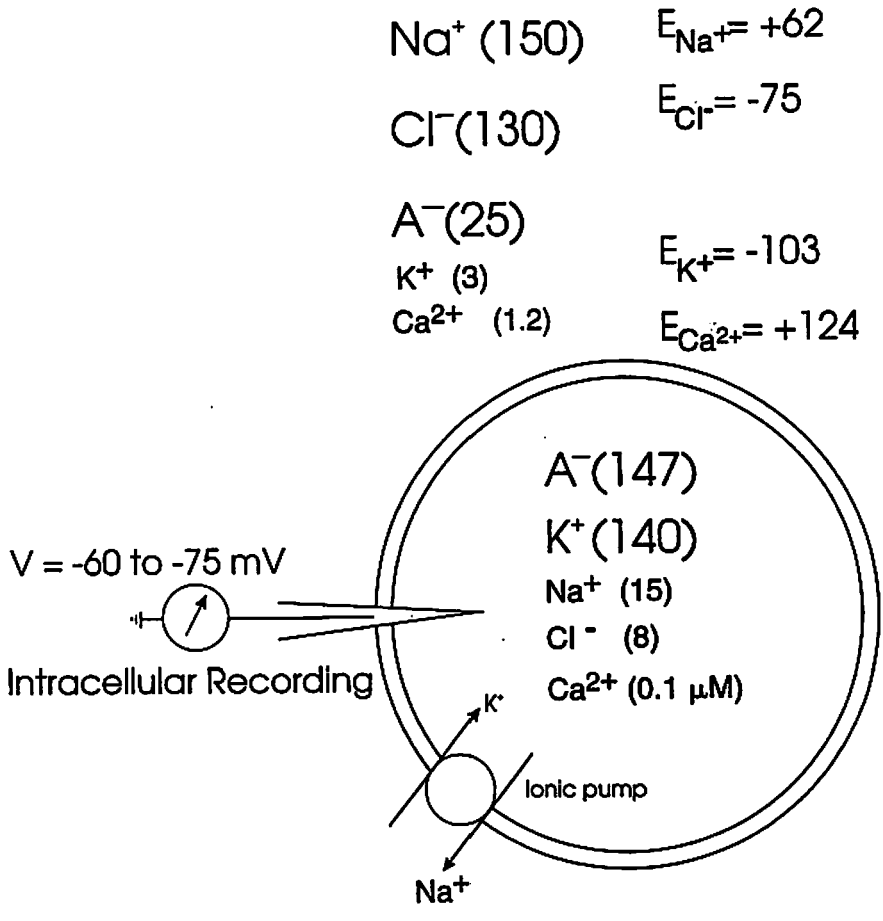

Fig. 2.1. Distribution of ions across neuronal membranes and their equilibrium potentials. At rest the cell membrane is permeable to K^+, Na^+, and Cl^- and exhibits a voltage difference (inside versus outside) of approximately −60 to −75 mV, as seen by an intracellular recording electrode.

Protein macromolecules in the membrane subserve a variety of functions. Those that underlie electrical signaling are large molecules that form ionic channels (see Ionic Channels, below). Membrane channels possess a number of important features, including the presence of a water-filled pore through which ions flow; selectivity for one or more types of ions (e.g., K^+, Na^+, Cl^-, Ca^{2+}); sensitivity to (i.e., opened or closed by) the electrical potential across the membrane or to a neurotransmitter substance, or both; and the ability to be modified by a variety of intracellular biochemical signals.

Because ions are unequally distributed across the membrane, they tend to diffuse down their concentration gradient through ionic channels. This tendency arises from the fact that the intrinsic movements of ions in a solution tend to disperse them from regions of higher to lower concentration. However, since ions are electrically charged molecules, their movements are dictated not only by concentration gradients but also

by the voltage difference across the membrane. For example, if the membrane of a neuron were made permeable to K^+ ions by opening K^+ channels, the higher concentration of these ions on the inside versus the outside of the cell would make it more probable that K^+ ions would leave, rather than enter the cell. As K^+ ions exit the cell they carry positive charge with them, thereby leaving behind a net negative charge (made up in part of impermeant anions; Fig. 2.1). However, this negative charge (expressed as a *voltage difference*) on the inside versus the outside of the cell will attract the potassium ions and slow down the rate at which they leave. At some point, the tendency for K^+ to flow out of the cell will be offset exactly by the attraction of the negative charge left inside the cell. The voltage difference at which this occurs is known as the *equilibrium potential* (denoted as E) and is different for each ionic species (see Figs. 2.1 and 2.2). It will be seen later in this and other chapters that the equilibrium potential is important for determining the effect of activation (synaptic or intrinsic) of an ionic current.

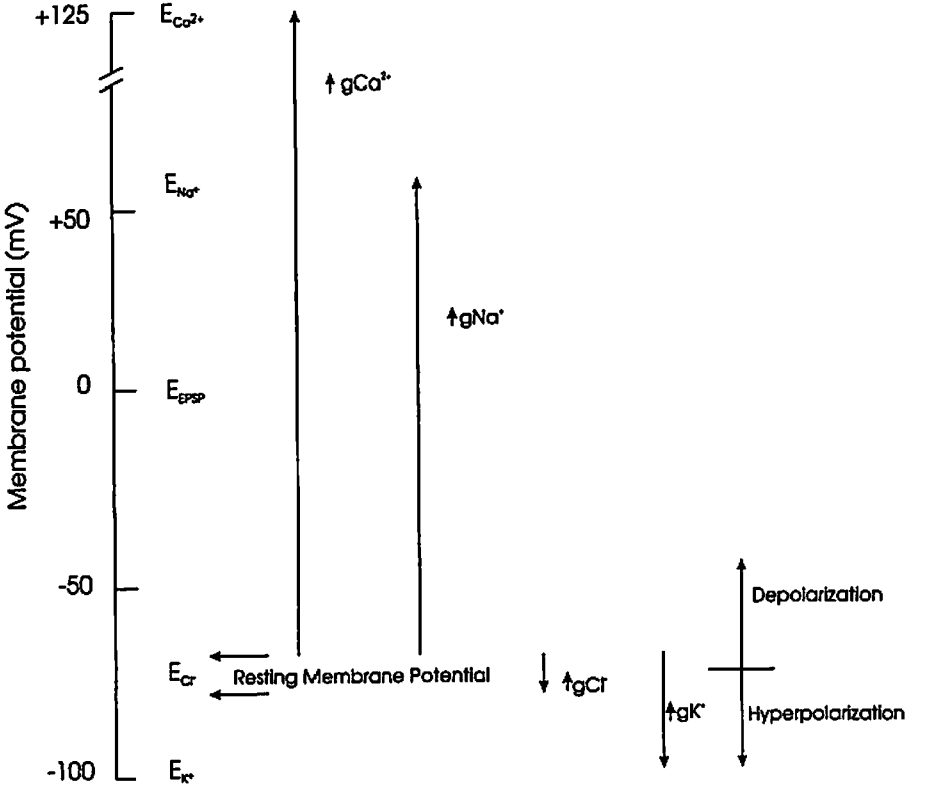

Fig. 2.2. Effect of increasing membrane conductance (denoted as g) to Ca^{2+}, Na^+, Cl^- or K^+. Increases in $g_{Ca^{2+}}$ or g_{Na^+} bring the membrane potential toward more positive values (depolarization), while increases in gCl^- or g_{K^+} brings it toward more negative values (hyperpolarization).

The flow of ions across the membrane obeys physical laws in a consistent and reproducible manner. Considering the basic forces involved in determining the passive distribution of ions (e.g., thermodynamic and electrical), it becomes clear that four of the important factors influencing the equilibrium potential are: *(1)* the concentration of the ion inside and outside the cell; *(2)* the temperature of the solution; *(3)* the valence of the ion; and *(4)* the amount of work associated with separating a given quantity of charge. A German physical chemist named Walter Nernst derived an equation in 1888 that related such factors, allowing for the calculation of the equilibrium potential (Nernst, 1888):

$$E_{\text{Ion}} = RT/zF \cdot \ln [\text{Ion}]_o/[\text{Ion}]_i \tag{2.1}$$

where E_{Ion} is the membrane potential at which the ionic species under consideration (e.g., K^+, Na^+, Cl^-) is at equilibrium, R is the gas constant (8.315 Joules/Kelvin·mole), T is the temperature in degrees Kelvin ($T_{\text{Kelvin}} = 273.16 + T_{\text{Celcius}}$), F is Faraday's constant (96,485 Coulombs/mole), z is the valence of the ion (typically $\pm$ 1 or 2), and $[\text{Ion}]_o$ and $[\text{Ion}]_i$ are the concentrations of the ion in question on the outside and inside of the cell. Substituting the appropriate numbers as well as converting from natural log (ln) to log-base-10 ($\log_{10}$) results in the following equation at room temperature (20°C) for a monovalent, positively charged ion (cation):

$$E_{\text{Ion}} = 58.2 \log_{10} [\text{Ion}]_o/[\text{Ion}]_i \tag{2.2}$$

and at a body temperature of 37°C the Nernst equation is:

$$E_{\text{Ion}} = 61.5 \log_{10} [\text{Ion}]_o/[\text{Ion}]_i \tag{2.3}$$

As an example, consider the passive distribution of K^+ ions in the squid giant axon as studied by Alan Hodgkin and Andrew Huxley (1952 a–d). The squid giant axon is very large—approximately 1 mm in diameter—as its name implies, and is used by the squid for the generation of escape reflexes. The large size and robust nature of the squid giant axon allowed Hodgkin and Huxley in the 1940s and 50s to perform many different experiments, such as intracellular recording, that could not be performed at that time on mammalian neurons.

The inside of the squid giant axon has a concentration of K^+ of about 400 mM, while the outside of the axon is exposed to about 20 mM K^+. Since there are many more potassium ions on the inside versus the outside of this axon, they will tend to flow down their concentration gradient, taking positive charge with them as they do. The membrane potential at which the tendency for K^+ to flow down its concentration gradient will be exactly offset by the attraction for K^+ to enter the cell, because of the negative charge on the inside of the cell, at a room temperature of 20°C is:

$$E_K = 58.2 \log_{10} (20/400) = -76 \text{ mV} \tag{2.4}$$

Therefore, at a membrane potential of −76 mV, there will be no net tendency for K^+ ions to flow either into or out of the axon.

The concentrations of K^+ experienced by mammalian neurons and glial cells are considerably different from that of the squid giant axon. In the mammalian brain the extracellular concentration of K^+ is approximately 3 mM whereas the intracellular concentration is approximately 140 mM (Fig. 2.1). Therefore, at a body temperature of 37°C, the equilibrium potential for K^+ in mammalian neurons is:

$$E_K = 61.5 \log_{10} (3.1/140) = -103 \text{ mV} \quad (2.5)$$

At membrane potentials positive to −103 mV in mammalian cells, K^+ ions will tend to flow out of the cell, down their concentration gradient (Fig. 2.2). Therefore, at these membrane potentials, increasing the ability of K^+ ions to flow across the membrane, in other words, increasing the *conductance* of the membrane to K^+ (abbreviated as *g*K), will result in the membrane potential becoming more negative, or *hyperpolarizing*, because of the exiting of positively charged ions from the inside of the cell.

The ease with which an ion diffuses across the membrane is expressed as the ion's *permeability*. Increasing the permeability of the membrane to a particular ionic species (e.g., by increasing the probability that membrane channels which conduct that ion will be open) increases the electrical conductance and will bring the membrane potential of the cell closer to the equilibrium potential of that ion. This is true whether the membrane potential becomes more negative (i.e., hyperpolarized) or more positive (i.e., depolarized) towards the equilibrium potential. Of course, if the membrane potential is already at the equilibrium potential, then its value will not change in response to a further increase in conductance. In this circumstance, the most significant change will be that the ability of other currents to move the membrane potential away from its present potential will be diminished. For example, if the membrane were only slightly permeable to Cl^- ions, then increases in membrane conductance to other ionic species could easily move the membrane potential away from E_{Cl}. However, if the permeability to Cl^- were greatly increased, the membrane potential would be effectively "clamped" close to E_{Cl}. In this instance, movements of other ions into or out of the cell would now largely be offset by compensating movements of Cl^- ions, thereby keeping the membrane potential close to E_{Cl}.

For example, presume that the membrane is highly permeable to Cl^- and that the membrane potential is at E_{Cl}. If the membrane is now also made permeable to sodium, Na^+ ions will enter the cell. However, as the positive ions enter the cell and move the membrane potential away from E_{Cl}, Cl^- ions will also move into the cell, bringing the cell back towards E_{Cl} and negating some of the depolarizing influence of the increased permeability to Na^+. If the permeability to Cl^- is much higher than that to Na^+, then the membrane potential will stay close to E_{Cl}. As we shall see below, this type of "shunting" of the membrane potential near E_{Cl} is important in the actions of some types of inhibitory neurotransmitters.

RESTING MEMBRANE POTENTIAL

When there is no synaptic input, or the neuron is "at rest," the cellular membrane is dominated by its permeability to K^+. This permeability to potassium ions draws the membrane potential of the cell towards approximately −103 mV (see Figs. 2.1 and

2.2). If the membrane were only permeable to K^+, then the membrane potential would be equal to E_K. However, even at rest, neuronal membranes are also permeable to other ions, Na^+ and Cl^- in particular, so that the membrane potential is pulled towards E_{Na} (+62 mV) and E_{Cl} (−75 mV). The point at which the movements of these varied ions come into equilibrium such that there is no *net* current (denoted as *I*) flow across the membrane corresponds to the resting membrane potential and is typically between −60 and −75 mV (see Fig. 2.2).

The weighted mixture of all of the ionic currents flowing across the membrane determines the resting membrane potential, as well as the membrane potential during nearly all types of activity. This principle allows the calculation of the membrane potential at any given point in time by means of the *Goldman–Hodgkin–Katz* (GHK) equation, which is based upon the concentration gradient and membrane permeability (P) of each ion (Goldman, 1943; Hodgkin and Katz, 1949):

$$V_m = RT/F \cdot \ln ((P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i)/(P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o)) \quad (2.6)$$

A consequence of this relationship of ionic currents and membrane potential is that, in general, the membrane potential of the cell will be closest to the equilibrium potential of the ion to which the membrane is most permeable (e.g., P_K, P_{Cl}, or P_{Na}). In this equation, each of the three different ions, K^+, Na^+, and Cl^- influence the membrane potential. The relative contribution of each is determined by the concentration differences across the membrane and the relative permeability (P_K, P_{Na}, P_{Cl}) of the membrane to each different type of ion. If the membrane were permeable to only one ion, for example K^+, then the GHK equation reduces to the Nernst equation. Experiments on the squid giant axon at resting membrane potential reveal permeability ratios of:

$$P_K{:}P_{Na}{:}P_{Cl} = 1/0.04/0.45 \quad (2.7)$$

In other words, the membrane of the squid giant axon at rest is most permeable to K^+ ions, followed by Cl^-, followed by a small permeability to Na^+. (Chloride appears to contribute considerably less to the determination of the resting potential of mammalian neurons.) These results indicate that the resting membrane potential is determined by the resting permeability of the membrane to K^+, Na^+, and Cl^-, and that this resting membrane potential may be, at least in theory, anywhere between E_K (e.g., −76 mV) and E_{Na} (+55 mV). Substituting in the values for the concentrations of Na^+, K^+, and Cl^- as well as their relative permeabilities into the GHK equation at a temperature of 20°C reveals:

$$V_m = 58.2 \log_{10} ((1 \cdot 20 + 0.04 \cdot 440 + 0.45 \cdot 40)/(1 \cdot 400 + 0.04 \cdot 50 + 0.45 \cdot 560)) = -62 \text{ mV} \quad (2.8)$$

This suggests that the squid giant axon should have a resting membrane potential of −62 mV. In fact, the resting membrane potential may be a few millivolts hyperpolar-

ized to this value through the operation of the electrogenic Na^+–K^+ pump (see below).

In the mammalian nervous system, the exact value of the resting membrane potential varies among different types of neurons and is very important in determining the manner in which a particular neuron behaves both spontaneously as well as in response to extrinsic inputs. For example, in the absence of synaptic input cortical pyramidal cells (Chap. 12) have a resting membrane potential of approximately −75 mV, thalamic relay neurons (Chap. 8) are at approximately −65 to −55 mV at rest in the waking animal, and retinal photoreceptor cells have a resting membrane potential of approximately −40 mV (Chap. 6). Some types of neurons do not have a true "resting" membrane potential in that they are spontaneously active even during the lack of all synaptic input (see below).

ACTION POTENTIAL

Rapid signaling in nerve cells is accomplished by brief changes in the membrane potential. Traditionally, the most characteristic type of signal has been considered to be the *action potential*, or *nerve impulse* (also referred to as a *spike*). Local action potentials in patches of dendritic membrane can also serve as boosters for the spread of synaptic potentials to the soma (as discussed in Chap. 1).

As with the resting membrane potential, the basic changes in membrane ionic permeability that underlie the action potential were first well characterized by Hodgkin and Huxley (1952a–d) using the squid giant axon preparation. The large size of the squid giant axon allowed Hodgkin and Huxley to thread a wire into the axon, giving them the ability to control accurately the membrane potential by a procedure known as *voltage clamp*. In this procedure, the amount of current injected into the cell is adjusted so that the voltage across the membrane is kept constant (i.e., the voltage is "clamped"). This technique not only allows one to observe directly the transmembrane currents responsible for the electrical behavior of the cell, but also to measure the current's kinetics and sensitivity to membrane potential. The isolation of the squid giant axon in vitro meant that Hodgkin and Huxley could also control the ionic composition of the medium on the outside as well as the inside of the axon. These experiments revealed that the rapid upswing of the action potential is mediated by a regenerative increase in a transient Na^+ current, denoted $I_{Na,t}$ (Fig. 2.3). Since $I_{Na,t}$ is rapidly activated by depolarization and is itself a depolarizing influence, it forms a positive feedback loop in the unclamped axon, as shown in Fig. 2.3A. Depolarization of the membrane causes a rapid increase in the number of Na^+ channels that are open, thereby allowing more Na^+ ions to enter the cell, resulting in even more depolarization of that portion of membrane, increased entry of Na^+, and so on.

Membrane currents that change their amplitude in response to changes in the membrane in a "nonlinear" manner, such as $I_{Na,t}$, are referred to as *voltage sensitive*, while the ionic channels that underlie these currents are said to be *voltage gated*. The depolarization caused by entry of Na^+ ions into the cell spreads to neighboring membrane by *electrotonic* current flow. The depolarization of one patch of membrane will also depolarize neighboring patches of membrane. The subsequent activation of the same

THE ACTION POTENTIAL

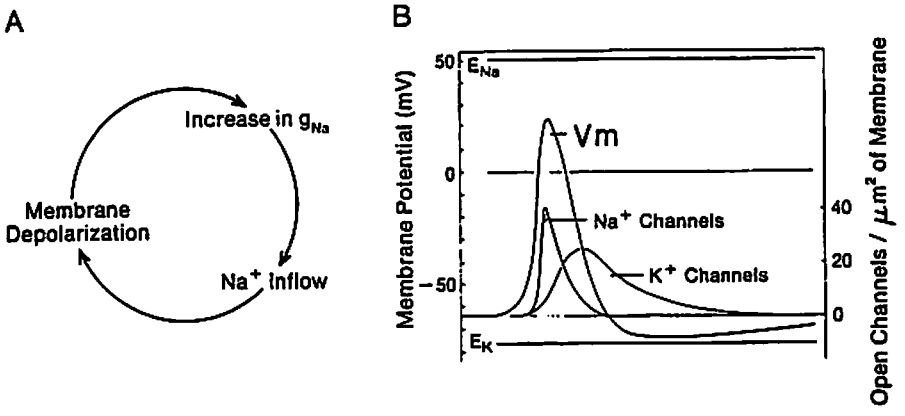

Fig. 2.3. **A:** Regenerative relation between membrane depolarization, increase in membrane conductance to Na^+ (gNa), and Na^+ current that underlies the action potential. **B:** Reconstruction of changes in ionic conductance underlying the action potential in squid giant axon; scale for the membrane potential (V_m) is shown on the left. The equilibrium potentials for Na (E_{Na}) and K^+ (E_K) are also indicated on the left. Changes in Na^+ and K^+ ionic conductances are scaled on the right in terms of calculated open channels per square micrometer of membrane. [Adapted from Hodgkin and Huxley, 1952d and Hille, 1992, with permission.]

regenerative mechanisms at these sites underlies the propagation of the action potential along the axon.

Repolarization of the action potential is very important not only because of the obvious need to be able to generate more than one action potential during the life of the cell, but also in determining the way the cell responds to repetitive inputs. Two processes are essential for the repolarization of the action potential in most neurons: the rapid inactivation of $I_{Na,t}$ and the activation of K currents. The rate of *inactivation* (termed *inactivation kinetics*) of $I_{Na,t}$ is only slightly slower than the rate of activation. Even during the rising phase of the action potential, the available Na^+ current becomes less and less because of inactivation. Simultaneously, but with a slower time course, a K^+ current, known as I_K, is activated by the membrane depolarization associated with the action potential, allowing K^+ to leave the cell. These two currents, flowing through their respective ionic channels, are indicated in Fig. 2.3B. At some point the hyperpolarizing influence of K^+ leaving overcomes the depolarizing influence of Na^+ entering, thereby terminating the action potential and repolarizing the membrane.

The triggering of an action potential occurs when the membrane potential of the neuron is depolarized sufficiently to reach action potential *threshold*. In many cells, this potential is approximately −50 to −55 mV. Action potential threshold is the membrane potential at which the regenerative activation of depolarizing currents (e.g., $I_{Na,t}$) is strong enough to overcome the inactivation of these currents as well as the activation of others that hyperpolarize the neuron back towards rest. At threshold, the generation of an action potential is an all-or-nothing event. If threshold is surpassed, an action potential is generated and the information is transferred down the axon to cause the release of neu-

rotransmitter at synapses. If firing threshold is not reached by a depolarizing event, an action potential is not produced and the event is not relayed to other cells. However, as we shall see, the depolarization can still serve to modify the probability that other postsynaptic potentials in the neuron may cause the cell to discharge.

Since 1952, it has become apparent that $I_{Na,t}$ is the dominant current in the generation of action potentials in axons and cell bodies. However, in somatic and dendritic regions, voltage-gated Ca^{2+} currents are also involved, as documented below and in ensuing chapters (e.g., see Fig. 2.5B,C,E). In mammalian somata and dendrites, in contrast to squid giant axon, repolarization of action potentials is accomplished not only by I_K but also by a complicated array of different K currents (see sections on I_K, I_C, and I_A below).

IONIC CHANNELS

The generation of ionic currents useful for the propagation of action potentials requires the movement of significant numbers of ions across the membrane in a relatively short period of time. The rapid rate of ionic flow occurring during the generation of an action potential is far too high to be achieved by an active transport mechanism, but rather results from the opening of ion channels. Although the existence of ionic channels in the membrane has been postulated for decades, their properties and structure have only recently become known in detail. The development by Erwin Neher and Bert Sakmann of the patch clamp technique, in which a small patch of membrane containing a single or small number of ionic channel(s) is drawn up into a blunt microelectrode allowed for the miniscule (10^{-12} or picoamperes) currents flowing through single channels to be recorded in intact biological membranes for the first time (Neher and Sakmann, 1976, 1992; Hamill et al., 1981; Sakmann, 1992). In addition, the rapid advances made by molecular biology in the isolation, cloning, and sequencing of the proteins making up ionic channels have revealed much about their primary structure. The powerful combination of electrophysiological and molecular techniques together has yielded valuable insights into the structure–function relationships of ionic channels (see reviews by Miller, 1989; Anderson and Koeppe, 1992; Catterall, 1988, 1992, 1995; Salkoff et al., 1992).

Voltage-sensitive ionic channels appear to have several shared features. First, they are large proteins that span the 6–8 nm of the plasma membrane and are typically made up of subunits. Second, through protein folding, they form a cylinder surrounding a central water-filled pore that permits the passage of only certain classes of ions between the inside and outside of the cell. The selection of which ions are allowed to pass through each different type of ionic channel is based upon the size, charge, and degree of hydration of the different ions involved (see Hille, 1992). Finally, voltage-gated ion channels possess one or more "gates," or voltage-sensing regions within the ionic pore, and the flow of ions through the channels is regulated by these gates.

IONIC PUMPS

The quantity of ions that enter and exit the cell during electrical activity is actually very small in comparison with the number of ions present. For example, the genera-

tion of a single action potential in a hypothetical spherical cell 25 μm in diameter should result in an increase of intracellular concentration of Na^+ of only approximately 6 μM (from approximately 15 mM to 15.006 mM)! This means that the action potential is an electrical event that is generated by a change in the distribution of charge across the membrane and not by a marked change in intracellular or extracellular concentration of Na^+ or K^+.

However, even these small exchanges of ions across the membrane coupled with a constant "leak" at rest can eventually destroy the correct ionic distribution and thereby render a neuron nonfunctional. To compensate for this "rundown," neuronal membranes possess specialized protein macromolecules known as *ionic pumps*. Ionic pumps maintain the correct distribution of all of the ions involved in electrical activity by actively transporting these ions "upstream" against their concentration gradient. The energy required to perform this task is sometimes obtained through the hydrolysis of ATP (adenosine triphosphate). The ionic pump that has been best characterized is the electrogenic sodium-potassium pump (see Thomas, 1972; Skou, 1988). This ionic pump carries approximately three Na^+ ions out for every two K^+ ions it brings in, thereby generating an electric current (Fig. 2.1). The exact amplitude of this current depends upon the rate at which the pump is active, which is in turn related to the intracellular concentration of Na^+ and the extracellular concentration of K^+.

Besides the electrogenic Na^+–K^+ pump, neurons and glia also contain many other types of ionic pumps in their membranes (e.g., Pedersen and Carafoli, 1987; Läuger, 1991). Many of these pumps are operated by the Na^+ gradient across the cell, while others operate through a mechanism similar to the Na^+–K^+ pump (i.e., the hydrolysis of ATP). For example, the calcium concentration inside neurons is kept to very low levels (typically 50–100 nM) through the operation of both types of ionic pumps as well as special intracellular Ca^{2+}-buffering mechanisms. Ca^{2+} is extruded from neurons through both a Ca^{2+}–Mg^{2+} ATPase as well as a Na^+–Ca^{2+} exchanger. The Na^+–Ca^{2+} exchanger is driven by the Na^+ gradient across the membrane and extrudes one Ca^{2+} ion for each Na^+ ion allowed to enter the cell.

The Cl^- concentration in neurons is actively maintained at a low level through the operation of a chloride-bicarbonate exchanger, which brings in one ion of Na^+ and one ion of HCO_3^- for each ion of Cl^- extruded (e.g., Thompson et al., 1988; Reithmeier, 1994). Finally, intracellular pH can also have marked effects on neuronal excitability and therefore pH is also tightly regulated, in part through the efforts of a Na^+–H^+ exchanger which, again, extrudes one proton for each Na^+ that is allowed to enter the cell.

Ionic pumps are essential and important constituents of neurons and neuronal membranes. Their time scale of action is seconds to minutes and they are therefore thought of as being more involved in long-term rather than short-term neuronal processing.

TYPES OF IONIC CURRENTS

Neurons in the nervous system do not simply lie at rest and occasionally generate an action potential. Rather, neuronal membranes are in a constant state of flux because of the presence of a remarkable variety of different ionic currents (Table 2.1). These cur-

Table 2.1. Neuronal Ionic Currents

Current	Description	Function
Na^+		
I_{Na} or $I_{Na,t}$	Transient; rapidly activating and inactivating	Action potentials
$I_{Na,p}$	Persistent; non-inactivating	Enhances depolarization; contributes to steady-state firing
Ca^{2+}		
I_T, low threshold	Transient; rapidly inactivating; threshold negative to −65 mV	Underlies rhythmic burst firing
I_L, high threshold	Long-lasting; slowly inactivating; threshold around −20 mV	Underlies Ca^{2+} spikes that are prominent in dendrites; involved in synaptic transmission
I_N	Neither; rapidly inactivating; threshold around −20 mV	Underlies Ca^{2+} spikes that are prominent in dendrites; involved in synaptic transmission
I_P	Purkinje; threshold around −50 mV	Underlies Ca^{2+} spikes that are prominent in dendrites
K^+		
I_K	Activated by strong depolarization	Repolarization of action potential
I_C	Activated by increases in $[Ca^{2+}]_i$	Action potential repolarization and interspike interval
I_{AHP}	Slow afterhyperpolarization; sensitive to increases in $[Ca^{2+}]_i$	Slow adaptation of action potential discharge; the block of this current by neuromodulators enhances neuronal excitability
I_A	Transient; inactivating	Delayed onset of firing; lengthens interspike interval; action potential repolarization
I_M	Muscarine sensitive; activated by depolarization; non-inactivating	Contributes to spike frequency adaptation; the block of this current by neuromodulators enhances neuronal excitability
I_h	Depolarizing (mixed cation) current that is activated by hyperpolarization	Contributes to rhythmic burst firing and other rhythmic activities
$I_{K,leak}$	Contributes to neuronal resting membrane potential	The block of this current by neuromodulators can result in a sustained change in membrane potential

rents are distinguished not only by the ions that they conduct (e.g., K^+, Na^+, Ca^{2+}, Cl^-) but also by their time course, sensitivity to membrane potential, and sensitivity to neurotransmitters and other chemical agents (for review see Llinás, 1988; Rudy, 1988; Storm, 1990; Hille, 1992; McCormick and Huguenard, 1992; Johnson and Wu, 1995; Stea et al., 1995). As the various ionic currents were discovered they were divided into two general categories: those that are sensitive to changes in membrane potential and those that are altered by neurotransmitters and internal messengers. However, with the recent discovery of a number of voltage-sensitive ionic channels they are also gated by neurotransmitters, and vice versa, it has become apparent that there is substantial overlap between these two groups. The currents that possess both voltage and neurotransmitter sensitivity have received much recent attention because of their ability to modulate the electrical behavior of neurons in unusual and interesting ways (see Chemical Synapses below).

Most currents that are sensitive to membrane potential are turned on (*activated*) by depolarization. The rate at which they activate as well as the membrane potential at which they start to become active (*threshold*) are important characteristics. Many voltage-dependent currents do not stay on once they are activated even during a constant shift in membrane potential. The process by which they turn off despite a stable level of membrane potential in their activation range is known as *inactivation*. Inactivation is a state of the current and ionic channels that is distinct from simple channel closure. Once a current becomes inactive, this inactivation must be removed before it can again be activated. *Removal of inactivation* is generally achieved by repolarization of the membrane potential. Like the process of activation, inactivation and removal of inactivation are time and membrane potential dependent. Together, all of these characteristics define the temporal and voltage domain over which the current influences the electrical activity of the neuron.

The names given to each ionic current often reflect one of the properties which distinguishes that current from the others. If the current is activated by relatively small deviations in the membrane potential (denoted as V_m) from rest, than it may be known as *low threshold* (for example, low-threshold Ca^{2+} current), whereas if the current is activated only at levels that are substantially positive (depolarized) from rest, the current may be known as *high threshold* (e.g., high-threshold Ca^{2+} current). In addition, if activation of the current through a constant and steady change in membrane potential (i.e., under voltage clamp conditions in which the membrane potential is held constant) leads to only a transient response, then it is known as *transient* or *rapidly inactivating* (examples are the A current and the T current). Likewise, a current that persists during constant activation (i.e., is noninactivating) is known as *sustained*, *persistent*, or *long lasting* (e.g., persistent Na^+ current and the L, or long-lasting, Ca^{2+} current).

The ionic currents that determine the neuronal firing behavior of neurons in different regions of the nervous system have been intensively investigated. To date, at least a dozen distinct neuronal currents, many of which are common to neurons at all levels of the neuraxis, have been identified (Table 2.1). We will briefly summarize these currents and the unique contribution that each makes to the firing behavior of neurons (see Fig. 2.4).

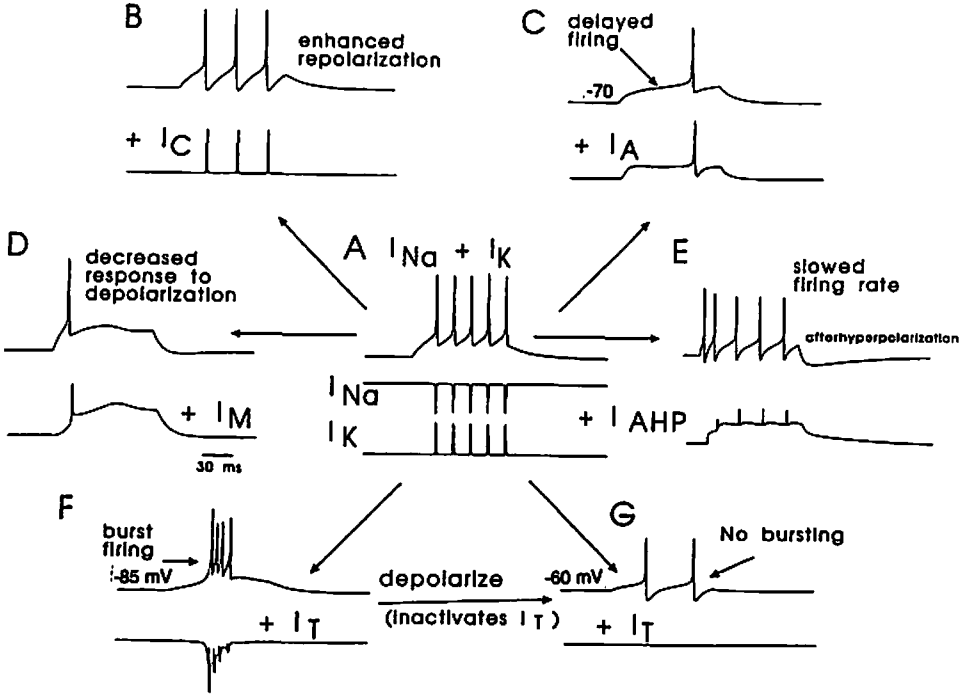

Fig. 2.4. A summary of different types of voltage-gated currents and the impulse firing patterns they produce in a neuron in response to steady injection of depolarizing current. At the center (A) is shown the repetitive impulse response of the classical Hodgkin-Huxley model (voltage recording above, current recordings below). Radiating out from this are changes in this pattern associated with the different types of ionic channels. **B:** Addition of the Ca^{2+}-activated K^+ current I_C (and the high-threshold Ca^{2+} current I_L) facilitates the repolarization of each action potential. **C:** Addition of the depolarization-activated but transient K^+ current I_A results in a delay to onset of action potential generation. **D:** Addition of the depolarization-activated but persistent K^+ current I_M results in a marked decrease in neuronal excitability. **E:** Addition of the slow Ca^{2+}-activated K^+ current I_{AHP} results in spike frequency adaptation and the generation of a slow hyperpolarization after the action potential train (afterhyperpolarization). **F:** Addition of the low-threshold and transient Ca^{2+} current I_T results in the generation of a burst of action potentials at -85 mV. **G:** Depolarization of the cell in F to -60 mV results in inactivation of I_T and now the cell generates a train of two action potentials. [Modified from Shepherd, 1994.] These traces are the result of computer simulations (Huguenard and McCormick, 1994).

SODIUM (NA) CURRENTS

Two sodium currents, $I_{Na,t}$ (transient) and $I_{Na,p}$ (persistent), are widely distributed in neurons from different regions of the nervous system. These two currents are distinguished from one another by their rate of inactivation, their threshold for activation, and their amplitude.

$I_{Na,t}$ Transient Sodium. As we have noted, the transient Na^+ ($I_{Na,t}$) current rapidly inactivates within a few milliseconds during steady depolarization. All central neurons stud-

ied to date possess a large $I_{Na,t}$, whereas $I_{Na,p}$ is considerably smaller in amplitude. The rapid activation and inactivation properties of $I_{Na,t}$ makes this current ideal for its role in the generation of action potentials (Fig. 2.4A).

$I_{Na,p}$ Persistent Sodium. In contrast to $I_{Na,t}$, the persistent Na^+ ($I_{Na,p}$) current shows little, if any, inactivation. This current is also rapidly activated by membrane depolarization, but its non-inactivating nature allows it to serve a very different role in neuronal function (Hotson et al., 1979; Llinás, 1981, 1988; Stafstrom et al., 1985). A large percentage of neuronal computations occur in a narrow range of membrane potential between approximately −75 and −50 mV. This range is between resting membrane potential and a level of depolarization at which the neuron is firing repeatedly at a high rate. The nature of $I_{Na,p}$ is such that it is activated by depolarizations, such as synaptic potentials, that bring the membrane potential from rest to near action potential firing threshold. The added depolarizing influence of the influx of Na^+ ions resulting from the activation of $I_{Na,p}$ serves to enhance markedly the response of the neuron to excitatory inputs. This may result in the generation of *plateau potentials* (Fig. 2.5C), which are prolonged depolarizations that persist despite the removal of all other depolarizing influences in the cell (such as a synaptic potential or the intracellular injection of current).

The amplitude and cellular distribution of $I_{Na,p}$ can therefore have an important role in determining the responsiveness of neurons. The persistent nature of $I_{Na,p}$ allows this current to participate in the determination of the baseline firing rate of neurons. $I_{Na,p}$ appears to be especially important to the ability of some neurons to maintain intrinsic "pacemaker" activity (e.g., the generation of action potentials in a repeated temporal pattern in the absence of synaptic input). In these cells, the steady influx of Na^+ ions into the neuron depolarizes the cell to above firing threshold, thereby triggering baseline activity. The membrane potential of these cells is in a state of constant change, cycling through the generation of an action potential to the repolarization of the cell (see Potassium (K) Currents below) to again the generation of an action potential. Examples of such neurons in the CNS are those of the locus coeruleus, dorsal raphe, and medial habenula (see Fig. 2.5F; Vandermaelen and Aghajanian, 1983; Williams et al., 1984; McCormick and Prince, 1987b).

CALCIUM (CA) CURRENTS

Ionic channels that conduct Ca^{2+} are present in all neurons. These channels are special in that they serve two important functions. First, Ca^{2+} channels are present throughout the different parts of the neuron (dendrites, soma, synaptic terminals) and contribute greatly to the electrophysiological properties of these processes (Llinás, 1988; Regehr and Tank, 1994; Markram et al., 1995). Second, Ca^{2+} channels are unique in that Ca^{2+} is an important second messenger in neurons and entry of Ca^{2+} into the cell can affect numerous physiological functions, including neurotransmitter release, synaptic plasticity, neurite outgrowth during development, and even gene expression.

Calcium currents have been separated into at least four separate categories based upon their voltage sensitivity and kinetics of activation and inactivation as well as their block by various pharmacological agents. Differences in the kinetics and pharmacology of three different categories of Ca^{2+} currents led Richard Tsien and col-

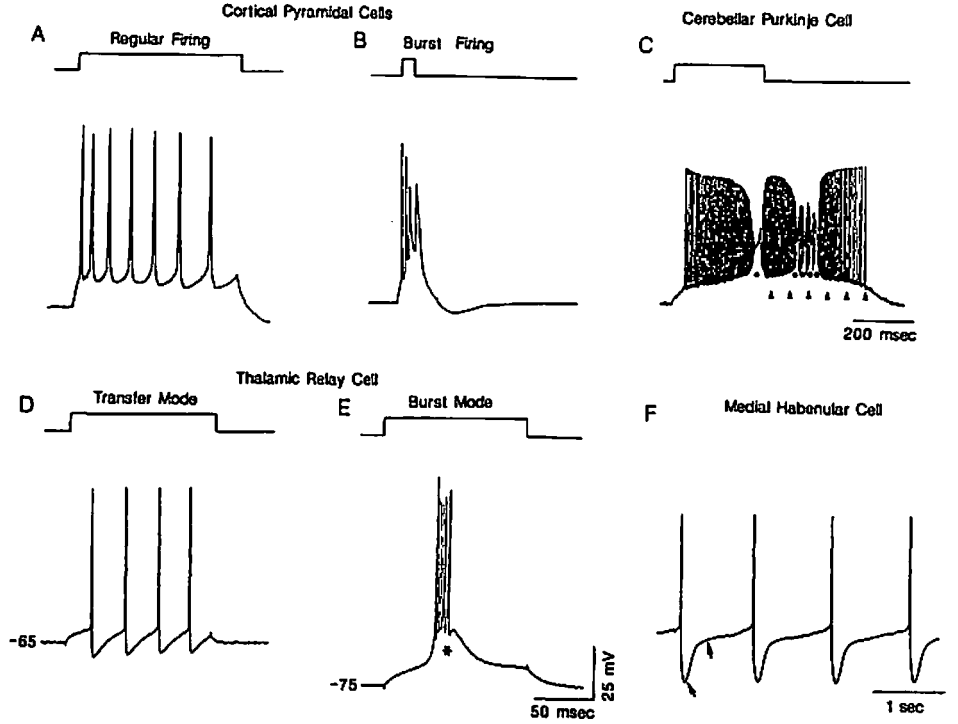

Fig. 2.5. Electrophysiological behavior of neurons in different regions of the mammalian brain. **A:** Example of a "regular" firing cortical pyramidal neuron. Intracellular injection of a depolarizing current pulse (top trace) results in the generation of a train of action potentials that occur at progressively slower frequencies (spike frequency adaptation). **B:** By contrast, intracellular injection of depolarizing current pulses into a "burst" generating cortical pyramidal neuron results in the clustering of action potentials together on top of a slow potential. **C:** Electrical activity of a cerebellar Purkinje cell in response to intracellular injection of a depolarizing current pulse. The cell generates initially a high-frequency discharge of fast Na^+-dependent action potentials (generated in the soma). This discharge is modulated by the occurrence of dendritic Ca^{2+} spikes (asterisks). The discharge outlasts the duration of the intracellular depolarizing pulse (top trace) because of the presence of a plateau potential mediated by $I_{Na,p}$ and calcium currents (arrowheads). **D:** Depolarization of thalamic relay neuron results in the generation of a train of four action potentials if the membrane potential is positive to approximately -65 mV, but a burst of action potentials if the cell is at or negative to -75 mV (**E**). The low-threshold Ca^{2+} spike underlying this burst discharge is indicated by an asterisk. **F:** Example of a neuron in the medial habenula that generates intrinsic "pacemaker" discharge. Intracellular recording reveals the presence of large hyperpolarizations after each action potential which are complicated in time course and help determine the rate at which the neuron fires (arrows).

leagues (Nowycky et al., 1985) to name them I_T ("transient"), I_L ("long-lasting"), and I_N ("neither") (see also Carbonne and Lux, 1984). More recent experiments by Rodolfo Llinás and colleagues (reviewed in Llinás et al., 1992) demonstrated that Purkinje cells of the cerebellum, as well as many different cell types of the CNS, also possess another Ca^{2+} current termed I_P. Molecular biology has revealed a wide variety of genes involved in the production of Ca^{2+} channels, and it is certain that more Ca^{2+}

currents are yet to be characterized (e.g., see Tsien et al., 1991; Birnbaumer et al., 1994).

High-threshold Ca^{2+} Currents. Most Ca^{2+} channels are activated at membrane potentials positive to approximately −40 mV; these are termed *high-voltage activated* (HVA). These Ca^{2+} channels include at least those underlying the currents I_L, I_N, and I_P. These three different ionic currents are separable from one another through examination of their voltage dependence and kinetics of activation and inactivation, and through their sensitivity to various Ca^{2+} channel blockers and neural toxins. The Ca^{2+} channel antagonists known as dihydropyridines, which clinically are useful for their effects on the heart and vascular smooth muscle (e.g., for the treatment of arrhythmias, angina, and migraine headaches), selectively block the L-type Ca^{2+} channels (reviewed by Bean, 1989; Stea et al., 1995). L-type calcium currents exhibit a high threshold for activation (around −10 mV) and give rise to rather persistent, or long-lasting, ionic currents. In contrast to I_L, I_N is not blocked by dihydropyridines, but rather is selectively blocked by a toxin found in Pacific cone shells (ω-conotoxin-GVIA). N-type Ca^{2+} channels have a threshold for activation of around −20 mV, inactivate with maintained depolarization, and are modulated by a variety of neurotransmitters. In at least some cell types I_N is involved in the Ca^{2+}-dependent release of neurotransmitters at presynaptic terminals (e.g., Wheeler et al., 1994).

P-type calcium channels are distinct from N and L in that they are not blocked by either dihydropyridines or ω-conotoxin-GVIA but by a toxin (termed ω-Agatoxin-IVA) present in the venom of the Funnel web spider (Llinás et al., 1992; Stea et al., 1995). P-type calcium channels activate at relatively high thresholds and do not inactivate. This type of calcium channel appears to be prevalent in Purkinje cells, as well as in other cell types, and participates in the generation of dendritic Ca^{2+} spikes, which can strongly modulate the firing pattern of the neuron in which they occur (see Fig. 2.5C).

Collectively, the high threshold–activated Ca^{2+} channels also contribute to the generation of action potentials in mammalian neurons. The activation of these Ca^{2+} currents adds a bit to the depolarizing portion of the action potential, but more importantly, they allow Ca^{2+} to enter the cell and this has the secondary consequence of activation of various Ca^{2+}-activated K^+ currents (reviewed in Latorre et al., 1989). The activation of these K^+ currents then modifies the pattern of action potentials generated in the cell, as mentioned above (Fig. 2.4B,E).

Molecular biological studies have demonstrated that high-threshold Ca^{2+} channels are similar to the Na^+ channel in that they contain a central α_1 subunit that forms the aqueous pore and several regulatory or auxiliary subunits. As in the Na^+ channel, the primary structure of the α_1 subunits of Ca^{2+} channels consists of four homologous domains (I–IV), each of which contain six regions (S1–S6) that may generate transmembrane α-helices. The genes for at least five different Ca^{2+} channel α-subunits have been cloned (α_{1A-E}), and the properties of the different products of these genes indicate that I_L is likely to correspond to α_{1C} and α_{1D}, while I_N corresponds to α_{1B} and I_P may be related to α_{1A} (reviewed in Birnbaumer et al., 1994; Stea et al., 1995).

Low-threshold Ca^{2+} Currents. Low-threshold Ca^{2+} currents, also known as the *transient Ca^{2+} current I_T*, are also present in many different cell types in the nervous system

and are often involved in the generation of rhythmic bursts of action potentials (Figs. 2.4F, 2.5E). The low-threshold Ca^{2+} current is characterized by a threshold for activation of around −65 mV, which is below the threshold for generation of typical Na^+/K^+-dependent action potentials (−55 mV). Another important feature of this current is that it inactivates with maintained depolarization. Owing to these properties, this Ca^{2+} current can perform a markedly different role in neurons from that of the high-threshold Ca^{2+} currents. Through activation and inactivation of the low-threshold Ca^{2+} current, neurons can generate slow (around 50–100 msec) Ca^{2+} spikes, which, because of their prolonged duration, can result in the generation of a high-frequency "burst" of short-duration Na^+/K^+ action potentials (Figs. 2.4F, 2.5E).

In the mammalian brain, this pattern is especially well exemplified by the activity of thalamic relay neurons, which in the visual system receive direct input from the retina and transmit this information to the visual cortex. During periods of slow-wave sleep, the membrane potential of these relay neurons is relatively hyperpolarized, resulting in the removal of inactivation (de-inactivation) of the low-threshold Ca^{2+} current. This allows these cells to spontaneously generate low-threshold Ca^{2+} "spikes" and bursts of 2 to 5 action potentials (Fig. 2.5E). The large number of thalamic relay cells bursting during sleep in part gives rise to the spontaneous synchronized activity that early investigators were so surprised to find upon recording activity in the brains of sleeping animals (reviewed in Steriade et al., 1993). It has even proved possible to maintain one of the sleep-related brain rhythms (spindle waves) intact in slices of thalamic tissue maintained in vitro, owing to the activation of low-threshold Ca^{2+} spikes and bursts of action potentials in networks of interacting thalamic cells (von Krosigk et al., 1993).

The transition to waking or the period of sleep when dreams are prevalent (rapid-eye-movement sleep) is associated with a maintained depolarization of thalamic relay cells to membrane potentials of around −60 to −55 mV. This maintained depolarization results in the inactivation of the low-threshold Ca^{2+} current and therefore an abolition of burst discharges in these neurons (see Figs. 2.4G, 2.5D). In this way, the properties of a single ionic current (I_T) help to explain in part the remarkable changes in brain activity occurring in the transition from sleep to waking!

Low-threshold Ca^{2+} channels have not yet been purified and sequenced, in part because of the lack of an agent that binds to these receptors with high affinity. Recent evidence suggests that some antiepileptic drugs may exert their therapeutic actions through a reduction in I_T. This is especially true of the drugs useful in the treatment of generalized absence (petit mal) seizures, which are known to rely upon the thalamus for their generation (see Coulter et al., 1990).

POTASSIUM (K) CURRENTS

Neuronal potassium currents form a large and diversified group. They are intimately involved in determining the pattern of activity generated by neurons. Because they are hyperpolarizing, they are responsible not only for the repolarization of the action potential, but also for the determination of the *probability* of generation of an action potential at any given point in time. As with other neuronal currents, potassium currents are distinguished by their voltage and time dependency, as well as by pharmacological techniques (reviewed in Jan and Jan, 1990; Storm, 1990; Salkoff et al., 1992; Johnston and Wu, 1995).

Recent molecular biological studies of voltage-sensitive K^+ channels, first done in *Drosophila* and later in mammals, have revealed the presence of four distinct gene families, *Shaker*, *Shab*, *Shaw*, and *Shal* (reviewed in Salkoff et al., 1992), that correspond to the newer nomenclature of Kv1, Kv2, Kv3, and Kv4 subfamilies (reviewed in Chandy and Gutman, 1995). These genes generate a wide variety of different K^+ channels through alternative RNA splicing and gene duplication. Functional expression of these different K^+ channels reveals remarkable variation in the rate of inactivation: *Shaker* channels are typically rapidly inactivating (A-current-like), *Shal* channels inactivate more slowly, *Shab* channels inactivate very slowly, and *Shaw* channels typically do not inactivate, in similarity with I_K. These studies indicate that each different type of neuron in the nervous system is likely to contain a unique set of functional voltage-sensitive K^+ channels, which are perhaps selected, modified, and placed in particular spatial locations in the cell in a manner to facilitate the unique role of that cell in neuronal processing.

I_K Delayed Rectifier. As we have seen, the early studies in the squid giant axon not only defined the role of the transient Na^+ current in the generation of the action potential but also identified an important outward potassium current known as the *delayed rectifier* or I_K. The activation kinetics of I_K are slower than those of the transient sodium current and therefore it appears somewhat "delayed" (Fig. 2.3B). This potassium current is voltage sensitive, being activated at membrane potentials positive to approximately -40 mV, and it only slowly inactivates. I_K is found in neurons throughout the nervous system and typically contributes to the repolarization of action potentials and the hyperpolarization that follows them (Figs. 2.3B and 2.4A).

Calcium-Activated Potassium Currents. An additional class of potassium currents that are important for determining the firing behavior of neurons are those that are Ca^{2+}-sensitive (denoted $I_{K,Ca}$). This family of potassium currents is activated by increases in the intracellular concentration of unbound Ca^{2+} ($[Ca^{2+}]_i$). Two $I_{K,Ca}$ currents have been widely identified in neurons: I_C and I_{AHP} (see Adams and Galvan, 1986; Rudy, 1988; Storm, 1990). I_C is not only sensitive to increases in $[Ca^{2+}]_i$ in the micromolar range but is also strongly voltage dependent, becoming larger with depolarization. I_C helps control the frequency of action potential generation during a steady depolarization by causing a marked hyperpolarization after the occurrence of each spike (Fig. 2.4B). I_C may even be important in some neurons in repolarization of the action potential. The voltage dependence of I_C results in its rapid inactivation once the membrane potential is repolarized. This inactivation constrains the influence of I_C in the temporal domain to tens of milliseconds or less.

I_{AHP}, in contrast to I_C, is much slower in time course and not very voltage dependent. Its influence on the membrane potential of the cell is best seen after the generation of a number of action potentials as a prolonged afterhyperpolarization, for which it is named. This potassium current contributes significantly to the tendency of the firing frequency of some types of neurons (e.g., cortical and hippocampal pyramidal neurons) to decrease during maintained depolarizations, a process known as *spike frequency adaptation* (Fig. 2.4E; see below).

The generation of action potentials, by increasing $[Ca^{2+}]_i$ through L- or N-type Ca^{2+} channels, triggers I_C and I_{AHP}. The hyperpolarizations of the membrane potential

resulting from K^+ leaving the cell during these currents regulate the rate at which the neuron fires. Due to its short time course, I_C contributes substantially to short interspike intervals. In contrast, because of its slow activation and prolonged time course, I_{AHP} contributes more to the overall pattern of spike activity. The relatively nonvoltage-dependent nature of I_{AHP} means that the influence of this current on the membrane potential is more closely related to changes in $[Ca^{2+}]_i$ than is I_C. Importantly, the amount of I_{AHP} appears to be under the control of putative neurotransmitters (see Decrease of I_{AHP} below).

Transient Potassium Currents. The first of a family of potassium currents that are activated by membrane depolarization and then undergo relatively rapid inactivation was discovered in molluscan neurons (Connor and Stevens, 1971; Neher, 1971) and termed I_A. The A current is a transient K current: after its activation by depolarization of the membrane potential positive to approximately −60 mV, it rapidly inactivates. Like other transient and voltage-activated currents (e.g., $I_{Na,t}$ and I_T), this inactivation is removed by repolarization of the membrane potential. I_A is involved in the response of neurons to a sudden depolarization from hyperpolarized membrane potentials and serves to delay the onset of the generation of the first action potential (Fig. 2.4C). I_A can also slow a neuron's firing frequency during a maintained depolarization and help to repolarize the action potential. For example, in a spontaneously active neuron, the hyperpolarization that occurs after the generation of an action potential will remove some of the inactivation of I_A. As the membrane potential depolarizes back towards firing threshold, I_A will be activated and slow down the rate of depolarization. Once firing threshold is reached and an action potential is generated, the rapid depolarization may activate more of I_A, which then helps to repolarize the cell. In this manner, I_A can be an important current in the determination of firing behavior of neurons.

Muscarine-Sensitive Potassium Currents. Another type of potassium current was discovered in sympathetic ganglion neurons of bullfrogs by David Brown and Paul Adams (1980). This potassium current is activated by depolarization of the membrane potential positive to approximately −65 mV, does not inactivate with time, and is blocked by stimulation of muscarinic cholinergic receptors (hence its name, I_M). I_M is found in neurons throughout the nervous system, including pyramidal cells of the cerebral cortex and hippocampus (reviewed in Brown, 1988; Nicoll et al., 1990; McCormick, 1992). Depolarizations that are large enough to result in the generation of action potentials also cause the activation of I_M. However, because of its relatively slow kinetics and modest amplitude, I_M probably does not affect substantially the waveform of a single action potential, but rather contributes to the slow adaptation of spike frequency seen during a maintained depolarization (Fig. 2.4D).

Currents activated by Hyperpolarization. Hyperpolarization of neurons in many regions of the nervous system results in the activation of a current that brings the membrane potential toward more positive values (e.g., back towards rest). This current, or family of currents, is generally referred to as I_h ("hyperpolarization-activated"), although it has also been given such lively names as I_Q ("queer") and I_f ("funny") (Halliwell and Adams, 1982; DiFrancesco, 1985; Crepel and Penit-Soria, 1986; McCormick and

Pape, 1990). The currents in this family are carried by both Na^+ and K^+ ions and are relatively slow in time course, although this varies widely between different cell types.

The activation of I_h has been demonstrated to be important in the generation of rhythmic oscillations in at least thalamic relay neurons and some types of cardiac cells (McCormick and Pape, 1990; DiFrancesco, 1985). The activation of the h current results in the slow depolarization of the cell, and in so doing, generates a "pacemaker" potential that can activate repetitive Na^+ and/or Ca^{2+} spikes (see Fig. 2.12B).

SUMMARY OF INTRINSIC MEMBRANE PROPERTIES

Neurons possess a virtual cornucopia of different ionic currents. The magnitude, cellular distribution, and sensitivity to pharmacological manipulation of each of these ionic currents is different for every major neuronal region in the central and peripheral nervous system. These differences result in widely varying electrophysiological properties and patterns of neuronal activity generated by cells in different parts of the brain. Each class of neuron is exquisitely "tuned" to do its particular task in the nervous system through its own special mixture of the basic ionic currents available and by the precise modulation of these currents by neuroactive substances. An analogy to this situation would be the "nature-versus-nurture" debate on determining human behavior. The cells are endowed with a particular mixture of ionic currents through genetic programming (nature) that can then be modified on either a short- or long-term basis through development or the actions of a number of substances impinging upon the cell (nurture).

Examples of the different electrophysiological "behaviors" of neurons due to different combinations of ionic currents are illustrated in Fig. 2.5. Cortical pyramidal neurons respond to a depolarizing current pulse with a train (Fig. 2.5A) or a burst (Fig. 2.5B) of action potentials (McCormick et al., 1985; Connors and Gutnick, 1990). The spike frequency adaptation of cortical pyramidal neurons (Fig. 2.5A) is due to the presence of I_{AHP} and I_M. In contrast to neocortical pyramidal neurons, the major output cell of the cerebellar cortex, the Purkinje cell, responds to a depolarizing current pulse with a high-frequency discharge of short-duration action potentials (Fig. 2.5C). This high-frequency discharge is modulated by dendritic calcium spikes (Fig. 2.5C, asterisks) as well as by prolonged sodium ($I_{Na,p}$) and calcium currents (I_P; Fig. 2.5C, arrowheads).

Thalamic relay neurons are unusual in that they possess two distinct modes of action potential generation: single spike activity when depolarized above -65 mV (Fig. 2.5D) and burst firing when depolarized at or negative to -75 mV (Fig. 2.5E). Thalamic neurons respond with a burst of action potentials at -75 mV because of the presence of a large I_T, which is completely inactivated at membrane potentials positive to -65 mV.

Some neurons display spontaneous activity in a regular and stereotyped manner, even in the lack of all synaptic input, such as the medial habenular neuron illustrated in Fig. 2.5F. These cells appear to possess prolonged and complicated spike afterhyperpolarizations (arrows) which help determine the rate at which the action potentials are generated.

Although the electrophysiological behavior of neurons can be markedly changed by the neurotransmitted "environment," they also remain distinct in that it generally is not possible to cause one class of neuron (e.g., cortical pyramidal neuron) to behave electrophysiologically identical to another (e.g., cerebellar Pukinje cell). However, substantial and interesting transformations take place in response to neuron-to-neuron communication.

TYPES OF NEURONAL COMMUNICATION

Communication from one neuron to another in the nervous system occurs through at least three different mechanisms: *(1)* gap junctions; *(2)* ephaptic interactions; and *(3)* the release of neuroactive substances.

GAP JUNCTIONS

Gap junctions are actual physical connections between neighboring neurons made by large macromolecules that extend through the membranes of both cells and contain water-filled pores (Fig. 2.6). Gap junctions allow for the direct exchange of ions and

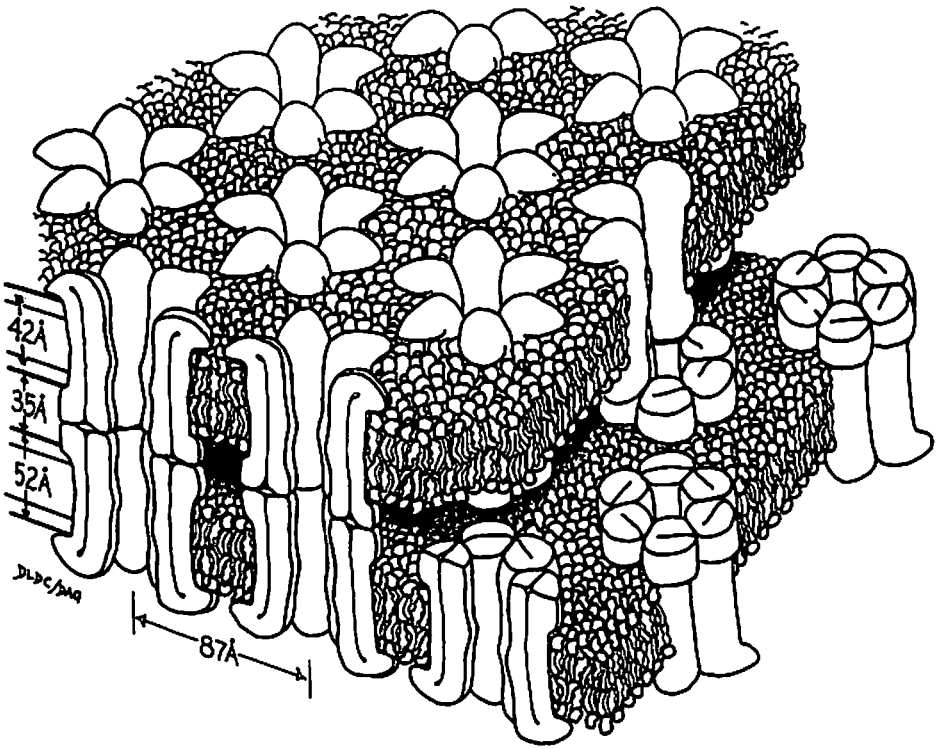

Fig. 2.6. Diagram of direct electrical connection between cells (gap junction). Channels provide for cell-to-cell exchange of low-molecular-weight substances and electric ionic current (in the form of ions). [From Makowski et al., 1977, with permission.]

other small molecules between cells. Ionic current through these channels directly couples the electrical activity of one cell to that of the other. Although in some cases gap junctions can be viewed as simple linearly conducting connections, in many other cases they are known to *rectify* (i.e., pass current in one direction much better than in the other). Gap junctions are known to be a prominent feature of neuron-to-neuron connections in many submammalian species, but in only a small number of regions in the mature mammalian nervous system (e.g., retina, inferior olive, vestibular nucleus, and the mesencephalic nucleus of the fifth cranial nerve). Gap junctions in these regions serve to synchronize the activity of individual elements with those of their neighbors. The ability of neurotransmitters to alter the conducting properties of gap junctions in some regions (e.g., retina) gives additional complexity to this system of communication.

EPHAPTIC INTERACTIONS

Ephaptic interactions refer to interactions between neurons based largely upon their close physical proximity (Fig. 2.7). The flow of ions into and out of one neuron will set up local electrical currents that can partially pass through neighboring neurons. The degree to which a neuron can be influenced by the activity of its neighbor is determined in part by the proximity of the cells and their processes (i.e., dendrites, cell bodies, and axons). In regions that possess closely spaced neuronal elements, such as the close packing of cell bodies in hippocampus and cerebellum or the bundling of dendrites in the cerebral cortex, there is the possibility of significant ephaptic interaction. Ephaptic interactions, like gap junctions, serve to synchronize local neuronal activity and may influence the general firing pattern of functionally related neurons (e.g., Taylor and Dudek, 1984).

CHEMICAL SYNAPSES

The release of neuroactive substances at the specialized connections called *synapses* is by far the most common method by which neurons influence other neurons. Some neuroactive substances can also diffuse over rather long distances to activate extrasynaptic sites, although it is not yet clear how common this type of transmission is.

As discussed in Chap. 1, neurotransmitters are released by neurons through exocytosis of packets (*vesicles*) of the substance from synaptic specializations into the space (*synaptic cleft*) between the cells. Examples of two of the most prevalent types of synapses are shown in Fig. 2.8. The release of transmitter is triggered by the entry of Ca^{2+} into the presynaptic terminal. This Ca^{2+} entry results from the depolarization associated with the arrival of the action potential. Once the neurotransmitter is released, it rapidly traverses the short distance between the neurons and binds to specific proteins (*receptor molecules*) on the postsynaptic cell. The activation of the receptors by the neurotransmitter may then cause a myriad of postsynaptic responses, many of which are expressed as an altering of the probability that a particular type of ionic channel will be open.

The actual receptor binding site may be part of, or separate from, the macromolecule making up the ionic channel. Examples of ionic channels to which the neurotransmitter directly binds include the glutamate and γ-aminobutyric acid (GABA)-activated channels (Fig. 2.8), and the nicotinic cholinergic receptor. The latter is acti-

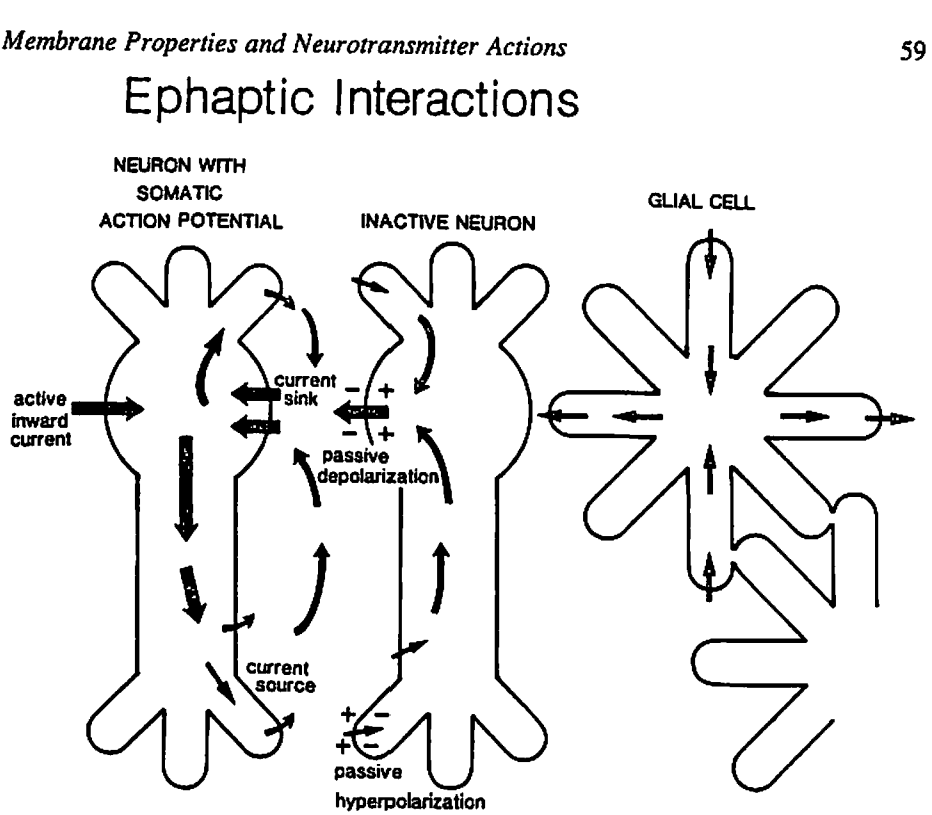

Fig. 2.7. Schematic diagram of current flow proposed to underlie excitatory electrical field effects between pyramidal neurons in the hippocampus (an example of ephaptic interactions). Arrows denote current flow of positive charges. The driving force of the ephaptic electrical field effect is the flow of positive current into somata produced by the synchronous firing if a population of hippocampal pyramidal cells (left). Positive current then flows passively out dendrites of active cells and returns through extracellular space. The relative decrease in positive charge in the extracellular space at the cell body layer causes the voltage on the inside of inactive cells (center) to appear relatively more positive (i.e., depolarized) than before. Likewise, the addition of positive current to the extracellular space at the levels of the dendrites by the neuronal activity causes the intracellular potentials of inactive dendrites to appear more negative (hyperpolarized) than before. Depolarization of the neuronal somata increases the probability that neighboring cells will generate action potentials in synchrony. Passive glial cell also develops transmembrane current flow within electrical field (right). [From Taylor and Dudek, 1984]

vated by acetylcholine (ACh) at the neuromuscular junction, in sympathetic ganglion neurons, and in many other regions of the nervous system. The binding of ACh to the nicotinic postsynaptic receptor induces a conformational change in the ionic channel, thereby opening the "gate" and allowing ions (in this case, Na^+, Ca^{2+}, and K^+) to flow through the pore (reviewed in Hille, 1992).

An example of a receptor site that appears to be separate from the channel molecule is the muscarinic receptor in the heart, which, when activated by acetylcholine, results in an increase in membrane potassium conductance. This response to ACh is associated with the receptor-mediated activation of an intracellular second messenger known as a *G-protein*. G-proteins are a class of molecule that require the binding of guanyl

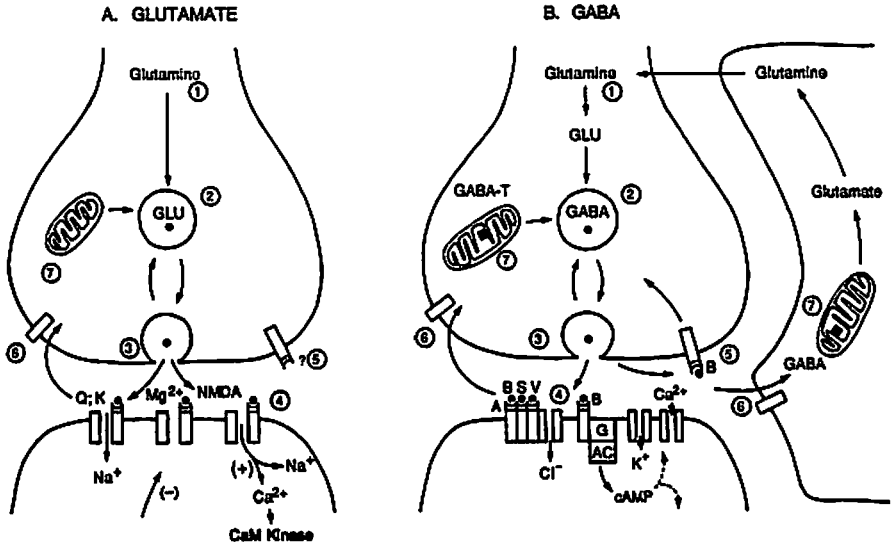

Fig. 2.8. Molecular mechanisms of ionotropic amino acid synapses. **A:** Glutaminergic synapses: (1) synthesis of glutamate (GLU) from glutamine; (2) transport and storage; (3) release of GLU by exocytosis; (4) binding of GLU to AMPA, kainate (K), and NMDA receptors. The Q (quisqualate or AMPA) and K receptors typically gate Na^+ and K^+ flux; the NMDA receptor also typically allows Ca^{2+} entry when the membrane potential is depolarized (+). When the membrane potential is hyperpolarized (−), Mg^{2+} blocks the channel. The release of glutamate may be regulated by presynaptic receptors (?5). Once GLU is released, it is removed from the synaptic cleft by re-uptake (6) and processed intracellularly (7). (From Shepherd, 1994; based on Cooper et al., 1987; Jahr and Stevens, 1987; Cull-Candy and Usowicz, 1987.) **B:** GABAergic synapse. (1) Synthesis of GABA from glutamine; (2) transport and storage of GABA; (3) release of GABA by exocytosis; (4) binding to a $GABA_A$ receptor that can be blocked by bicuculline (B), picrotoxin or strychnine (S) and can also be modified by benzodiazepines, such as valium (V); $GABA_B$ receptors, by contrast, are linked via a G-protein to the opening of K^+ or the reduction of Ca^{2+} channels. (5) Release of GABA is under the control of presynaptic $GABA_B$ receptors; GABA is removed from the synaptic cleft by uptake into terminals or glia (6); (7) processing of GABA back to glutamine. [From Shepherd, 1994; modified from Cooper et al., 1987; Aghajanian and Rasmussen, 1987, Nicoll, 1982, with permission.]

nucleotides in order to be active. The active component (catalytic subunit) of the G-protein is then thought to act as an intermediary between the receptor molecule and the ionic channel (reviewed by Neer, 1995).

Once a neurotransmitter is released, the length of time that it is present in the synaptic cleft is controlled by either hydrolysis of the transmitter, reuptake into the presynaptic terminal, uptake into neighboring cells, or by diffusion out of the cleft.

NEUROTRANSMISSION VERSUS NEUROMODULATION

Neuroactive substances in the nervous system have often been classified as either "neurotransmitters" or "neuromodulators" according to the duration and the functional implications of their actions. Substances released by neurons that have typical neuro-

transmitter roles cause postsynaptic responses that are both quick in onset (e.g., <1 msec) and relatively short in duration (e.g., <tens of milliseconds). The summation of phasic excitatory and inhibitory postsynaptic potentials and the way they interact with the intrinsic electrophysiological and morphological properties of the neuron forms to a large extent the manner in which neuronal computations occur.

In contrast, modulatory actions of neuroactive substances are characterized by their prolonged duration and the ability to *modulate* the response of the neuron to other, perhaps more phasic, inputs. Although the distinction between these two types of neurotransmitter actions is not always easy, it is nonetheless useful. Receptors acted on directly by a neurotransmitter are called *ionotropic*, whereas those acted on indirectly by second messengers are sometimes referred to as *metabotropic*.

It is probably safe to say that most neurons in the brain are under the influence of as many as a dozen or more neuroactive substances (see Table 2.2). The wide range of cellular responses (ionic as well as biochemical) to these substances adds great depth and richness to the possible behavior or individual neurons and consequently to a neuronal circuit as a whole. For example, it is the job of neurotransmitters not only to al-

Table 2.2. Common Neurotransmitter Responses in the CNS

Response	Neurotransmitter	Receptor
$\uparrow I_{Na}$, $\uparrow I_K$	Glutamate	AMPA/kainate
$\uparrow I_{Na}$, $\uparrow I_K$, $\uparrow I_{Ca}$	Glutamate	*N*-methyl-D-aspartate (NMDA)
	Acetylcholine	Nicotinic
	Serotonin	5-HT_3
$\uparrow I_{Cl}$	γ-aminobutyric acid	$GABA_A$
	Glycine	
$\uparrow I_{K,IR}$	Acetylcholine	M_2
	Norepinephrine	α_2
	Serotonin	5-HT_1
	γ-aminobutric acid	$GABA_B$
	Dopamine	D_2
	Adenosine	A_1
	Somatostatin	
	Enkephalins	μ, δ
$\downarrow I_{AHP}$	Acetylcholine	Muscarinic
	Norepinephrine	β_1
	Serotonin	(?)
	Histamine	H_2
	Glutamate	Glu metabotropic
$\downarrow I_{K,leak}$	Acetylcholine	Muscarinic
	Norepinephrine	α_1
	Serotonin	(?)
	Glutamate	Glu metabotropic
$\downarrow I_{Ca}$	Multiple transmitters	

low neurons to communicate accurately and quickly the exact details of a complicated visual scene (e.g., the reading of this page), but also to control the proper level of arousal of the nervous system (e.g., awake and attentive) for efficient and accurate processing of the information, as well as to cause the generation of relatively permanent cellular changes (memory) through which the contents of the written page can be recalled. Considering the wide range of involvement of neurotransmitters in simple (e.g., reflexes) as well as complicated (e.g., emotions, psychiatric disorders) behavioral attributes, it is not surprising that one should find that there is an equally wide range of neurotransmitter actions on single neurons.

IONIC ACTIONS OF NEUROTRANSMITTERS

A large number of substances exist in the nervous system that are thought to be released by neurons in order to modify the electrophysiological properties of other neurons (Table 2.2). Many of these substances can cause more than one postsynaptic response. Most if not all of these various responses are mediated by pharmacologically distinct receptor molecules. In this manner, a neuroactive substance released onto a pyramidal neuron in the cerebral cortex may have a very different effect from the release of the same neurotransmitter onto a relay neuron in the thalamus (see below). Indeed, the same neurotransmitter may have very different, or even opposite, postsynaptic effects on neighboring neurons in the same neuronal region, depending upon the particular function of the neuron in the local circuit.

Many of the ionic currents in neurons are under the control of neuroactive substances. Recently, it has become apparent that different neurotransmitters, each acting through its own distinct class of receptor molecules, can modify the same ionic current. For this reason, I will review here the more common postsynaptic actions of neurotransmitters in terms of the physiological action rather than the type of neurotransmitter.

FAST POSTSYNAPTIC POTENTIALS

The classical postsynaptic potential (PSP) occurs through a temporally (e.g., milliseconds or less) and spatially (i.e., local) limited increase in membrane ionic conductance. The relatively brief time course of these postsynaptic potentials allows neurons to perform a large number of computations within short time periods, limiting the interactions between events that are widely separated in time. Synaptic potentials, especially those brief in duration, are usually classified by whether they increase (excitatory) or decrease (inhibitory) the probability of action potential discharge. However, it is always better to know the actual biophysical and biochemical actions of the neuroactive substance than to refer to them as being just "excitatory" or "inhibitory," especially when considering the *modulatory* actions of many putative neuroactive substances (see below).

Fast Excitatory Postsynaptic Potentials. Two main types of brief-duration PSPs have been identified in the nervous system: those due to the activation of nicotinic receptors by ACh, and those caused by the release of excitatory amino acids.

Nicotinic Cholinergic Responses. Fast nicotinic excitatory PSPs mediated by ACh have so far been shown to occur in the spinal cord, peripheral nervous system, and skeletal

muscle. Nicotinic receptors are also located throughout the central nervous system (e.g., Albuquerque et al., 1995).

The activation of the nicotinic receptor-ionic channel complex by ACh results in a conformational change in the shape of critical portions of this macromolecule, thereby allowing ions to flow through. The nicotinic ionic channel is a "nonselective" cation channel, meaning that positively charged ions (e.g., Na^+, Ca^{2+}, and K^+) pass through the channel with about equal proficiency. Because of the mixed nature of the ions flowing through the nicotinic channel, the equilibrium (reversal) potential of the nicotinic response, approximately -5 mV, lies between the equilibrium potentials of the various cations (see Fig. 2.2).

The nicotinic receptor channel is a pentameric structure composed of, in order of mobility on SDS polyacrylamide gels, two α, one β, one γ (expressed in development; replaced by ϵ in adults) and one δ subunit surrounding a water-filled pore. Amino acid sequencing of α subunits, which contain the binding site for receptor activation, revealed the presence of at least eight distinct subtypes, termed α_1–α_8. These eight different α subunits (α_1, muscle; α_2–α_8, neural) differ not only in their primary structure, but also in their pharmacological properties and their distribution in the CNS.

The actions of ACh through nicotinic receptors in the nervous system is of particular interest, since nicotine, in the form of tobacco products, is still one of the most widely used drugs of addiction. Recent proposals suggest that the activation of dopaminergic neurons in the basal forebrain (ventral tegmental area) may be important in the pleasureful, and addictive, aspects of nicotine use (e.g., Calabresi et al., 1989).

Excitatory Amino Acid Responses. A substantial portion of the fast excitatory PSPs in the brain, particularly those in the cerebral cortex and hippocampus, are believed to be due to the release of an excitatory amino acid such as glutamate or aspartate. Postsynaptic ionotropic receptors for glutamate have been categorized according to their affinity for three different exogenous agonists: (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) (reviewed by McLennan, 1983; Watkins and Olverman, 1987; Westbrook, 1994). More recent molecular biological studies of glutamate receptors have revealed that each of these three subgroups is encoded for by a number of different genes, including *GluR1–4* for AMPA receptors, *GluR5-7*, *KA1* and *KA2* for kainate receptors, and *NMDAR1* and *NMDAR2A-D* (also known as *NR1* and *NR2A-D*) for NMDA receptors (for review see Barnes and Henley, 1992; Hollmann and Heinemann, 1994; Schoepfer et al., 1994). Hetero-oligomers formed by the different subunits generated by these genes are of a wide variety and exhibit varying electrophysiological and pharmacological properties, depending upon the combinations of subunits expressed.

Activation of excitatory amino acid receptors underlies fast glutamatergic excitatory postsynaptic potentials (EPSPs). The postsynaptic potentials mediated by AMPA and kainate receptors, like those associated with nicotinic channels, are caused by an increase in a mixed cation conductance (mainly Na^+ and K^+, but sometimes Ca^{2+} as well) such that the reversal potential is approximately 0 mV (see MacDermott and Dale, 1987; Hollmann and Heinemann, 1994). These synaptic potentials have a very short delay from the arrival of the action potentials at the presynaptic terminal to the appearance of the postsynaptic potential, and a rapid rate of rise. The falling phase is

much slower, being determined in large part by the membrane properties of the neuron (see Fig. 2.9B).

In contrast to the fast PSPs mediated by AMPA-kainate receptors, the action of glutamate through NMDA receptors is more complicated (reviewed by Ascher and Nowak, 1987). Stimulation of NMDA receptors results in the activation of a voltage-dependent current that is carried not only by Na^+ and K^+ but also importantly by Ca^{2+}. The voltage-dependent nature of this NMDA receptor–mediated current is due to the differential block of the ionic channel by magnesium ions (Mg^{2+}) at different membrane potentials (Mayer et al., 1984). At resting membrane potential (e.g., −75 mV) the driving force on Mg^{2+}, which is concentrated on the outside of the cell, to enter the neuron is quite high. Because of this, magnesium ions compete with Ca^{2+} and Na^+ ions for access to the pore of the channel. Since Mg^{2+} ions cannot flow through the pore, the channel is effectively blocked whenever one of the ions enters, thereby reducing the amount of time that the channel is open and conducting (see Fig. 2.9C).

When the cell is depolarized, the tendency for Mg^{2+} to fill the pore is substantially reduced, thereby lessening the block and allowing a larger $Na^+/Ca^{2+}/K^+$ current to flow. Because of this voltage dependence, activation of a glutaminergic synapse onto a neuron at resting membrane potentials may result in a fast EPSP mediated through the activation of kainate and AMPA (also known as quisqualate) receptors with little contribution of NMDA receptor mediated current, even though glutamate may be binding to these receptors (Fig. 2.9C). However, repetitive activation of the same synapse may cause a large depolarization of the cell through temporal summation of the unitary PSPs. The more these PSPs depolarize the cell, the more the degree of magnesium block will be removed, and thus the larger the activation of the NMDA current (Fig. 2.9D). Since NMDA channels conduct Ca^{2+} as well as Na^+ and K^+, calcium will flow into the postsynaptic cell and, by activating further biochemical mechanisms, can result in a *potentiation* of the strength of the unitary excitatory PSP. This enhancement of the PSP can last for prolonged periods (hours, days, longer?) and therefore is known as *long-term potentiation* (LTP) (see Collingridge and Bliss, 1987; Malenka and Nicoll, 1993; Nicoll and Malenka, 1995 for review). LTP is currently one of the leading models of the mechanisms by which synapses change their efficacy in order to participate in the encoding of memories in the nervous system (see Chap. 10, Olfactory Cortex; Chap. 11, Hippocampus; Chap. 12, Neocortex).

In addition to the activation of fast EPSPs, glutamate may also activate slow (seconds to minutes) EPSPs through the activation of glutamate "metabotropic" receptors (see below).

Fast Inhibitory Postsynaptic Potentials. Postsynaptic potentials that are quick in onset and inhibit the postsynaptic activity of the neuron are known to be mediated by two different neurotransmitters in the CNS: γ-aminobutyric acid (GABA) and glycine.

GABA-Mediated IPSPs. Gamma-aminobutyric acid is the major inhibitory neurotransmitter of the nervous system. GABA-releasing cells are present throughout all levels of the neuraxis. In the cerebral cortex and thalamus, they account for approximately 20–30% of all neurons. Neurons utilizing GABA as a neurotransmitter form a diverse

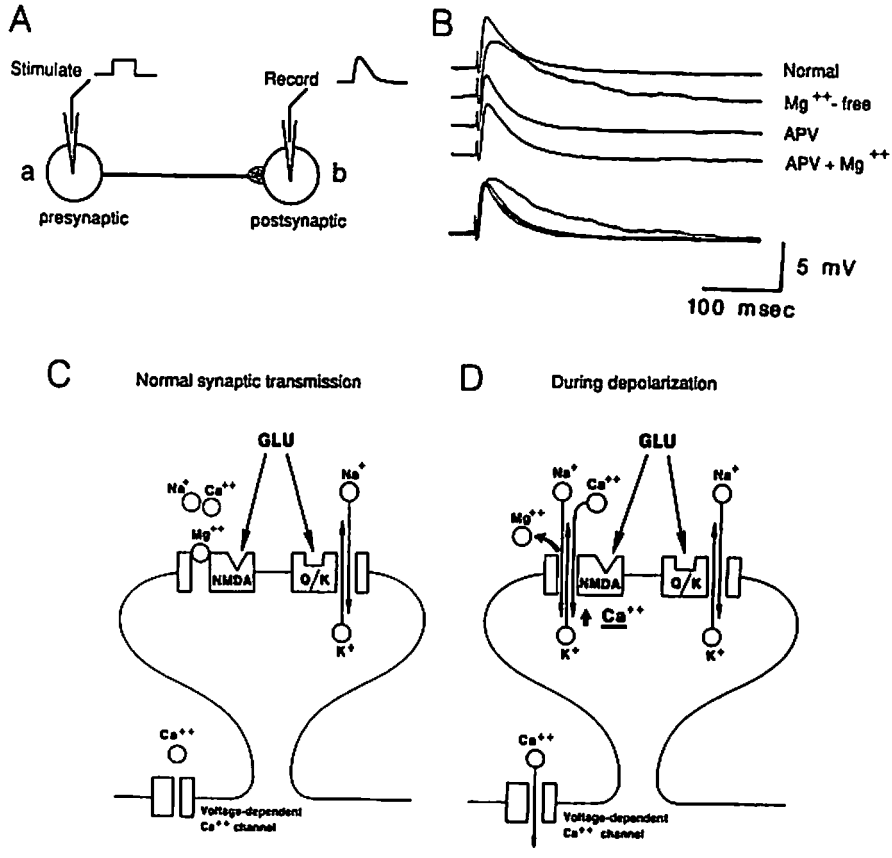

Fig. 2.9. Synaptic potentials mediated by the release of glutamate. **A:** Schematic diagram of experimental protocol in which the actions and pharmacology of monosynaptic connections between cultured cortical pyramidal cells is investigated. Intracellular recordings are used to stimulate a generator cell (a) that is monosynaptically connected to a follower cell (b). **B:** Activation of an action potential in the generator cell (a) causes a monosynaptic EPSP in the follower cell (b) through the stimulation of AMPA and kainate receptors (top trace, normal). Removal of Mg^{2+} from the medium bathing the cultures enhances the late components of this EPSP (second trace, Mg^{2+}-free). Addition of the NMDA receptor antagonist APV abolishes this late component indicating that it was due to the activation of NMDA receptors (third trace, APV). Returning Mg^{2+} to the bathing medium now has no additional effect on the EPSP (fourth trace, Mg^{2+}). At the bottom of B the traces are superimposed for comparison. These data illustrate that the release of glutamate can activate AMPA/kainate and NMDA receptors and that NMDA, but not AMPA/kainate, ionic channels can be blocked by Mg^{2+} ions. **C:** Schematic summary diagram illustrating that glutamate release from the presynaptic terminal at a low frequency (normal synaptic transmission) acts on both the NMDA and AMPA/kainate type of receptors. Na^+ and K^+ flow through the AMPA/kainate channel, but not through the NMDA receptor channel because of Mg^{2+} block. **D:** Depolarization of the membrane potential, or activation of the glutamatergic inputs at a high frequency, relieves the Mg^{2+} block of the NMDA channel, thereby allowing Na^+, K^+, and importantly, Ca^{2+} to flow through the channel. Depolarization due to the synaptic potential now also activates other voltage-dependent channels, such as those that conduct Ca^{2+}. [B from Huettner and Baughman, 1988; C and D from Nicoll et al., 1988, with permission.]

group, with several different morphologies specific for their own role in neuronal processing. They are instrumental in defining and confining the response properties not only of single neurons but also of large neuronal circuits. They figure prominently as interneurons in the types of inhibitory circuits illustrated previously in Chapter 1. It would be fair to say that without GABAergic neurons, the nervous system would not function in any logical manner.

There are three major types of GABA receptor, which are referred to as $GABA_A$, $GABA_B$, and $GABA_C$ (Bowery et al., 1987; Bormann and Feigenspan, 1995). Here we consider only the $GABA_A$ receptor ($GABA_B$- and $GABA_C$-mediated responses are discussed below). Many fast inhibitory PSPs in the brain are believed to result from the release of GABA acting upon the $GABA_A$ subclass of receptor (see early IPSP, Fig. 2.10). Binding of GABA to this class of receptor opens ion channels that are selective for Cl^- ions, and therefore the reversal potential of $GABA_A$-mediated responses is at the equilibrium potential for chloride (i.e., approximately -75 mV). Like the fast EPSPs in the nervous system, fast $GABA_A$-mediated inhibitory postsynaptic potentials (IPSPs) possess a rapid rising phase and a slower decay. These IPSPs are only tens of milliseconds in duration and are involved in rapid computations by neuronal networks (see Chap. 1).

$GABA_C$ receptors also conduct Cl^- ions and are most pronounced in the retina, although it is likely that they will be found in other parts of the CNS (see Bormann and Feigenspan, 1995).

Glycine-Mediated IPSPs. Glycinergic interneurons were first identified in the spinal cord and the brainstem. Glycine inhibits neuronal activity by increasing a Cl^- conductance similar to that activated by GABA (reviewed in Hamill et al., 1983; Kuhse et al., 1995). Indeed, it has been proposed that the glycine and GABA receptors may couple to the same Cl^- ionic channels (Hamill et al., 1983). Recent evidence suggests that glycine also serves as a classical neurotransmitter function in the forebrain (Betz, 1991; Trombley and Shepherd, 1994). Very low doses of glycine can greatly potentiate the actions of glutamate at NMDA receptors (Johnson and Ascher, 1987). This potentiating action occurs at low enough doses that even the concentrations of glycine occurring in the extracellular fluid are large enough to have a significant effect.

SLOW SYNAPTIC POTENTIALS

Like fast PSPs, slow PSPs are found at all levels of the nervous system. They have a large variety of sizes, shapes, and effects on the functional properties of neurons and neuronal circuits. Because of their delayed onset and prolonged duration, these PSPs are probably more involved in the regulation of the *excitability* of single neurons and neuronal circuits as opposed to underlying the relatively high frequency transfer of information (see Hartzell, 1981; Adams and Galvan, 1986).

Increase in Potassium Conductance. Applications onto neurons of a large variety of putative neurotransmitters, including acetylcholine, adenosine, norepinephrine, serotonin, γ-aminobutyric acid, dopamine, and various peptides, have been found to cause an increase in membrane potassium conductance (g_K; Fig. 2.10, and Table 2.2) (reviewed by North, 1987; Nicoll, 1988; Nicoll et al., 1990; McCormick, 1992). This occurs

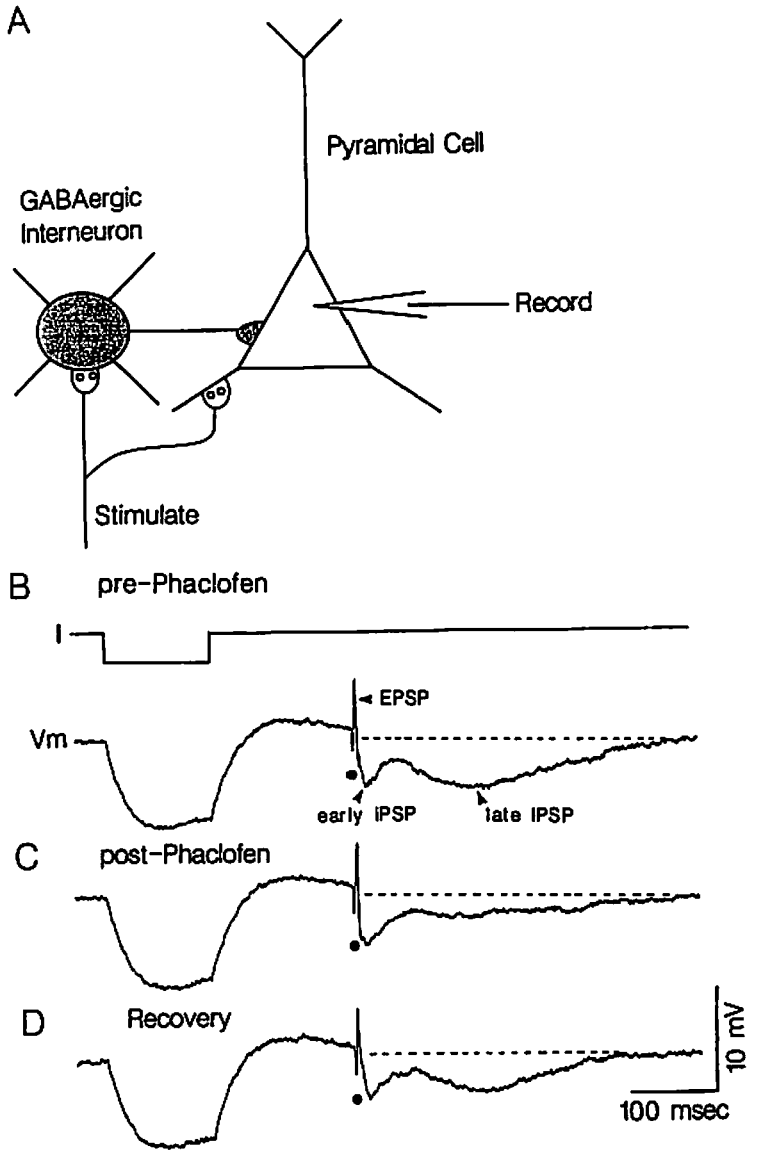

Fig. 2.10. Synaptic potentials generated in cortical pyramidal cells. **A:** Schematic diagram of stimulation and recording situation. Stimulation of afferent fibers activates both pyramidal cells and GABAergic interneurons that subsequently inhibit pyramidal cells. **B:** Intracellular recording from a human cortical pyramidal cell during stimulation of ascending axons. Injection of a hyperpolarizing current pulse (I) is used to investigate the apparent input resistance of the neuron. Electrical stimulation (dot) results in the generation of a fast EPSP followed by an early (fast) and late (slow) IPSP. Activation of the axons excites local GABAergic interneurons that subsequently release GABA onto the recorded pyramidal cell. GABA then activates both $GABA_A$ and $GABA_B$ receptors. Activation of $GABA_A$ receptors causes an increase in Cl^- conductance and underlies the early IPSP, while activation of $GABA_B$ receptors causes an increase in K^+ conductance and is responsible for the generation of the late IPSP. **C:** Local application of the $GABA_B$-specific antagonist phaclofen substantially reduces the late IPSP, confirming that this PSP is due to the activation of $GABA_B$ receptors. The effect of phaclofen is reversible (**D**).

through a specific subtype of neuronal receptor for each neuroactive substance. Although all of these substances have the ability to increase potassium conductance in some region of the nervous system, the nonhomogeneous distribution of receptors that mediate this response means that some neurons exhibit it and others do not. For example, application of acetylcholine to GABAergic interneurons in the feline thalamus results in an *increase* in g_K, whereas in neighboring thalamocortical relay cells this putative neurotransmitter causes a *decrease* in g_K (McCormick and Prince, 1987a; Pape and McCormick, 1995; see Chap. 8). Furthermore, in many regions of the nervous system there is convergence of different neuroactive substances with each one generating an increase in g_K in the same postsynaptic neuron. For example, hippocampal pyramidal cells respond to serotonin, GABA (through $GABA_B$ receptors), and adenosine with an increase in the same potassium conductance (Nicoll et al., 1990; see Chap. 11). In this manner, a variety of neuroactive substances can activate or inactivate the same ionic currents in a given neuron and perhaps even converge onto the same ionic channel.

Functionally, an increase in membrane potassium conductance is considered inhibitory in that it usually decreases the probability of action potential discharge, and this can have important functional consequences. For example, GABA can increase both g_{Cl} (through $GABA_A$ or $GABA_C$ receptors) and g_K (through $GABA_B$ receptors); the result is a fast $GABA_A$-mediated increase in g_{Cl} followed by a slow $GABA_B$-mediated increase in g_K in the postsynaptic neuron (see late IPSP, Fig. 2.10; Newberry and Nicoll, 1985; Dutar and Nicoll, 1988a,b; McCormick, 1989b). In addition, there are many differences between the fast and slow GABA-mediated IPSPs other than just their time course. The conductance increase associated with the late IPSP is much smaller than that associated with the fast IPSP even though the amplitude of the voltage deviation associated with each may be similar. Indeed, if the membrane potential is negative to E_{Cl}, the fast IPSP will be *depolarizing* (although it is still inhibitory), while the late IPSP will still be hyperpolarizing. In addition, the GABA-activated late IPSP is mediated through a second messenger system (G-proteins) while the fast IPSP is the result of GABA binding to a receptor located directly on the ion channel.

These physiological differences make fast IPSPs more of a shunting inhibition (i.e., the membrane potential of the cell is held close to E_{Cl} and the input resistance of the cell is "shunted"), whereas the late IPSP operates more through the hyperpolarization of the neuron. Fast IPSPs are useful for local (e.g., particular subparts of the cell) "yes-no" decisions, whereas the late IPSP is useful for the modulation of the overall excitability of the neuron. The restricted time and space domains of the fast IPSPs allow them to participate in relatively high-frequency neuronal processing, whereas the slow IPSP is important for setting a particular level of excitability in the neuron for more prolonged periods of time.

The postsynaptic morphological locations of IPSPs are also very important in determining their consequences for processing within synaptic circuits. Many types of GABAergic neurons form synaptic contacts at specific locations of the postsynaptic neuron. For example, *chandelier cells* of the cerebral cortex give rise to chains of synaptic terminals on the axon hillocks of cortical pyramidal cells (see Chap. 12), while *basket cells* give rise to a "basket" or "pericellular nest" of terminals around the cell bodies of pyramidal neurons. In this way both of these inputs have powerful effects on the

output of the entire neuron. It may even be possible for the chandelier cell to prevent the propagation of an action potential down the axon after its generation in the cell body and/or dendrite, or to determine the precise timing of action potential generation.

The opposite extreme of the above two examples of a rather global inhibition by GABA of the output of the neuron is found in the very localized synaptic processing in dendritic microcircuits (see Chap. 1). At this level of organization, individual GABAergic terminals may have effects that are relatively independent of one another, as well as independent of the output activity of the neuron itself. In these situations, the GABAergic process may affect only a particular portion of the postsynaptic dendritic tree, or perhaps, only particular synaptic terminals. Numerous examples of GABAergic contributions to processing in synaptic glomeruli, dendritic trees, and other types of microcircuits will be discussed in subsequent chapters.

Decrease in Potassium Currents. Neuroactive substances can decrease as well as increase neuronal potassium currents. To date, there are four different potassium currents that can be decreased in amplitude in response to various neurotransmitters: I_{AHP}, I_M, I_A, and a resting "leak" potassium current which I shall denote as $I_{K,leak}$.

Decrease in I_{AHP}. I_{AHP} has been shown to be decreased by a number of putative neurotransmitters (norepinephrine, acetylcholine, serotonin, histamine, glutamate, etc.) (Charpak et al., 1990; reviewed in Nicoll et al., 1990; McCormick, 1992). In the case of norepinpehrine, the decrease in I_{AHP} is achieved through an increase in the intracellular activity of a second messenger, cyclic adenosine monophosphate (cAMP) (Madison and Nicoll, 1986b; Pedarzani and Storm, 1993).

As stated previously, I_{AHP} contributes substantially to spike frequency adaptation. Therefore, block of this current greatly reduces the tendency for cells to slow down their firing rate during maintained depolarization (Fig. 2.11). This is an important effect, for it allows a neurotransmitter to increase the response of a cell to barrages of excitatory PSPs with little or no change in the resting membrane potential, or the response to inhibitory PSPs. Indeed, if the putative neurotransmitter simultaneously increases membrane conductance to K^+ or Cl^- while blocking I_{AHP}, the result may actually be an increase in "signal-to-noise" ratio. The baseline spontaneous firing of the cell will be reduced by the increase in potassium and/or chloride currents, while the response of the cell to barrages of large EPSPs may actually be enhanced by the decrease in I_{AHP} (see below).

Decrease in I_M. As stated above, I_M is a potassium current that is slowly (tens of milliseconds) activated by depolarization of the membrane potential above approximately −65 mV (Brown and Adams, 1980; Brown, 1988). This current has been shown to be potently reduced by stimulation of muscarinic receptors by acetylcholine. Like I_{AHP}, I_M contributes to spike frequency adaptation; blocking it subsequently increases the response of a neuron to barrages of excitatory PSPs. Because I_M is active only at depolarized potentials, its blockade may have little effect on the cell's resting membrane potential or response to IPSPs.

The M current may be reduced following the activation of a variety of receptors, including serotoninergic and glutamatergic receptors, and some types of peptide

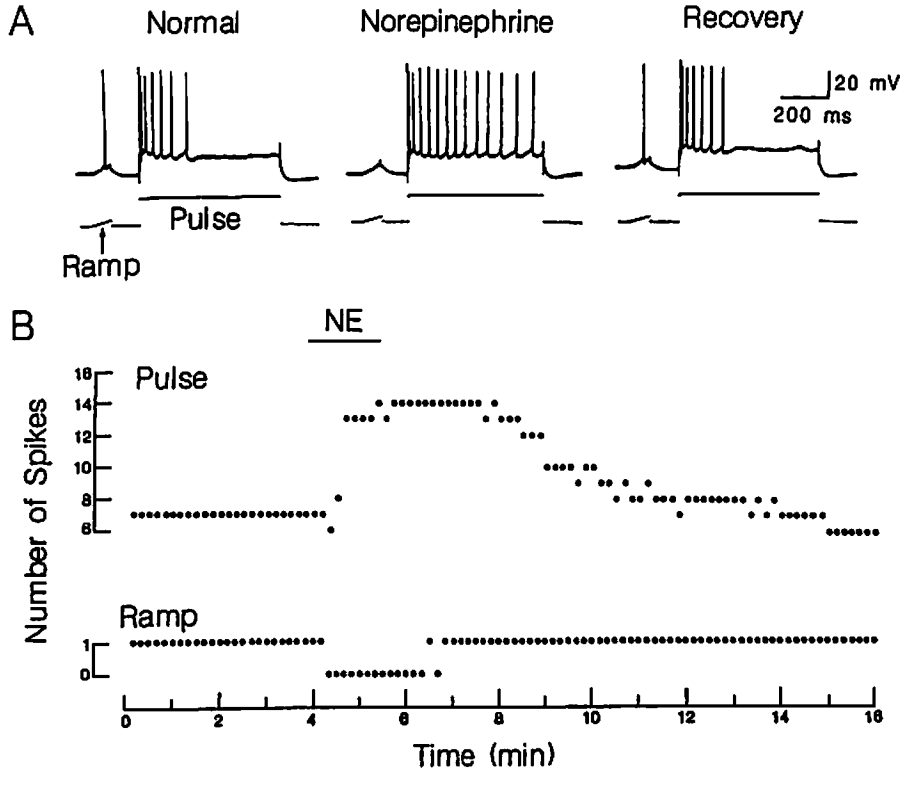

Fig. 2.11. Effect of norepinephrine on the excitability of cortical pyramidal neurons. The response of this hippocampal pyramidal neuron to two different types of input was examined: a small depolarizing ramp (to mimic weak EPSPs) and a prolonged depolarization (to mimic a train of strong EPSPs). A: In normal conditions, the small ramp input causes the generation of a single action potential while the prolonged depolarization results in a train of action potentials that show strong spike frequency adaptation (A, left). Addition of norepinephrine to the bathing medium results in a small hyperpolarization of the membrane potential (not shown). During the hyperpolarization the small depolarizing input no longer generates an action potential while the response to the prolonged input is actually potentiated because of the block of spike frequency adaptation (A, norepinephrine). The reduction in spike frequency adaptation is a secondary effect due to the block of I_{AHP} (not shown). This effect of norepinephrine is fully reversible (A, recovery). B: Graphic representation of the data in A. The generation of an action potential by the small ramp input is blocked, while the response to the prolonged input is greatly enhanced. In this manner, norepinephrine can increase the "signal-to-noise" ratio of the cell. [From Madison and Nicoll, 1986a, with permission.]

receptors (McCormick and Williamson, 1989; Charpak et al., 1990; Nicoll et al., 1990).

Decrease in I_A. Many of the different K^+ currents in neurons are differentiated by different rates of activation and inactivation. One of these, the A current, and probably others, can be modulated by the application of neurotransmitters (Aghajanian, 1985). Since I_A contributes to an increase in the interval between action potentials during cer-

tain types of neuronal activity, the blocking of I_A will enhance the response of the neuron by increasing the frequency of action potential discharge.

Decrease in calcium currents. Numerous putative neurotransmitters, including acetylcholine, norepinephrine, serotonin, and GABA, can reduce the flow of Ca^{2+} across the membrane (see Tsien et al., 1988; Stea et al., 1995). The functional consequences of neurotransmitter suppression of calcium currents has not been well studied in the CNS. One possible effect is related to the actions of neurotransmitters at presynaptic terminals. The amount of transmitter that is released after the invasion of the terminal by an action potential is under the control of neuroactive agents binding to receptors located on these terminals. In most (perhaps all) systems, the binding of the transmitter that is released by the terminal *reduces* the quantity released by subsequent action potentials. This *auto-inhibition* then forms a negative feedback loop that is useful for regulating the concentration of transmitter in the area of the synaptic cleft. The ionic mechanisms of this negative feedback are not known. However, since neurotransmitter release is highly dependent upon Ca^{2+} entry, transmitter-mediated decreases in Ca^{2+} currents may be involved.

Possible Gating Actions of Neurotransmitters. As discussed previously, many different types of neurons in the nervous system possess two intrinsic and physiologically distinct firing modes: single-spike and burst activity (e.g., Llinás and Yarom, 1981a,b; Jahnsen and Llinás, 1984a,b; Llinás, 1988). The cell's membrane potential determines in part which of these two firing patterns the neuron will exhibit. Burst firing occurs in response to excitatory inputs whenever the membrane potential is negative to approximately −65 mV, whereas single-spike activity occurs at membrane potentials positive to approximately −55 mV (Fig. 2.5D,E). Therefore, a neuroactive substance that activates a potassium conductance can actually increase the probability of a neuron firing by hyperpolarizing the cell into the burst firing mode of action potential generation (e.g., from −60 to −70 mV). In this situation, the increase in membrane conductance is acting more as a "switching" or modulatory mechanism than as a strict "yes-no" inhibition (McCormick, 1992). Likewise, decreasing resting conductance to K^+ is an effective mechanism by which a neuron can be tonically depolarized out of the burst firing mode and brought closer to threshold for generation of the more unmodulated single-spike discharge (Fig. 2.12). Such changes in membrane potential have been found to occur during shifts in arousal (Hirsch et al., 1983) and may underlie the well-known shift in the characteristics of the electroencephalogram (EEG) from synchronized slow waves to desynchronized, higher frequencies during increases in arousal (e.g., Moruzzi and Magoun, 1949; Steriade et al., 1993).

INTRINSIC AND SYNAPTIC CURRENTS: PUTTING IT ALL TOGETHER

With our new armament of knowledge of the intrinsic properties of neurons and how they may be affected by neurotransmitters we can proceed (with due caution) to propose a scenario of how synaptic computations may be implemented and modulated in a representative neuron. We take as our example one of the most abundant and important neuronal cell types in the human brain: the cerebral cortical pyramidal cell (see Chaps. 1, 10–12).

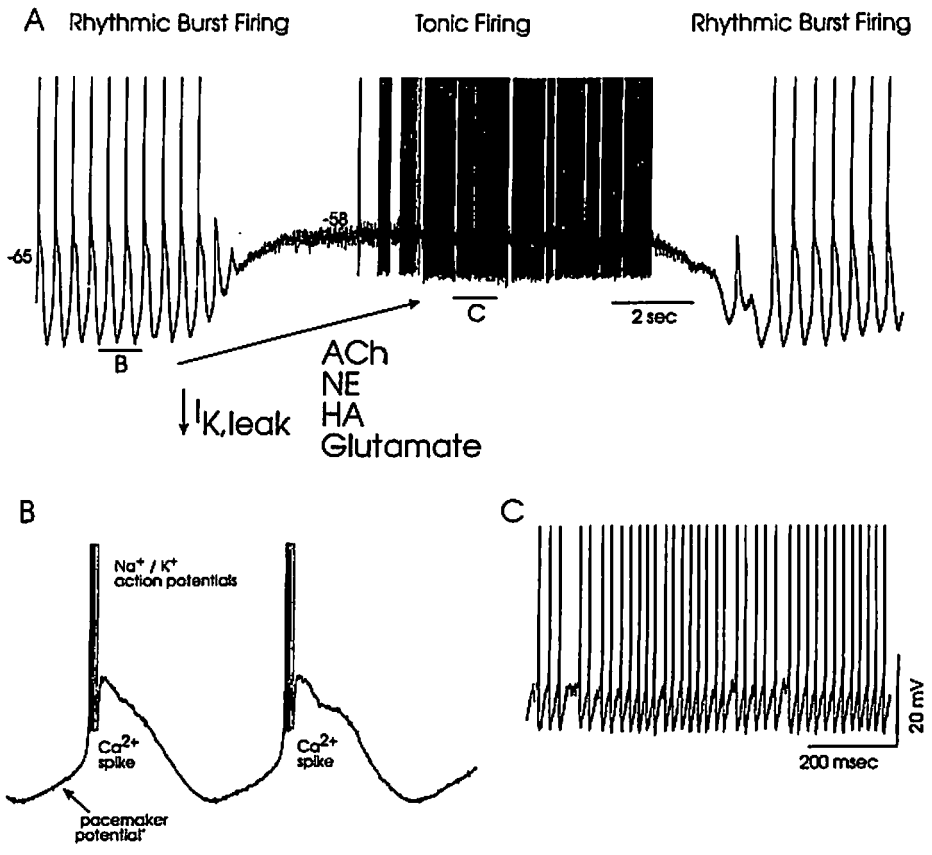

Fig. 2.12. Neurotransmitters control the firing mode in thalamic relay neurons. **A:** Thalamic relay neurons can spontaneously generate rhythmic bursts of action potentials (rhythmic burst firing) through the generation of repetitive low-threshold Ca^{2+} spikes (see B). Application of a variety of neurotransmitters, including acetylcholine (ACh), norepinephrine (NE), histamine (HA), and glutamate, can reduce a "leak" K^+ current, $I_{K,leak}$, and therefore depolarize the thalamic cell. This depolarization inactivates the low-threshold Ca^{2+} current and therefore blocks rhythmic burst firing. Now the cell generates tonic trains of action potentials. Once the block of $I_{K,leak}$ wears off, the cell returns to rhythmic burst firing. **B:** Expansion of the rhythmic burst firing in A illustrating the rhythmic Ca^{2+} spikes interspersed by a "pacemaker potential" generated by the activation of I_h. **C:** Expansion of part of the tonic firing in A. [Modified from McCormick and Pape, 1990, with permission.]

Cortical pyramidal cells, like neurons in most other parts of the brain, receive excitatory, inhibitory, and modulatory inputs from a variety of sources. Putative *glutamatergic* synapses, which have typical fast excitatory actions, are found on the spines of apical and basilar dendrites (Fig. 2.13). Notable sources of excitatory inputs are other pyramidal cells (located in neighboring or distant cortical regions), spiny stellate neurons of layer IV, and inputs from the thalamus (see Chap. 12). In contrast to excitatory inputs, *GABAergic* inhibitory synapses are found on the soma, proximal and distal dendrites, and initial segment of the axon; they arise largely from intrinsic cortical

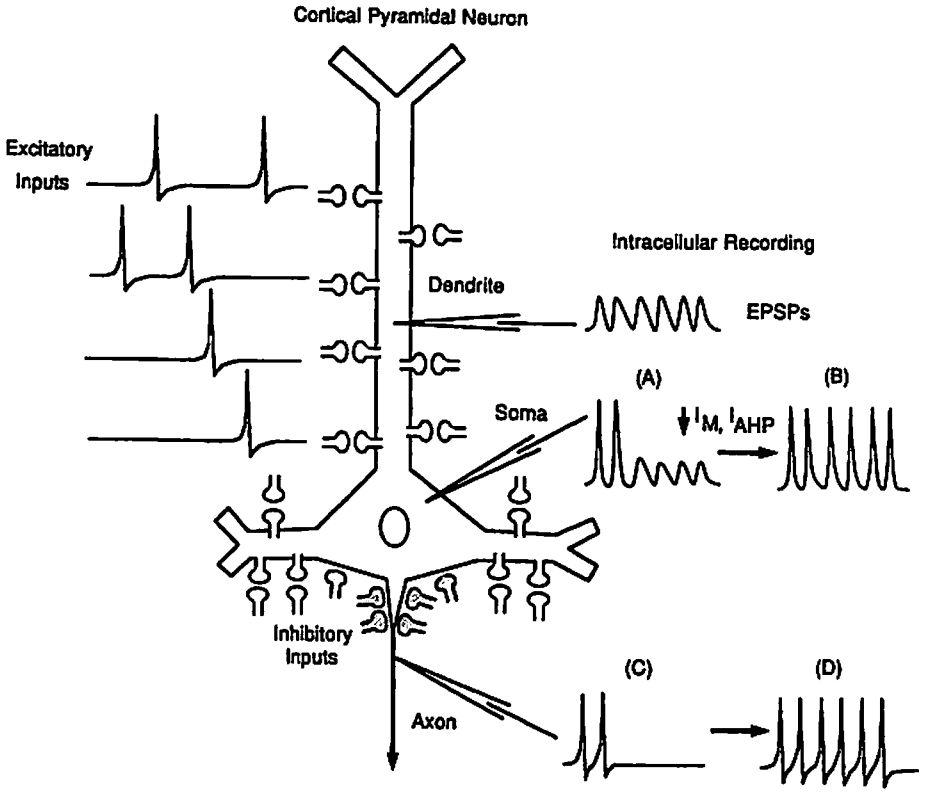

Fig. 2.13. Effect of activation of excitatory inputs to a cortical pyramidal cell. A train of action potentials arriving at different synaptic endings on the apical dendrite of the pyramidal cell results in the generation of a train of EPSPs. The first two EPSPs generate action potentials in the somatic region, while the last four fail because of activation of I_M and I_{AHP} (A). This is further reflected in the axonal output of the neuron (C). Block of these two currents reduced spike frequency adaptation and allows all six EPSPs to generate action potentials (B and D). See text for details.

interneurons, which are morphologically and functionally heterogeneous. Putative *neuromodulatory* substances arrive from a variety of subcortical (cholinergic, noradrenergic, serotonergic) and intracortical (cholinergic and peptidergic) neurons. Their synaptic contacts on pyramidal neurons are found largely on dendrites. Some types of GABAergic neurons also contain, and may release, one or more peptides. The ionic actions of these peptides and how they interact with the actions of GABA are not yet known.

Let us imagine that our cortical pyramidal cell is in the visual cortex and that, although the animal is awake and attentive, the cell is not yet receiving any specific visual input. The resting potential of our hypothetical cell will probably be somewhere around −65 mV, depending upon the state of input from the slowly acting neurotransmitters, especially those (e.g., acetylcholine) that can alter the level of resting potassium conductance. This resting potential is about 10 mV below (more hyperpo-

larized than) the threshold of around −55 mV for the generation of action potentials by a cortical pyramidal neuron.

Now let us stimulate the visual receptive field of our cell with an adequately adjusted light stimulus to the retina (for example, a moving bar of light). This input will first cause excitation of the thalamic neurons (see Chap. 8). Since the animal is awake and attentive, the thalamic neurons respond to the input in a one-spike-out-per-spike-in fashion (e.g., Fig. 2.5D) and in turn give rise to a train of action potentials that reach some of the presynaptic terminals onto our cell. Each action potential causes an increase in $[Ca^{2+}]_i$ in the presynaptic terminal that in turn causes the release of excitatory transmitter from a variable number of synaptic vesicles in a probabilistic manner (see Chaps. 1 and 3). The transmitter travels across the synaptic cleft and binds to specific receptor molecules on the postsynaptic spine, increasing the probability that certain ionic channels (assume they conduct Na^+ and K^+ ions) will be in the open and conducting state. In this manner, each presynaptic spike will cause an EPSP in the postsynaptic dendrite (Fig. 2.13). The exact amplitude–time course of each EPSP depends upon a large number of factors, including the amount of transmitter released, the density of postsynaptic receptor molecules, the sensitivity of the postsynaptic element to the transmitter, the size and shape of the postsynaptic element, and finally, the amplitude and distribution of active currents that the postsynaptic element possesses. Indeed, the "efficacy" of each synaptic connection is not a static number, since it is probably modified during the acquisition of new information, as well as new strategies to analyze that information, perhaps through a process similar to LTP (see Fast excitatory postsynaptic potentials above).

In order for the barrage of EPSPs generated by the train of inputs from the thalamus to cause our cell to fire, it must cause the output decision point of the cell (the cell body and *axon hillock* in this case) to rise above firing threshold (e.g., −55 mV). To do this, the EPSPs must spread from their points of generation in the dendrites, through the cell body, to the axon hillock. What happens to these EPSPs as they make this trip is determined by the intrinsic properties of our cell and the state of other neuroactive substances impinging upon it. The dendritic EPSPs will probably be large enough to activate $I_{Na,p}$, or a Ca^{2+} current and thereby receive an extra "boost" from these depolarizing currents. This enhancement is needed to help overcome the fact that cell membranes are not perfect insulators and some of the current will leak out, thereby reducing the size of the EPSP as it travels toward the cell body. If the train of EPSPs comes at a high enough frequency, they will exhibit temporal summation, while EPSPs that arise from more than one point in the cell will also exhibit spatial summation. If the summated EPSP is large enough, it may be capable of causing the generation of a dendritic Na^+/Ca^{2+}-mediated action potential that will, of course, greatly enhance and transform the response of the cell to the synaptic input (Fig. 2.5C). However, for simplicity, assume that the threshold for the generation of a dendritic Na^+/Ca^{2+} spike is not reached.

Now consider the situation in which many of the EPSPs in the train are large enough to cause the generation of an action potential in the cell body and axon hillock. In this circumstance, the initial EPSPs in the train will be more likely to cause the generation of spikes than the latter ones because of the progressive activation of I_{AHP} and I_M, both of which contribute to spike frequency adaptation (Fig. 2.13A,C). Thus, although the

cell may fire to the initial few EPSPs, the later ones will not reach firing threshold and the cell's firing will cease. This is where our modulatory transmitters come into play. If we were to arouse our animal such that there were an increase in the release of, for example, norepinephrine and acetylcholine, then I_{AHP} and I_M (and perhaps $I_{K,l}$) would be reduced. Reduction of these potassium currents would enhance the response of the neuron by reducing spike frequency adaptation as well as by moving the cell's membrane potential closer to firing threshold (Fig. 2.13B,D).

As the visual stimulus moves out of the cell's excitatory receptive field and into those of neighboring cortical neurons, our pyramidal cell may now be actively inhibited through the connections of intrinsic GABAergic neurons. These barrages of IPSPs will meet with many of the constraints as did the previous EPSPs, although they may occur in a more "linear" portion of the membrane potential (i.e., between -65 and -75 mV). The fast GABAergic IPSPs will be important in terminating the residual excitation from the previous barrage of EPSPs by causing an increase in Cl^- conductance. The influential position of the IPSPs on or near the soma and initial portion of the axon (axon hillock) make them particularly effective.

Now let's consider the situation when the animal or person falls to sleep. As drowsiness sets in, the rate of release of the ascending modulatory neurotransmitters, such as acetylcholine, norepinephrine, and histamine, will decrease. The decreased release of these modulatory transmitters will result in a hyperpolarization of many cell types owing to increases in various K^+ conductances. For example, thalamocortical neurons in the thalamus (Chap. 8) may hyperpolarize by up to 20 mV because of the increase in a resting K^+ conductance that is normally reduced by the release of these agents. This hyperpolarization of neurons in the CNS and the increase in amplitude of various K^+ currents results in a decreased excitability of these cells. In addition, the hyperpolarization also results in the removal of inactivation of some ionic currents, most notably, the low-threshold Ca^{2+} current I_T. The removal of inactivation of I_T allows for the generation of low-threshold Ca^{2+} spikes, and the activation of these in thalamocortical networks results in the generation of the spontaneous rhythms of sleep (Steriade et al., 1993).

The presentation of a visual (or other sensory) stimulus to our drowsy or sleeping friend will now result in a reduced response in the visual cortex: his or her brain will be less responsive and less able to respond quickly. This reduction in responsiveness becomes more and more pronounced as the person falls deeper and deeper to sleep.

Many of the properties outlined for our hypothetical cortical pyramidal and thalamic neurons can be generalized to neurons in all regions of the nervous system. However, each type of neuron is unique, and generalizations must be used with caution so as not to neglect the important features of each neuronal type that allow it to perform its own brand of cellular processing and thereby make its specific contributions to the synaptic circuits of which it is a part.