NEUROBIOPHYSICS

ANDREW A. MARINO,* *Department of Orthopaedic Surgery and Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, Louisiana, U.S.A.*

ILKO G. ILIEV,, *Department of Orthopaedic Surgery, Louisiana State University Medical Center, Shreveport, Louisiana, U.S.A.*

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INTRODUCTION

Neurobiophysics is the application of basic physical principles to the operation of the nervous system. The methods of neurobiophysics are identical to those of other branches of quantitative science:

i. observations of phenomena under controlled conditions and subsequent replication of the phenomena;
2. elaboration of models to which physical laws can be applied and unambiguously shown to explain pertinent quantitative aspects of the observations.

A basic assumption in neurobiophysics is that all neuronal activity is susceptible of an explanation based on the application of known physical laws. In this view, the morphological complexity of the neuron and the structural complexity of neuronal interconnections are practical barriers to an understanding of the nervous system, but it is not expected that as-yet-undiscovered laws will be needed to explain nervous-system activity. Because electricity is the currency of the nervous system, most models of neural activity are electrical in nature.

In 1902, Julius Bernstein hypothesized that cells were ionic solutions surrounded by thin membranes having permeability properties that resulted in the establishment of an electrical potential across the membrane. Further, during nervous-system activity, the permeability of the membrane changed in such a way as to lower the membrane potential. These ideas were subsequently developed by many investigators, culminating in the fluid-mosaic model of the membrane (Singer and Nicolson, 1972) and in the work of Alan Hodgkin and Andrew Huxley on the biophysical basis of the time-dependent permeability changes in nerve axons (Hodgkin and Huxley, 1952). The quantitative description of synaptic transmission at the neuromuscular junction, and Wilfrid Rall's development of methods for describing the flow of electrical activity in nerve dendrites (Rall, 1960), were further important contributions to the present-day theoretical framework of neurobiophysics. Beginning around 1975, the experimental technique *patch-clamping* was developed; it permitted direct observation of the kinetics of the *ion channel*, the membrane-level effector in the nervous system.

*Name and address for all communications.

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Much *activity* is presently devoted to characterizing the broad range of ionic channels present in the nervous system, finding both new channels and previously unrecognized functions of known channels, and establishing the mechanisms by which ion channels are regulated and synchronized to produce the specific phenomena observed in the nervous system.

1. STRUCTURE AND FUNCTION OF THE NERVOUS SYSTEM

The complexity of the nervous system varies with the degree of development of the organism; in the human being, it is a structure composed of more than 10^{10} cells that carries out sensory and regulatory functions, facilitates a variety of behaviors, and subserves what we recognize as memory, emotion, will, and intellect. The overall organization of the vertebrate nervous system is depicted in Fig. 1.

The *neuron* is the electrically active cell of the nervous system. There are many types of inputs to neurons, including deformation, light, and temperature, but probably the most important neuronal input signal consists of a flow of ions that enters the cell via a *synapse*, a specialized junction with a neighboring neuron. Two general types of synapses are recognized, depending on the source of the ions (Fig. 2). In an electrical synapse, the presynaptic and postsynaptic cells are linked by conducting channels that permit ionic flow between them. Electrical synapses (also called gap junctions) are rare in mammals, but are found between neurons in lower vertebrates and in invertebrates. The chemical synapse is a more complicated junction, and is the characteristic linkage between neurons in the mammalian nervous system. In chemical synapses, a 1—2 μm diameter region of the plasma membranes of the cells is separated by a 20—30-nm gap that must be traversed by a *neurotransmitter*, a chemical agent synthesized and secreted by the presynaptic cell, to effect a communication between the cells. The neurotransmitter diffuses across the gap and binds to receptors on the postsynaptic cell, resulting in the opening or closing of membrane channels and thereby altering ion flow between the interstitial fluid and the neuron. In some cases, the neurotransmitter receptor and the pore through which the ions pass are each part of a unitary transmembrane protein. In other cases, however, the receptor and the pore are distinct proteins, and the events at each site are coupled by an intracellular second messenger (the neurotransmitter is the first messenger). Since direct gating of ion channels involves only a change in the conformation of a single macromolecule, it can occur on the order of milliseconds. In contrast, channels activated by second messengers are slow (seconds to minutes) because they involve a series of sequential reactions. In both cases, the net result of the ion flow is to produce a change in the membrane potential of the neuron in the vicinity of the point of entry of the ions into the cell. If Cl^- enters the cell, the resting membrane potential (typically about —70 mV) usually becomes more negative, resulting in a hyperpolarization; entry of cations—Na^+, for example—produces a depolarization. For reasons that will be discussed, hyperpolarization of the neuronal membrane inhibits neuronal activity,

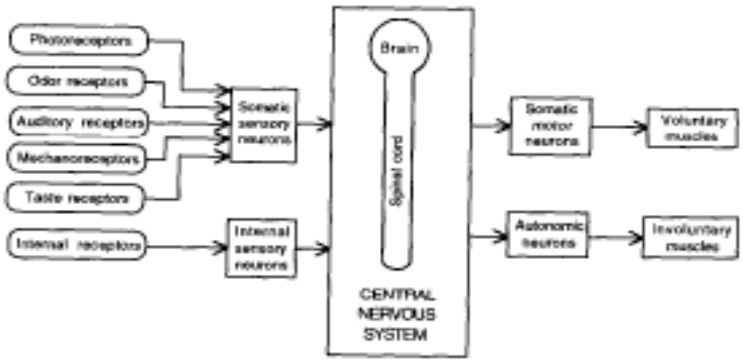

FIG. 1 General organization of the vertebrate nervous system. The input to the central nervous system (CNS) Consists of propagating electrical signals called action potentials that convey information to the brain from the sensory organs or from receptors located in internal tissues. The CNS output consists of action potentials that propagate along the motor neurons from the brain to the muscles. Additionally, the ONS communicates with the endocrinological and immune systems via chemical signals. (Adapted from Alberts *et al.*, 1989.)

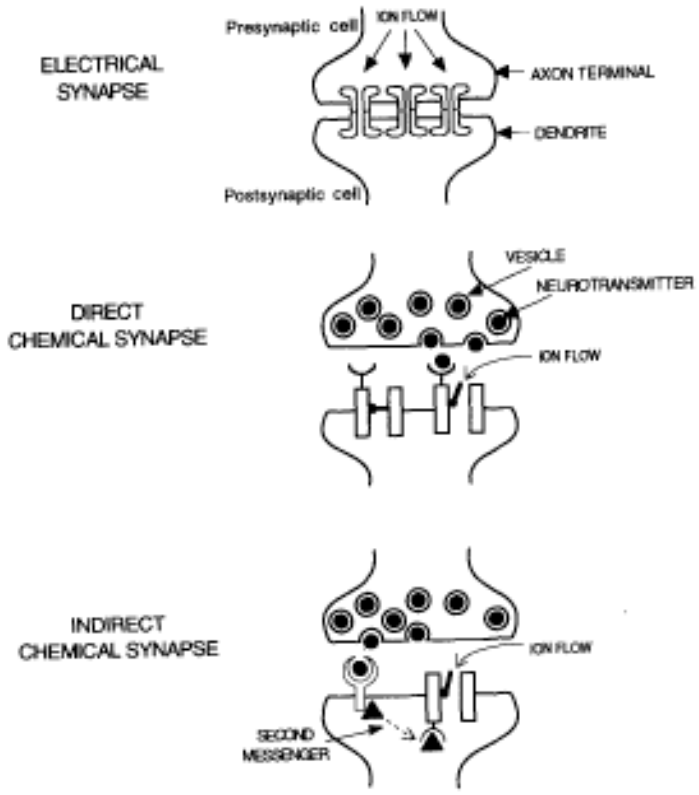

FIG. 2. Types of synapses in the nervous system. A synapse is a functional)unction between two neurons, and it is their defining characteristic. The presynaptic and postsynaptic neurons are defined by the direction of information transfer between them. The signal to a postsynapfic neuron consists of a flow of ions that enters the cell in the region of the dendrites or cell body. In the chemical synapse, the cells are separated by a narrow gap across which a neurotransmitter diffuses; it binds to receptors on the postsynaptic cell, thereby triggering the opening of otherwise closed membrane channels that permit the flow of ions into the postsynaptic cell from the interstitial fluid. The receptor, membrane pore, and gate may consist of a unitary protein structure *(direct chemical synapse),* or the receptor and pore portions of the channel may be physically separated, linked by various intermediary substances known as second (or higher-order) messengers (indirect chemical *synapse).* The conductance of individual channels at electrical synapses is relatively large (100—200 pS) compared with those at chemical synapses. Typically, directly gated channels mediate neuronal activity, whereas indirectly gated channels modulate the excitability of neurons.

neuronal activity, whereas depolarization produces the opposite effect.

The signal transmitted by the neuron has several different forms, depending on the morphological region of the cell (Fig. 3). A typical neuron simultaneously receives numerous excitatory and inhibitory input signals at synapses on the dendrites or cell body, but it responds only to the instantaneous sum of the individual changes in membrane potential. The components of the summation signal propagate passively from their point of initiation to the cell's trigger zone, usually the axon hillock; consequently, the contribution of a particular input signal to the summation signal is inversely proportional to the distance between the location of the input and the axon hillock. If the net change in the membrane potential at the axon hillock induced by the summed inputs is a depolarization that exceeds a threshold value, an *action potential* is generated, which propagates along the cell axon.

The action potential does not undergo amplitude diminution such as occurs during propagation of the postsynaptic potential to the axon hillock. Action potentials are all-or-none phenomena in the sense that their occurrence depends upon whether the cell threshold is exceeded. The electrical characteristics of individual propagating action potentials are determined by the physical properties of the neuronal axon, not by the characteristics of the stimulus; thus, within particular neurons, the action potentials are essentially identical in amplitude, pulse width, and propagation velocity. Quantitative information regarding the stimulus intensity is coded by the repetition rate of the action potential: An increase in the magnitude of the stimulus results in an increase in the repetition rate. Some axons are wrapped with an

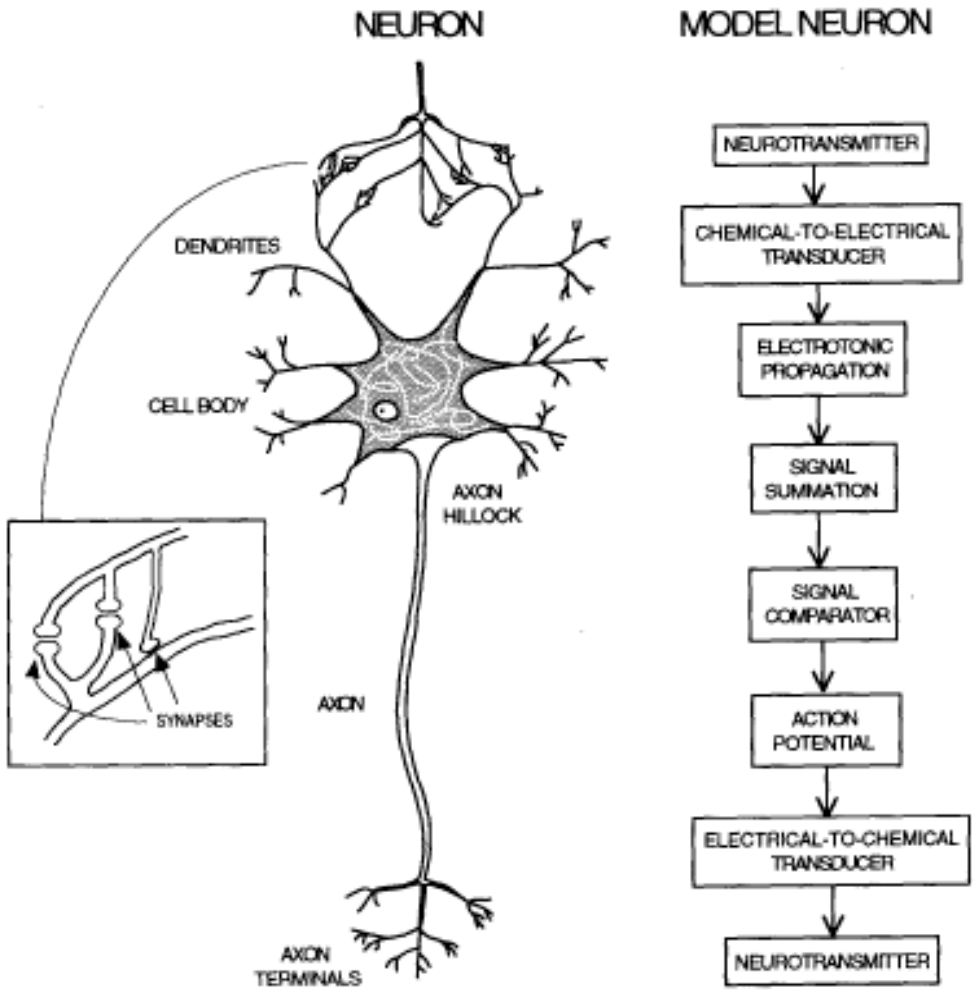

FIG. 3. Information transfer in the neuron. The initial signal consists of vesicles containing a neurotransmitter (NT) that are received by the neuron at synapses in its dendritic region. A neuron may have many dendrites, but only one axon.

insulating material called *myelin* that is interrupted at intervals by the *nodes of Ranvier;* electrical conduction in such neurons is the result of a combination of the processes underlying propagation of the summation signals and the action potential, and it proceeds more quickly than would be the case in the absence of myelin (Deutsch and Micheli-Tzanakou, 1987). Arrival of the action potential in the terminal portion of the axon triggers a series of biochemical events leading to secretion of the neurotransmitters that function as the input signal for the next neuron. Thus, in a typical mammalian neuron, neurotransmitters constitute the signal conveyed to and emitted by the neuron, but intaneuronal transmission is essentially electrical in nature.

The biophysical mechanisms underlying the complementary transduction events involving the interconversion of chemical and electrical signals are not well understood, but adequate descriptions of the phenomena that must be explained are now available. Cable theory provides a good description of the passive propagation of the postsynaptic potentials and the subsequent formation of the summation signal. The physical substrate of the signal comparator (Fig. 3) is the density and functional characteristics of the ion channels located at the axon hillock. The bestunderstood aspect of neuronal signaling is the

formation and propagation of the action potential; the explanation of this phenomenon on the basis of ionic permeability changes was a significant development in neurobiophysics.

2. ION CHANNELS

2.1 Overview

The electrical activity of neurons arises from the flow of ions through transmembrane proteins called channels. There are probably several hundred different channel proteins in the nervous system, but an individual neuron contains only some of them. A gated channel is a functional unit containing a pore through which ions may pass, a gate, and a sensor capable of opening or closing the gate in response to a signal; there are two classes of channels, depending on the nature of the signal to which they are responsive. *Voltage-gated* channels have an ion conductance that depends on the membrane potential, whereas the conductance of *ligand-gated* channels is dependent, directly or indirectly (Fig. 2), upon the binding of a neurotransmitter (the most important class of ligands in the nervous system) to the sensor portion of the channel (which, in the case of chemical signals, is called a *receptor).* Most gated channels exhibit only one form of gating behavior. Nongated channels are essentially membrane pores lacking gates and sensors.

The *selectivity* of a channel refers to the ion species that will pass through the channel pore. Voltage-gated channels are denoted by the ion that passes through most readily; the main types are Na^+, K^+, Ca^{2+}, Cl^-, and all small cations. Ligand-gated channels are labeled by a ligand that is effective in opening the channel. Thus, a Na^+ channel has Na^+ as the main permeant ion, and a nicotinic acetylcholine channel is a transmembrane protein having a receptor capable of binding the neurotransmitter acetylcholine, resulting in the passage of ions (Na^+ and K^+) through the channel pore. The response of a neuron to its environment is mainly determined by the gating and selection characteristics of its ion channels, and by the density and distribution of each channel type in the neuronal membrane. Neurons contain

1. nongated channels that serve to establish the membrane potential;
2. ligand-gated channels that subserve reception of the input signal;
3. voltage-gated Na^+ and K^+ channels that function in a synchronized fashion to permit propagation of an action potential; and
4. voltage-gated Ca^{2+} channels that participate in the transduction of the action potential into the chemical signal that constitutes the neuron's output.

In addition, there is an important class of channels that are sensitive to various intracellular chemical signals (Hall, 1992). Elucidation of the mechanisms underlying channel permeability (a measure of the selectivity of an ion pathway), channel conductance (a measure of the interaction between the ion and the channel structure), and channel gating are the central problems of neurobiophysics.

2.2 Methods of Study

The patch-clamp technique permits measurements of currents from individual ion channels in biological membranes (see BIOPHYSICS). This is achieved using a heat-polished micropipette having an opening of about 0.5—1 μm, and filled with a solution whose composition is compatible with the cell cytoplasm and the particular purposes of the measurement. The micropipette is gently placed against the cell membrane under direct visualization, and application of a slight negative pressure through the pipette results in an intimate contact between the membrane and the micropipette tip (Fig. 4). The electrical resistance of the seal is about 10—100 GΩ, indicating that the membrane is firmly attached to the pipette, thereby ensuring that the measured current actually flows between the cell interior and the micropipette, and is not shunted to the bath solution. Several measurement configurations—relationships between the micropipette and the membrane under measurement—are employed for the study of membrane kinetics. In the *cell-attached* (also called *on-cell)* configuration, the micropipette is sealed onto an intact cell. Several mechanical manipulations can be performed at this stage. The membrane patch may be ruptured by a brief pressure pulse applied through the

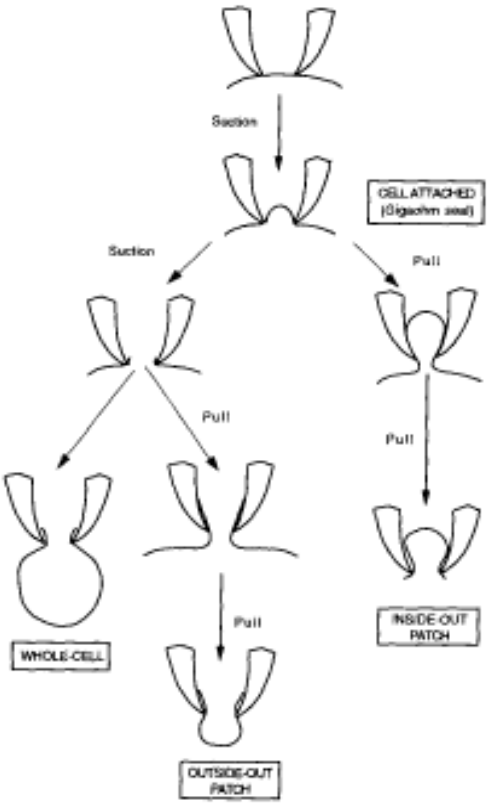

FIG. 4. Procedures leading to the various patch-clamp configurations (adapted from Neher, 1992).

micropipette, thereby establishing an electrical connection between the interior of the micropipette and the cell cytoplasm; the resulting *whole-cell* configuration is electrically equivalent to a conventional microelectrode penetration. Withdrawal of the pipette from the cell after the gigohm seal has been formed usually leads to one of two other configurations, depending on the mechanics of the process; the seal can be formed in such a way that the cytoplasmic surface of the membrane comes to face the bath solution or the micropipette solution respectively termed *inside-out* and *outside-out* patches (Fig. 4).

Each of the four patch-clamp configurations has properties that are useful for studying different kinds of problems. Recording from cell-attached patches provides a minimal perturbation of the cell under study, and allows single-channel currents from nearly all known types of ion channels to be resolved. The inside-out and outside-out patches permit high resolution of channel currents and provide the opportunity to control the ion milieu on both sides of the membrane. The whole-cell configuration (and a recently developed modification using the antibiotic nystatin) allows conventional voltage-clamp and current-clamp measurements to be performed on small cells.

In principle, any ion channel can be isolated, purified, chemically modified, and reinserted into artificial lipid bilayers. The result is a well-characterized membrane system in which the biochemical properties likely to be important in regulation and transport can be monitored, controlled, and studied with patch-clamp techniques. In another molecular approach, mRNA encoding a channel protein is injected into the oocyte of the frog *Xenopus*, which then synthesizes the protein and inserts it into the cell membrane, where it becomes accessible for study using patch-clamp techniques. The relation between structure and function can be systematically studied by mutating the mRNA and observing the resulting effect on the encoded protein after its insertion in the membrane. This strategy can be used to identify the portions of the proteins that are directly involved in voltage-dependent and ligand-dependent interactions. Recombinant DNA techniques permit creation of chimeric genes that code for channels made up of subunits from differing species; such preparations permit study of the conservation of the determinants of ion transport.

The various methods described above can be combined to permit

1. analysis of the kinetic pattern of specific channels during normal and pharmacologically induced activity;
2. study of the molecular structure of ion channels;
3. studies of the mechanisms of the regulation of the ion channel activity and identification of the cellular components involved in the regulation; and
4. identification and description of the roles of the channels in cellular processes.

2.3 Gating and Structure

The electrophysiological and molecular paradigms have not yet been fully carried out for any channel, but intensive work is under way, and good progress has been achieved for several

channels in the voltage-gated and in the ligand-gated families (Hille, 1992).

The Na^+ channels respond rapidly to depolarization; their basic role in the nervous system is to generate the initial portion of the action potential, and neither their pharmacology nor their structure varies significantly from tissue to tissue. The Na^+ channel consists of three subunits (Fig. 5); the a subunit has four homologous domains, each of which contains 6—8 hydrophobic amino-acid sequences, and an occludable pore selective for the passage of Na^+ It appears from site-directed mutagenesis studies that the locus of the voltage sensitivity is contained in an amino-acid sequence known as S4, one of which is contained in each of the four-domains. When amino acids in S4 are replaced by either neutral or negatively charged residues, the reduction in positive charge decreases the relative sensitivity for activation, which is direct evidence that the positive charge in S4 forms part of the voltage sensor for channel activation.

The K^+ family of voltage-gated transmembrane proteins is the largest and most diverse of the voltage-gated channels. The K^+ channels subserve a variety of functions, including return of the membrane potential to a pre-existing level, formation of trains of action potentials, and the occurrence of rhythmic activity. There are at least three major types of voltage-gated K^+ channels in the nervous system and perhaps ten times that number of K^+-channel subtypes. The main types are

1. the *delayed rectifier*, which is the axonal K^+ channel that opens with depolarization and is largely responsible for repolarizing the axon membrane following an action potential;
2. the K^+ channel, an axonal channel that opens rapidly upon depolarization and then quickly closes; and
3. the inward-rectifying K^+ channel, which opens only with hyperpolarization.

Another important class of K^+ channels is sensitive to levels of intracellular Ca^{2+}; the channels appear to be voltage-gated, with the Ca^{2+} acting to shift the voltage dependence (Hall, 1992; Hille, 1992). The K^+ channel has been well studied and is believed to be a prototype for the other K^+ channels; there is only about a 10% variation in the total amino-acid sequence among the various K^+ channels. The molecular weight of the protein encoded by K^+ DNA is about 25% of that of the Na^+ channel protein, and consists of six transmembrane segments; consequently, by analogy with the known structure of the Na^+ channel, each K^+ channel is thought to be a tetramer (Fig. 5).

Among the more remarkable features of some voltage-gated K^+ channels is the combination of high conductance and high ionic selectivity. The conductance of some K^+ channels appears to exceed the value associated with movement in aqueous solutions.

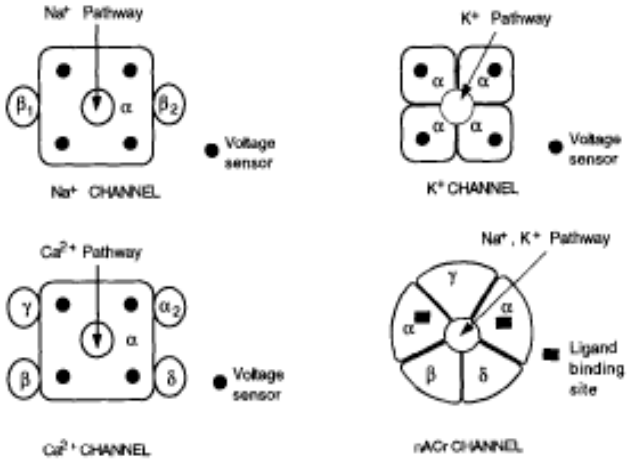

FIG. 5. Subunit composition of typical voltage-gated and ligand-gated ion channels. The channels are depicted as they appear when viewed from above the plasma membrane. (Na^+, K^+, and Ca^{2+} channels adapted from Catterall, 1988; nACr channel adapted from Alberts *et al.*, 1989.)

It is difficult to understand a mechanism that could be faster than the free-diffusion process, and yet could facilitate high ion selectivity. One possibility is that the channel can accommodate more than one ion at a time. If so, the presence of the first ion might reduce the electrostatic forces on the second ion, thereby facilitating its passage through the pore. It is not possible to calculate the magnitude of such an effect because K^+ channel structure is not known in sufficient detail.

Axons from mollusks, arthropods, annelids, and vertebrates have been found to contain essentially identical Na^+ and K^+ channels participating in conduction of the action potential. Thus, the evolution of these channels was essentially complete at the time of the common ancestor of these phyla, approximately 500 million years ago (Hille, 1992).

There is a wide range of voltage-gated Ca^{2+} channels within the nervous system; they all open with depolarization and appear to have a common subunit composition (Fig. 5), but they differ in voltage dependence, ionic selectivity, and pharmacology. Neurotransmitter secretion at nerve terminals is a well-studied Ca^{2+}-dependent process. Voltage-gated Ca^{2+} channels in the presynaptic terminal open following the depolarization produced by the arrival of the action potential, thereby triggering some of the membrane-bound yesicles containing neurotransmitter to fuse with the membrane surface, releasing neurotransmitter into the synapse. The Ca^{2+} channels in the axon terminal provide the only mechanism whereby the action potential can be transduced into a form capable of carrying information across a chemical synapse.

What mechanism might explain voltage-dependent activation of ion channels? A voltage sensor is the part of the transmembrane protein that undergoes a conformational transformation as a consequence of a change in membrane potential. From measurements of the time dependence of the response of channel proteins to changes in membrane potential, it can be inferred that such a conformational change is equivalent to the movement of 2—6 positive charges from the inner to the outer surface of the membrane (the *gating current).* Flow of the gating current is completed before ions flow through the channel. The S4 segments consist largely of positively charged amino acids (usually arginine), followed by two hydrophobic amino acids. In a proposed model (Catterall, 1988), the S4 segment is viewed as consisting of a helix in which each peptide bond is regularly hydrogen-bonded to nearby peptide bonds in the helix (α helix), with the positive charge of the arginine residue forming a spiral pattern around the core of the helix. Prior to activation, each positive charge is paired with a negatively charged amino acid residue located in another transmembrane segment. The ion pairing is in equilibrium with the resting membrane electric field, but, upon depolarization, the stabilizing field is reduced, resulting in rotation of the S4 segment about its axis in an outward-going direction. This helical motion corresponds to a 600 rotation and a 5 Å outward translation, and is equivalent to the movement of one positive charge a distance of 5 Å**.** When this process occurs in each of the four S4 segments (transfer of four gating charges or more, depending on the number of positively charged residues), the transmembrane pore is created. Upon return to the initial resting membrane potential, the sets of ion pairs formed as a consequence of depolarization are broken, and the original ion pairing is reestablished.

The helix-screw gating model is speculative and is only one of a number of possibilities for gating. Whatever the actual mechanism, it is important to note that, in general, ion channels are voltage dependent for two reasons:

1. The voltage provides the driving force for each ion;
2. the probability that a channel is open depends on the voltage.

Ligand-gated ion channels are specialized for converting neurotransmitters into graded electrical signals; the similarity in structure of ligand-gated channels suggests that they are a superfamily of proteins. The channels open transiently following binding of a neurotransmitter, thereby producing a postsynaptic potential as a consequence of the ion flux; ligand-gated channels are usually voltage insensitive. Each ligand-gated channel (whether gated directly or indirectly; see Fig. 2) has one or more binding sites for a particular neurotransmitter or second messenger, and a characteristic ion selectivity. Neurons contain four general classes of ligand-gated ion channels, distinguished on the basis of the chemical class of

neurotransmitter to which they are responsive (Table 1).

The most studied ligand-gated channel is the nicotinic acetylcholine receptor (nACr) channel (Fig. 5), which is found at the neuromuscular junction and at other locations in the nervous system; the nACr channel serves as a prototype for the less studied ligand-gated channels with regard to function, structure, and underlying biophysical mechanisms. When two acetylcholine molecules bind to the nACr, a conformational change is induced that opens an aqueous pore for an average of about a millisecond. Thereafter, the acetylcholine molecules disassociate from the receptor and are hydrolyzed by acetylcholinesterase. Each channel is probably about 9 nm in diameter, and protrudes from the membrane surfaces about 6 nm into the extracellular space and about 2 nm into the cytosol; when activated, the pore itself is about 2—3 nm in diameter. The nACr channel excludes anions, possibly because of the negatively charged amino acids at its mouth. The channel is formed from five subunits having the stoichiometry shown in Fig. 5; the a subunits bind acetylcholine with high affinity, one molecule of which must bind to each a subunit for the channel to open efficiently. Site-directed mutagenesis of the cDNAs of the a subunits has led to the identification of the binding sites for acetylcholine near two cysteine residues on the extracellular portion of the subunit. The four subunits are encoded by different but homologous genes; each subunit appears to consist of four membrane-

Table 1. The four major classes of neurotransmitters. Acetylcholine is the only member of its class; representative neurotransmitters in the other three classes are listed. The localization, gating, subunit composition, and amino-acid sequence are known to varying degrees for many of the neurotransmitters in the first three classes. Much less is known regarding the larger class of peptide neurotransmitters. In many cases, one or more peptide neurotransmitters are colocalized at synapses with a classical neurotransmitter (as indicated). Although many substances can act as neurotransmitters by activating specific receptors on the cell surface, there are fewer second-messenger pathways (Fig. 2).

Transmitter class	Neurotransmitter	Gating	Permeant ion
Acetylcholine	Acetylcholine (A)	Direct (nicotinic)	Na^+/K^+
		Indirect (muscarinic)	K^+, Ca^{2+}
Amino acids	GABA (B)	Direct	Cl^-
		Indirect	K^+,Ca^{2+}
	Glycine	Direct	Cl^-
	Glutamate	Direct	Na^+, K^+, Ca^{2+}
Monoamines	Epinephrine (C)	Indirect	K^+, Ca^{2+}
	Norepinephrine (D)	Indirect	K^+, Ca^{2+}
	Dopamine (E)	Indirect	K^+, Ca^{2+}
	Serotonin (F)	Indirect	K^+, Ca^{2+}
Peptides	ACTH		
	Angiotensin		
	ß-endorphin		
	Bombesin		
	Camosine		
	Cholecystokinin, +E		
	Endorphins		
	Dynorphin		
	Luteinizing-hormone releasing hormone		
	Enkephalins, +A, +D, +F		
	Motilin		
	Neuromedins		
	Neuropeptide Y, +D		
	Neurotensin, +C, +E		
	Oxytocin		
	Prostaglandin		
	Somatostatin, +B		
	Substance K		
	Substance P, +A, +F		
	Thyroid-hormone releasing hormone, +F		
	Vasoactive intestinal peptide, +A		
	Vasopressin		

spanning regions arranged in such a way that specific regions of each of the subunits face each other to create the membrane-spanning pore.

3. BIOPHYSICS OF NEURONS

3.1 Membrane Potential

The membrane potential of the neuron plays an important role in its bioelectric processes; instantaneous deviations from the steady-state membrane potential can be viewed as the basic physical change in the neuron underlying information transfer. The mechanism responsible for the establishment of the steady-state membrane potential in the neuron is the same as that in other cells (Fig. 6). The Na^+-K^+ pump is a transmembrane energy-consuming enzyme that moves three Na^+ out of the cell and two K^+ into the cell for each molecule of ATP that is converted to ADP. Nongated ion channels in the cell membrane permit transmembrane flow of ions, principally Na^+ and K^+ down their concentration gradients. Since the cell membrane is more permeable to K^+ than to N^+, a membrane potential is generated oriented to retard K^+ diffusion and enhance Na^+ diffusion, resulting in equal diffusion rates and thereby preserving electroneutrality. Under steady-state conditions (no net current) with no applied voltage V_m, the membrane potential is called the *resting membrane potential*

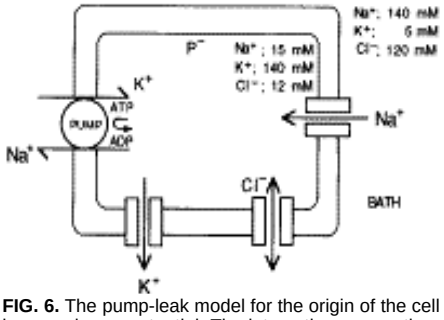

FIG. 6. The pump-leak model for the origin of the cell resting membrane potential. The interaction among the pump and leak channels results in a negative membrane potential (cell interior with respect to bath solution). P^-, negatively charged impermeant proteins. Typical intracellular and extracellular concentrations of several important ions are shown.

and is given by the Goldman—Hodgkin—Katz (GHK) equation (Hille, 1992):

$$E_m = \frac{RT}{F} \ln \frac{P_{Na}[Na]_0 + P_K[K]_0 + P_{Cl}[Cl]_i}{P_{Na}[Na]_i + P_K[K]_i + P_{Cl}[Cl]_0} \quad (1)$$

where R is the universal gas constant, T is the absolute temperature, F is Faraday's constant, the internal and external concentrations of Na^+ are represented by [Na], and $[Na]_0$, respectively, and the membrane permeability of Na is P_{Na} The K^+ and Cl^- concentrations and permeabilities are represented in a similar fashion. If the permeability of any two of the ions is zero, the GHK equation reduces to the Nernst equation for the third ion, and the resulting potential is the *reversal potential* for that ion.

The GHK equation describes the resting membrane potential of the neuron in terms of the various concentrations and permeabilities. An equivalent-circuit model of the neuronal membrane permits consideration of other conditions, such as the response of a cell to an applied voltage or to injection of current from a microelectrode or from a natural cellular event (Kuffler and Nicholls, 1976). In this case, the membrane potential departs from that predicted by the GHK equation, and the equivalent circuit can be used to analyze the resulting time-dependent and steady-state current changes that occur: Assume, for example, that a step voltage is applied to the membrane. Application of Ohm's law to the membrane equivalent circuit (Fig. 7) yields $I_m = g_m(V_m - E_m)$, where V_m is the total potential across the membrane, g_m is the membrane conductance, and I_m, is the current flowing across the membrane, and where it is assumed that enough time has passed following a voltage step for the system to have reached steady state (zero capacitive current); $V_m - E_m$ is known as the *driving force*. The permeability mechanisms can be investigated by studying the relationship between membrane current and voltage. If $g = g(V)$, the current-voltage relationship of a membrane will be nonlinear; if multiple conductance pathways exist in the membrane, complex relationships may be observed. Under conditions of zero net membrane current, the membrane potential is that derived from the GHK equation.

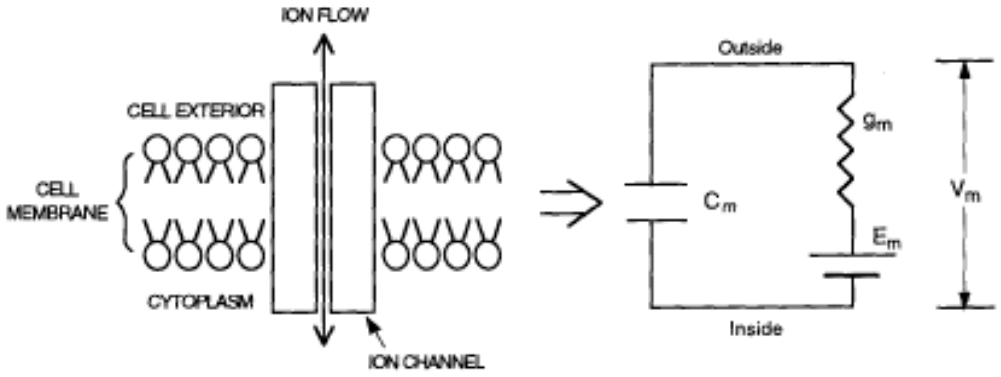

FIG. 7. A membrane equivalent circuit. Each conducting pathway contributes to the membrane potential: a lumped presentation of the permeability pathways is depicted.

3.2 Chemical-to-Electrical Transduction

Signal transduction at the postsynaptic membrane is mediated by transmembrane ion flow occurring in response to the interaction of a neurotransmitter (NT) secreted by the presynaptic cell and a receptor in the membrane of the postsynaptic cell. Neurotransmitter binding to the receptor results in either formation of a conductance pathway in the receptor, or initiation of an intracellular second messenger, which, in turn, leads to transmembrane ion flow (Fig. 2). The ion flow produces a transient change in the resting membrane potential at its point of entry into the neuron.

The nACr channel is a representative neurotransmitter-gated ion channel. It shows little selectivity among cations, and, consequently, the relative contributions to the channel current are determined by the cationic driving forces. Here K^+ is near Nernst equilibrium for a typical neuron, but the Na^+ concentration gradient and the membrane voltage both act to drive Na^+ into the cell; thus, nACr current is carried mostly by Na^+.

In a membrane region containing both nACr channels and nongated Na^+, K^+, and CL^- channels, the membrane potential at no net current is

$$V_m = \frac{g_K}{g_T} E_K + \frac{g_{Na}}{g_T} E_{Na} + \frac{g_{Cl}}{g_T} E_{Cl} + \frac{g_{nACr}}{g_T} E_{nACr}, \quad (2)$$

where g_T is the sum of the individual channel conductances. Under resting condition, the membrane potential is about —70 mV, and $g_{\mathrm{nACr}} = 0$. Following activation of the acetylcholine receptor, g_T increases significantly because of the large contribution of g_{nACr}; since E_{nACr} (the reversal potential of the nACr channel) is near 0 (Kandel *et al.*, 1991), the net result of the acetylcholine-induced conductance is the production of an electrical depolarization in the membrane containing the nACr channels. The total transmembrane current at the synapse is the sum of the ion flow through several hundred thousand such transmitter-gated channels, each of which has an identical conductance, but an open time that is governed by stochastic processes that render progressively longer open times correspondingly less likely.

The biophysical principles governing synapses at the neuromuscular junction also apply to synapses in the central nervous system (CNS). However, signal transduction at central synapses is complicated by several factors.

1. A typical CNS neuron receives many simultaneous excitatory and inhibitory inputs (synapses involving vertebrate skeletal muscle are always excitatory).
2. Many different neurotransmitters may be involved in signal transduction by one neuron, and a given neurotransmitter may have more than one kind of receptor at the cell membrane.
3. The actual role of the neuron in the signaling pathway is determined by the sum of its excitatory and inhibitory inputs, and not merely by the occurrence of the inputs

(at the neuromuscular junction, each synaptic potential produces an action potential).

4. The indirect mechanism (Fig. 2) for the effect of neurotransmitters on membrane potential can result in (a) channels that open or close at the resting potential; (b) transient changes in membrane voltage that last much longer than those caused by directly gated channels; or (c) second (and higher-order) messengers that cause effects in addition to those on channel conductance (alterations in receptors for other neurotransmitters, and in gene expression, for example).

3.3 Signal Summation

The transient voltage changes that occur at synapses as a consequence of the action of neurotransmitters have no individual significance with regard to the information actually transferred by the postsynaptic neuron: Physiological significance resides in the relative versions of the transients that propagate to the axon hillock. The essential features of the propagation process in the dendritic portion of the neuron can be modeled in terms of the response of a lossy insulated wire embedded in a conducting medium (Fig. 8) (Deutsch and Micheli-Tzanakou 1987; Shepherd, 1990). The dendrite is conceptually divided into a series of isopotential segments represented by the membrane capacitance per unit length, C, in

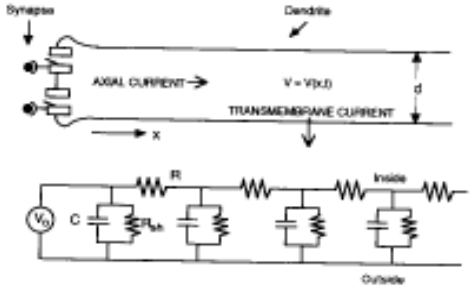

FIG. 8. Electrotonic spread of voltage in a dendrite and the corresponding equivalent circuit. At $t = 0$, a transient change V_o in the membrane potential is induced by ion flow through neurotransmitter-gated channels in the den-drite. V_o can be modeled as a voltage spike having a dura-tion that is negligible with respect to typical propagation times. Thereafter, dendritic membrane potential is given by $V(x,t)$.

parallel with the transmembrane resistance R_{sh} (expressed in units of ohms x distance), with each adjacent pair of segments connected by a resistor R determined by the axonal resistance, where $R = 4\rho/\pi d^2$ (units of ohms/distance) and ρ is the resistivity of the cytoplasm. A voltage transient occurs at a synapse on the distal portion of the dendrite as a result of an ion flux, and a portion of the resulting current charges the capacitance of the membrane adjacent to the synapse, and thereby increases or decreases the membrane potential, depending on the charge of the permeant ion. The remaining current splits and either charges the capacitance of the second segment, passes through the membrane, and completes the circuit back to its source, or continues on to the next segment. This process continues until all the current has leaked out and returned to its source via the extracellular fluid, which is assumed to have negligible resistance.

From Ohm's and Kirchoff's laws, the equation governing the spread of the potential $V(x, t)$, measured from the resting potential, is

$$\lambda^2 \frac{\partial^2 V}{\partial x^2} = \tau_m \frac{\partial V}{\partial t} + V, \tag{3}$$

where $\lambda = \sqrt{R_{sh} / R}$ is the length constant, and $\tau_m = R_{sh}C$ is the time constant. In terms of C_m and R_m, which are the capacitance and resistance per unit area, respectively, we have $\lambda = \sqrt{R_m d / 4\rho}$ and $\tau_m = \sqrt{R_m C_m}$; C_m is usually assumed to be about 1 μF/cm^2. Both λ and τ_m depend on the type of neuron; for the hippocampal pyramidal cell, typical values are τ_m = 15—70 msec, and λ = (0.5—l.5)l_0, where l_0 is the length from the tip of the dendrite to the cell body.

The general solution for $V(x,t)$ has been given (Rall, 1960), but the behavior of the potential can be inferred from a consideration of the simplest special cases. The steady-state solution for an infinitely long dendrite (this model is applicable to slow synaptic potentials and to background depolarizations, as in cells in the retina) is $V = V_o e^{-x/\lambda}$. Since ρ ≈ 100 Ω cm in all neurons, the spatial characteristics of the potential are determined by the membrane resistance and the dendrite diameter.

The spread of the potential in finite dendritic systems

depends on the nature and extent of the branching that occurs. If the diameters of the dendrites at a branching point are such that (Rall, 1960)

$$d_0^{3/2} = \sum_i d_i^{3/2}, \qquad (4)$$

where d_0 is the diameter of the parent dendrite and d_i are the diameters of each of the daughter branches, then the branches are electrically equivalent to the stem, and the potential spreads through the entire system as it would in an infinite dendrite. This *equivalent cylinder* model, developed by Rail, is useful in modeling the passive spread of potential in dendritic systems *(electrotonus);* in conjunction with measurements of transient responses, it can be used to estimate λ of the dendritic tree.

The membrane time constant is an important determinant of the time course of the postsynaptic potential, but a precise description depends upon many factors, including the structural model chosen for consideration. The essential feature of all such models is the prediction that the postsynaptic potential diminishes in peak amplitude and increases in pulse width as it propagates from its point of origin. Thus, synapses near the cell body facilitate relatively large and rapid responses, whereas distant inputs lead to weaker and slower changes in the membrane potential at the cell body. Neurons have evolved mechanisms by which they can receive synapses at their distal dendrites but, nevertheless, transmit large and rapid post-synaptic potentials to the region of the axon hillock (Fig. 3), which is the site of generation of the action potential. These mechanisms include

1. the presence of a high specific membrane resistance;
2. production of a particularly large post-synaptic potential; and
3. the presence, in dendrites, of active membrane processes such as voltage-gated ion channels similar to those that participate in production of the action potential.

When two neighboring synapses on a cell are activated simultaneously, the conductance changes interact nonlinearly, thereby precluding a general analysis of the response as a superposition of the effects associated with the individual post-synaptic potentials; postsynaptic potentials produced at widely separated synapses may behave linearly because spatial separation lessens mutual interaction. Thus, the net effect of simultaneously activated synapses is greater if the synapses are located in different regions of the dendritic tree.

Dendritic spines are narrow projections from the dendrites; they are found on many types of neurons (see Fig. 9, for example), and can be the locus of synaptic inputs. The structural and electrophysiological characteristics of spines are not well understood; they may exhibit properties and functions not occurring elsewhere in the dendritic system. For example, the dendritic spine might provide a high-resistance path for a postsynaptic potential into the dendritic tree; this would have the effect of electrically isolating its postsynaptic potential from those induced at synapses on the dendrite itself. Consequently, the dendrite-synapse postsynaptic potential and the spine-synapse postsynaptic potential would add in a more nearly linear fashion than if both potentials occurred side by side directly on the dendrite.

Although the basic response of each portion of the dendritic membrane to a transient voltage change consists of the reasonably well understood phenomena of a propagating and diminishing voltage transient, the overall response of the neuron is difficult to characterize because of its highly complex morphology. A typical spinal neuron from a monkey is shown in Fig. 9; such a cell may contain 20 000—30 000 synapses, any combination of which may simultaneously transmit either inhibitory or excitatory postsynaptic potentials. Since the input and output of each region of the dendritic tree are processed in parallel, as in a network, the computation carried out in the dendritic tree is determined by dendritic morphology and the specific spatial and temporal relations of the synaptic sites to each other and to the axon hillock. Specialized computer programs have been developed to accommodate the many degrees of freedom needed to apply the cable equation to realistic models of actual neurons (McKenna *et al.*, 1992).

3.4 Action Potential

The biophysical process underlying development of the action potential was elucidated by Hodgkin and Huxley in a

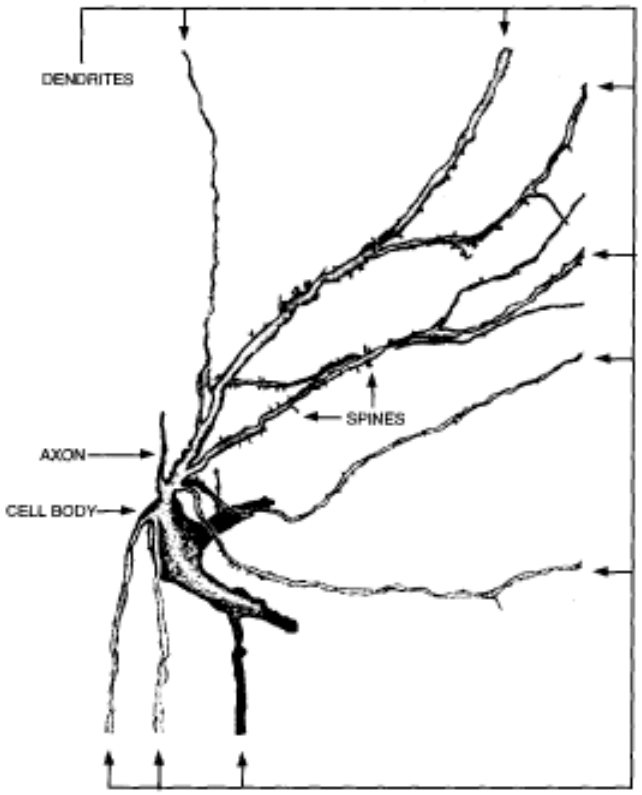

FIG. 9. Drawing of a sensory neuron from the dorsal horn of the spinal cord of a Macaque monkey. Only a portion of the dendritic tree and the axon is shown. The cell morphology was determined from serial reconstructions. using the Golgi technique; the shading indicates relative depth. A single such neuron can be affected by simultaneous excitatory and inhibitory stimuli from more than 20 000 axons. (Courtesy of John A. Beal.)

classic series of studies on the giant axon of the squid, employing the *voltage-clamp* technique (Fig. 10). Since voltage dependence of membrane conductance proved to be the basic explanation for the action potential, the voltage clamp was particularly useful because it permitted direct control over the fundamental variable.

The current across the membrane was described in terms of a capacitive and three ionic components (Noble, 1966) (Fig. 11):

$$I_m = C\frac{dV_m}{dt} + g_{Na}\left(V_m - E_{Na}\right) + g_K\left(V_m - E_K\right) + g_L\left(V_M - E_L\right), \tag{5}$$

where g_L is a leakage conductance of undetermined ionic basis; the electrical excitability of the membrane is contained in the time-and voltage-dependent conductances, g_{K} and g_{Na}. By varying ion concentrations in the bath solution and in the axon and the magnitude of the displacement of the membrane poten

tial, and by using radioactive K^+, Hodgkin and Huxley showed that the early inward current in the voltage-clamped squid axon was due to Na^+ entering the axon, and the later-appearing outward current was due to K^+ leaving the axon (Fig. 12).

To provide a basis for reconstructing the action potential, Hodgkin and Huxley measured the variations of g_{Na} and g_{K} with time for various values of the membrane potential, using an ion-substitution method (pharmacological methods are now used for dissecting the Na^+ and K^+ currents). The mathematical description of the observations was based on the maximum possible conductance for both Na^+ and K^+. The K^+ conductance was expressed as a constant, $\bar{g}_K$ (the maximum conductance value), multiplied by a coefficient whose magnitude varied between 0 and 1, and which contained all of the voltage- and time-dependent characteristics of the K^+ conductance. The data showed that depolarization of the membrane

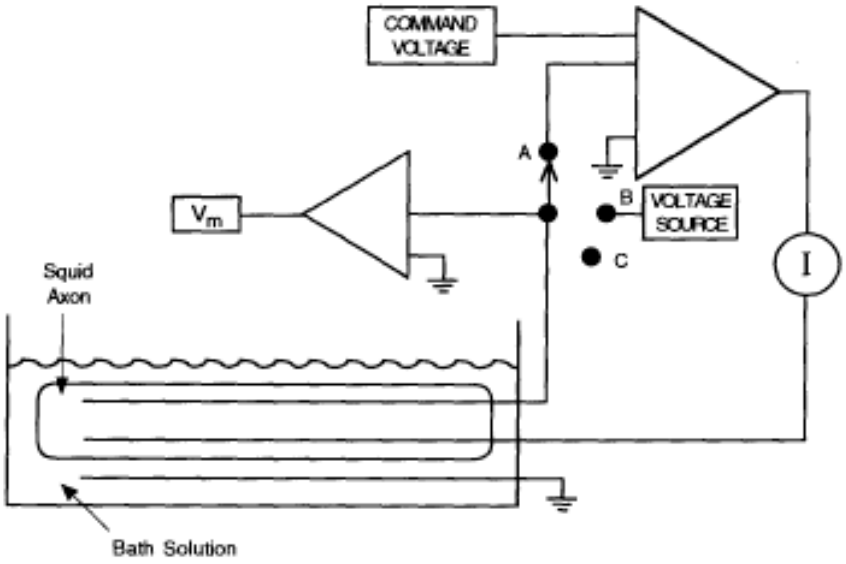

FIG. 10. The voltage-clamp technique and measurement of the action potential in the squid axon. For voltage-clamp measurements (switch position A), a voltage-recording electrode and a current-delivering electrode are placed intracellularly. The output of the feedback amplifier is determined by the difference between the command voltage and the membrane potential. For nonzero differences, the amplifier drives a current through the membrane in such a direction as to reduce the difference to zero. When a voltage step is applied, the membrane capacitance becomes charged in a time on the order of microseconds; thereafter, the capacitive current does not contribute to the membrane current. The circuit permits an abrupt displacement of the membrane potential that can be maintained indefinitely at the new value as the membrane current is measured. The design of the electrodes prevents the flow of longitudinal currents. In switch position B, the voltage clamp is removed, and a brief (100 μs) voltage pulse is applied between the voltage-measuring and bath-solution electrodes; if the switch is then moved to the open-circuit position C, the axon develops an action potential or returns to baseline, depending on whether the depolarizing pulse reached the threshold level.

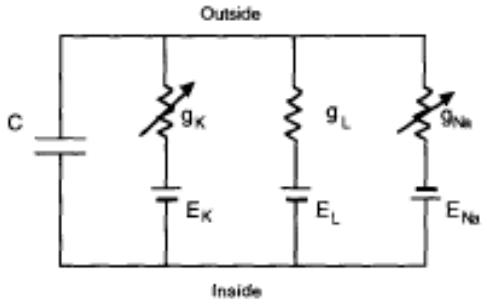

FIG. 11. Pathways for current flow in the Hodgkin—Huxley model of the axon. In voltage-clamp studies, capacitive current flows only during the time the potential is changing; thereafter, the membrane current consists solely of an ion flow through the three ion-conductive branches represented by Na^+, K^+, and leak conductances. The batteries depict the Nernst equilibrium potentials; time- and voltage-dependent conductances are employed to explain the response of the membrane to changes in voltage.

changed K^+ conductance such that both the amount and rate of change increased with depolarization. One interpretation was that the K^+ conductance was determined by a first-order process whose rate constants depended on the membrane potential. If α and ß are two possible conformational states of a molecule, and *n* is the fraction in the active state (α), then

$$\frac{dn(V,t)}{dt} = \alpha_n(V)\left[1 - n(V,t)\right] - \beta_n(V)n(V,t), \tag{6}$$

where α_n is the rate constant for conversion from ß to α and $ß_n$ is the rate constant for the reverse process. Hodgkin and Huxley modeled the observed data as $g_K \propto n^x$ and found that the best fit occurred for $x = 4$.

Consequently, they chose $g_K = n^4 \bar{g}_K$.

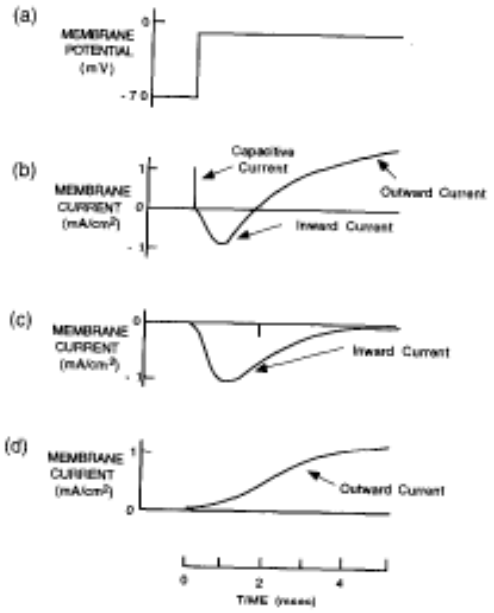

FIG. 12. Current flow across the squid axon under voltage-clamp conditions. During a depolarization of the membrane potential (a), an early inward and later outward current is observed following the capacitive transient. The ionic current flow (b) can be resolved into (c) an inward Na^+ current and (d) an outward K^+ current. (Adapted from Kuffler and Nicholls, 1976.)

Except for the rate of increase, the initial behavior of the Na^+ conductance following depolarization was similar to that of K^+. Consequently, g_{Na}. could also be modeled in terms of a first-order reaction:

$$\frac{dm(V,t)}{dt} = \alpha_m(V)\left[1 - m(V,t)\right] - \beta_m(V)m(V,t). \tag{7}$$

In contrast with the behavior of g_K, the increased value of g_{Na} was not maintained, even though the membrane was clamped at the depolarized value (Fig. 12). This effect was accounted for by assuming a voltage-dependent reaction in the opposite direction to the *m* and *n* processes,

$$\frac{dh(V,t)}{dt} = \alpha_h(V)\left[1 - h(V,t)\right] - \beta_h(V)h(V,t), \tag{8}$$

where α_h decreased and ß$_h$ increased on depolarization of the membrane, in contrast to the changes in the rate constants of the other two processes. The Na^+ conductance was assumed to be determined by both *m* and *h:* $g_{Na} = \bar{g}_{Na} m^3 h$. The processes that determine the K^+ and Na^+ conductances in the chosen model are depicted schematically in Fig. 13.

In this manner, the voltage-clamp data were converted into analytical expressions for g_{Na} and g_K as functions of membrane potential and time. Then the change in membrane potential that would occur with time following a brief superthreshold voltage pulse was calculated, using a piecewise-linear approximation at successive O.O1-msec intervals (Fig. 14); the calculation was shown to account for the time course of the action potential, thereby establishing that voltage-dependent permeabilities and ionic gradients were sufficient to explain electrical excitability.

The voltage-clamp data consisted of curves that described the voltage- and time-dependent membrane conductances of Na^+ and K^+. The measurements were made under highly controlled conditions, in the absence of longitudinal currents (hence, no propagation), with the membrane potential held at one or another specific value, at various Na^+ and K^+ concentrations. In a remarkable effort, employing some *ad hoc* but plausible assumptions, Hodgkin and Huxley synthesized the data obtained under the disparate experimental conditions, and succeeded in reconstructing the action potential. The Hodgkin—Huxley model, together with the cable equation, is sufficient to explain propagation of the action potential, the existence of the voltage threshold, and the velocity of propagation. Despite some limitations, the model is the generally accepted explanation of the origin and characteristics of the action potential in mammalian peripheral nerve, and in the central neurons of invertebrates and vertebrates; the mathematical variables employed in the model appear to have molecular structural counterparts. The complex pattern of electrical activity found in most neurons arises, ultimately, from the Hodgkin—Huxley mechanism, neuronal structural complexity, and ion-channel diversity.

3.5 Electrical-to-Chemical Transduction

When the action potential arrives at the axon terminal, the depolarization opens voltage-gated Ca^{2+} channels, which are concentrated in the terminal region. Neither the influx of Na^+

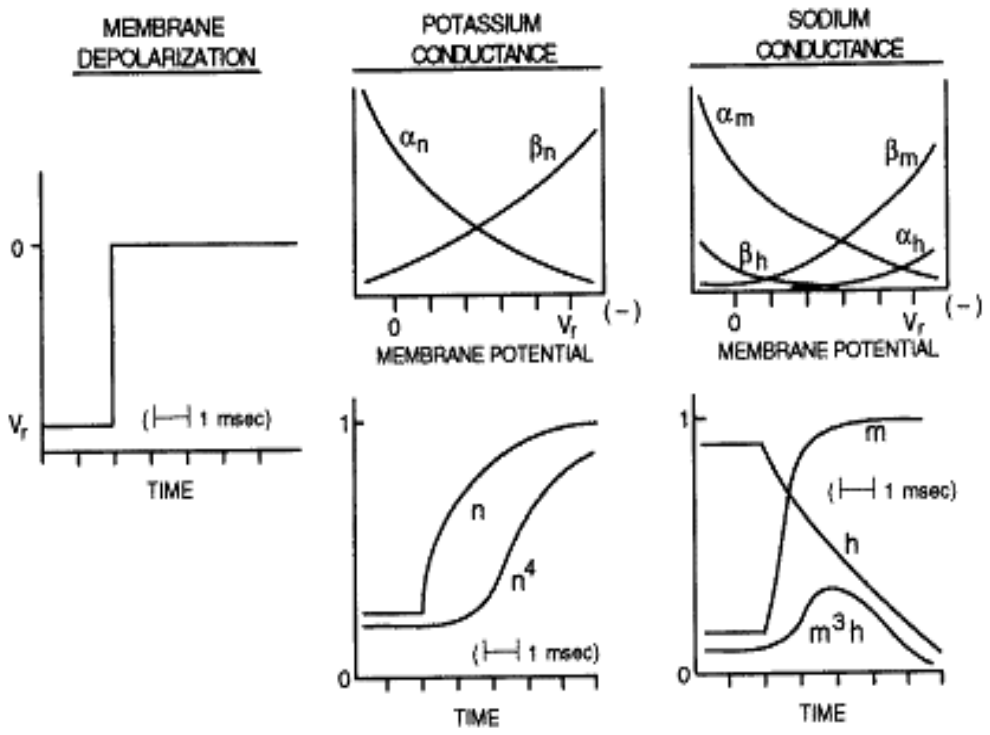

FIG. 13. Schematic representation of the processes assumed by Hodgkin and Huxley to determine the K^+ and Na^+ conductances. The K^+ conductance is determined by *n*, the K^+ activation variable (the traction of activated molecules), which is itself determined by the rate constants for the forward and reverse processes; α_n is the rate constant for conversion from the inactive to the active state, and $ß_n$ is the rate constant for the reverse process; both constants were assumed to follow first-order kinetics (see CHEMICAL KINETICS). At the resting potential, $ß_n$ is greater than α_n; consequently, the fraction of molecules in the active state is small. Depolarization increases α_n and decreases $ß_n$; consequently, for any specific depolarization step, *n* rises exponentially to a larger value. Hodgkin and Huxley employed n^4 because it provided a better fit to the experimentally observed conductance changes. Modeling of the Na^+ conductance changes requires both an activation and an inactivation variable, and both processes are assumed to follow first-order kinetics. V_r, membrane resting potential. (Adapted from Noble, 1966.)

nor the efflux of K^+ is necessary for transmitter release. A higher frequency of action potentials results in a longer time during which the membrane is depolarized, thereby permitting a greater Ca^{2+} influx; thus, neurotransmitter release is a graded response.

The neurotransmitter is stored in specialized organelles called synaptic vesicles. Under resting conditions, a baseline level of neurotransmitter release occurs spontaneously. The entry of Ca^{2+} increases the amount of released neurotransmitter by increasing the probability that an individual intracellular vesicle will be released. The effect of the arrival of each action potential at the axon terminal can be viewed as a Bernoulli trial with regard to the fate of individual vesicles. If *p* is the probability that a given vesicle will be released, then the probability that *x* vesicles of a total population of *n* will be released is

$$p(x) = \frac{n!}{(n-x)!\,x!}\,p^x q^{n-x} \tag{9}$$

where q = 1 - *p*. Such a relationship has been established at the neuromuscular junction (del Castillo and Katz, 1954) and is thought to be a model applicable to the central nervous system.

When the vesicles fuse with the cell membrane, the resulting increase in membrane area results in an increased membrane capacitance; that increase, and the decrease that occurs as the excess membrane is retrieved, can be measured (Fernandez *et al.*, 1984). The mechanism by which the vesicles

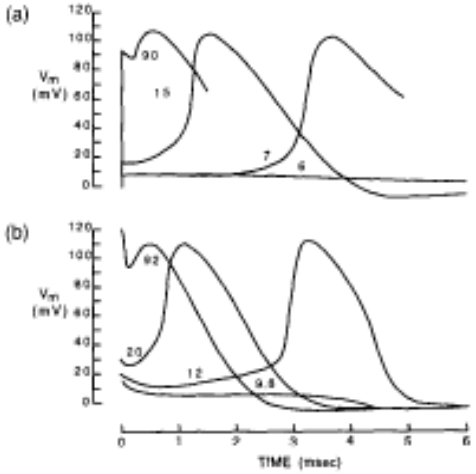

FIG. 14. Comparison of observed and calculated action potentials in squid axon. (a) Action potentials seen by Hodgkin and Huxley in the squid action preparation. The data were obtained by applying a brief depolarizing potential of the magnitude indicated on the various curves, and then measuring the subsequent time course of the membrane potential (Cf. Fig. 10). (b) The predicted time course of the action potential based on the assumed model for current flow (Fig. 11) and the analytical expressions for g_K and g_{Na}, derived from modeling the conductance data under voltage-clamp conditions. Small depolarizations of the membrane have a marginal effect on g_{Na} for large depolarizations. g_{Na} increases to the point that the inward Na^+ current overcomes the outward K^+ current, thereby permitting the regenerative Na^+ response to occur. V_m, membrane potential measured from the resting potential. (Adapted from Kuffler and Nicholls, 1976.)

fuse with the membrane is unknown, but it may involve formation of an electrical synapse between the vesicle and the cell membrane (Breckenridge and Almers, 1987).

4. NEURONAL SYSTEMS

4.1 Overview

Neurons receive inputs from receptors that detect stimuli external to the nervous system (Fig. I). Following transduction into the language of the nervous system (Fig. 3), the information is processed by groups of neurons in local neuronal networks whose activity may be synchronized with that occurring in other regions of the nervous system. Synchronization is accomplished by neurons that are hard-wired between local networks, and possibly by *volume transmission,* mediated by electrical and chemical messengers. The ultimate result of the sensory inputs and subsequent regional interactions is the behavior manifested by the organism. Not surprisingly, the extent of knowledge of the biophysical basis of the activity of neuronal systems is inversely related to the complexity of the level of the activity under consideration: There is convincing evidence that receptor function is mediated by changes in conductance of ion channels, but the physical basis of higher functions, such as behavior, memory, and consciousness, is poorly characterized.

4.2 Sensory Systems

Information enters the nervous system via *receptor* cells, which are specialized neurons or cells in intimate contact with neurons. At these sites, objectively characterizable chemical, mechanical, or energetic stimuli are transduced into electrical activity that controls the autonomic regulatory systems in the body, or that serves as the basis of conscious perception of the environment. The main types of receptor cells in mammals and the particular sense to which each corresponds are listed in Table 2. Other life forms possess sensory capabilities not shown to occur in human beings. Examples include the ability of some fish to detect weak electric fields, and the ability of some birds and bacteria to detect weak magnetic fields.

The mechanism underlying transduction by sensory receptors is the change in conductance of membrane-bound ionic channels (Fig. 15). The membrane receptor protein is indirectly coupled to a channel protein via a second-messenger system in the cases of chemical and light stimuli, but mechanical forces act directly on the channel to produce a deformation that alters conductance. In all three cases, the stimulus transduction ultimately produces a change in channel conductance that gives rise to a change in membrane potential called the *receptor potential* (also called a *generator potential).* The receptor potential is smoothly graded in proportion to the strength of the stimulus, and propagates electrotonically to the site of impulse generation. Thus, it is the receptor that determines the specificity of the sensory response, because the

Table 2. Main types of mammalian sensory modalities. Modified from Shepherd (1988).

Sense	Stimulus	Receptor location	Receptor cell
		Chemical	
Arterial oxygen	0_2 tension	Carotid body	Cells and nerve terminals
Toxins (vomiting)	Molecular	Medulla	Chemoreceptors
Glucose	Glucose	Hypothalamus	Glucoreceptors
pH (cerebrospinal fluid)	Ions	Medulla	Ventricle cells
Taste	Ions and molecules	Tongue and pharynx	Taste-bud cells
Smell	Molecules	Nose	Olfactory receptors
Pain	Various	Skin and various organs	Nerve terminals
		Mechanical	
Osmotic pressure	Osmotic pressure	Hypothalamus	Osmoreceptors
Pressure	Mechanical	Skin and deep tissue	Nerve terminals
Vascular pressure	Mechanical	Blood vessels	Nerve terminals
Muscle stretch	Mechanical	Muscle spindle	Nerve terminals
Muscte tension	Mechanical	Tendon organs	Nerve terminals
Joint position	Mechanical	Joint capsule and ligaments	Nerve terminals
Balance (linear and angular acceleration)	Mechanical	Vestibular organ	Hair cells
Hearing	Mechanical	Cochlea	Hair cells
		Energetic	
Vision	Photons	Retina	Photoreceptors
Temperature	Temperature	Skin, hypothalamus	Nerve terminals and central neurons

change in channel conductance, whether induced by the natural stimulus for the channel or by an agonist, results in the same electrical consequences.

4.3 Neural Networks

Receptor cells have no functional significance within nervous systems unless they are part of a network. Network complexity varies across the phylogenetic scale from a simple two-cell reflex response to the highly complex neural networks that mediate human consciousness and behavior. Various mathematical models of neuronal networks have been proposed to explain the learning abilities of the human brain (see NEURAL NETWORKS). In most such networks, the neuron is modeled as a summing node that weights each input and transforms the sum in a predetermined manner to yield an output activity level (Fig. 16). A model network typically consists of three or more layers of model neurons interconnected in such a way that the outputs of the model neurons in one layer are the inputs to the next layer. By following a systematic iterative procedure (the *back-propagation algorithm),* the weights and transfer function of each model neuron can be adjusted so that the input pattern is reproduced at the network output. Thus, the artificial network can mimic the learning behavior of biological neurons.

Artificial neural networks have considerable practical utility, but it is doubtful that they simulate the actual learning procedures used by the brain. Artificial networks operate via backward flow of information in the network, in distinction to the forward flow of information in biological neurons. The time required for an artificial network to learn increases rapidly as the size of the network increases, and there seems to be no biological parallel to this property of artificial neural networks. Finally, artificial networks require specification of both the input and output before learning can be accomplished. Again, this is not a property of biological networks, because no agency provides specific instructions regarding the particular pattern of receptor or action potentials that must occur before learning occurs.

Actual neuronal networks are spatially localized functional units; in addition to input and output neurons, such units

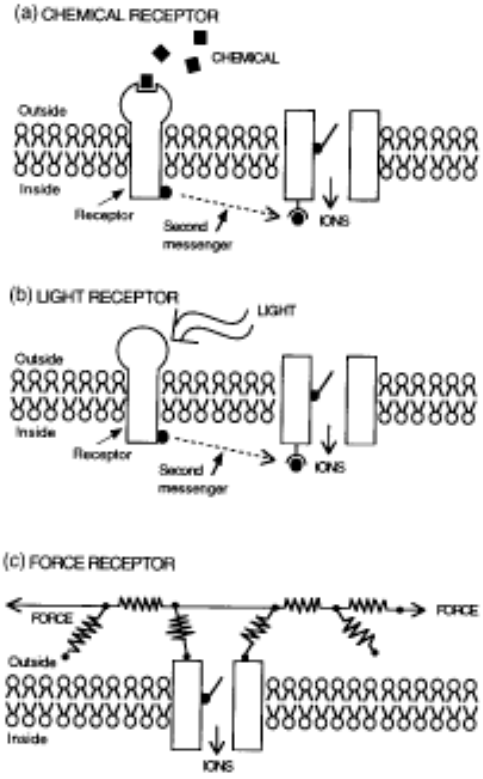

FIG. 15. Signal transduction in the three main classes of sensory receptors. (a), (b) In detection of chemicals or light, channel conductance is altered by intracellular second (or higher-order) messengers released following molecular events triggered by the interaction of the stimulus and the receptor. (c) In contrast, force transduction is directly coupled to channel conductance via a mechanically induced conformational change of the channel. (Adapted from Shepherd, 1988.)

usually contain local neurons that are involved exclusively in information processing within the network. The operations within the local networks are mediated by a combination of basic excitatory and inhibitory synaptic patterms (Fig. 17). Synaptic divergence is a morphological pattern that amplifies neuronal activity by distributing it to many cells. Neurotransmitter release does not necessarily occur at each synapse within a particular morphological unit; in the neuromuscular junction, for example, only about 10% of the synapses are activated by entry of the action potential into the presynaptic axon terminal (about 90% are *silent synapses)* (Shepherd, 1990). Synaptic convergence [Figs. 17(b) and 17(c)] is the pattern that makes possible the spatial and temporal integrative function of the dendritic tree. Presynaptic inhibition is a neuronal connectivity pattern in which a specific cell may be simultaneously presynaptic to one cell and postsynaptic to a second cell [Fig. 17(d)]; this arrangement permits a neuron to modify the activity of a cell without actually synapsing with it. The combination of the dendritic architecture and the types of possible synapses provides for a wide range of computational possibilities.

A local network that is characteristic of the cerebral cortex is depicted in Fig. 18. Various combinations of the elements depicted have been shown to result in rhythmic activity, directional sense, spatial contrast, and many other behavioral and physiological manifestations; presumably, they also underlie more complicated behaviors. Even though the network (Fig. 18) is highly simplified, it nevertheless exemplifies several important properties of biological neuronal networks:

1. A variety of specific biophysical processes that interact nonlinearly are responsible for the function of the local network;
2. performance of the network is degraded if one or more of the elements fail but catastrophic failure does not occur.

Networks of biological neurons differ fundamentally from the models developed thus far; analysis of real neuronal networks

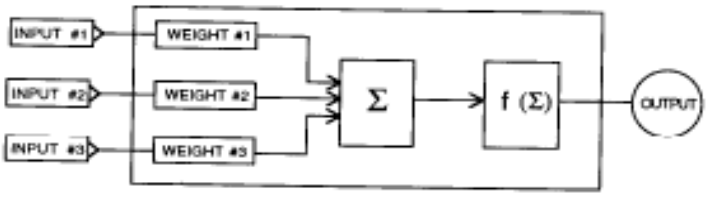

FIG. 16. Typical model neuron in an artificial neural network. The output is the transformed sum of the weighted inputs. A threshold is a common transfer function; in such a case, the weighted sum becomes the output only if it exceeds a specified level.

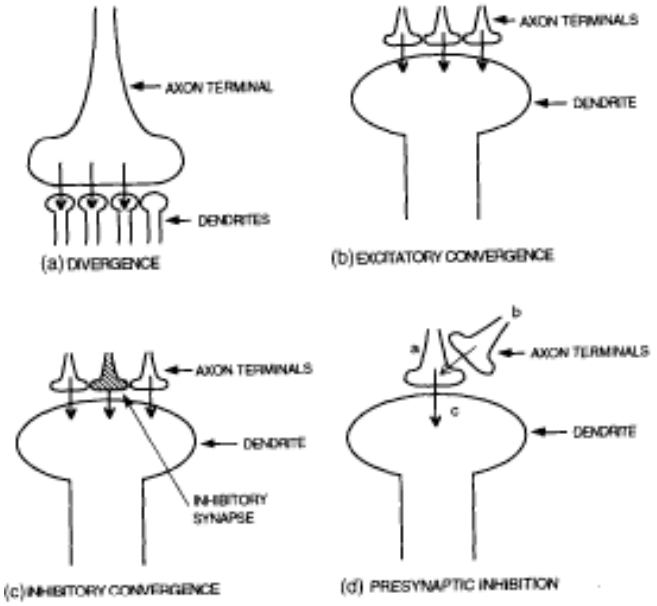

FIG. 17. Basic form of neuronal connectivity. (a) A divergent pattern produces a gain in activity unless all but one of the synapses are silent. (b), (c) The convergent patterns mediate neuronal integration. (d) In presynaptic inhibition, the effect of cell *a* on cell *c* is modified by cell *b,* which does not directly affect cell *c.* (Adapted from Shepherd, 1988.)

is a prerequisite to formation of more realistic models (Shepherd, 1990).

4.4 Volume Transmission

In the hard-wired model of the central nervous system, information transfer is viewed as being mediated by synapses between adjacent cells (Fig. 2), and neuronal complexity is ascribed to the complexity of the interconnections (Fig. 9) and their connectivity patterns (Fig. 17). Neurotransmitters (Table 1), in addition to their recognized role of diffusion across the synaptic cleft, may have other physical consequences. Some molecules may be released in a region not containing clefts, or may escape from the cleft region and diffuse to other parts of the central nervous system through the cerebrospinal fluid in the extracellular space of the brain (which occupies about 20% of the brain's volume). In this manner, a neurotransmitter could reach many different and distant targets, thereby constituting a parallel information transfer

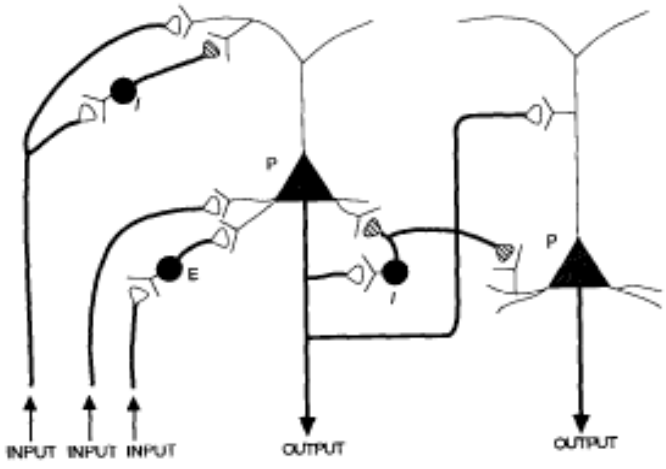

FIG. 18. Basic circuit organization of the cerebral cortex. Pyramidal neurons *P* receive input, generate outputs, and interact with one another. Local neurons may be inhibitory (0 or excitatory *(E).* Because of the cell density, dendritic architecture, and synaptic organization, there exist a vast number of computational possibilities. lAdapted from Shepherd, 1988.)

system within the central nervous system based on *volume diffusion.*

Neuroactive substances such as neurotransmitters can migrate through brain tissue from their point of secretion to distant binding sites, and theoretical models for the extracellular diffusion have been proposed to describe the concentration profiles as functions of time and position (Fuxe and Agnati, 1991). For example, neuropeptide Y is a neurotransmitter that is released in certain regions of the thalamus and hypothalamus; the receptors for neuropeptide Y are located several mm from the points of release. Since neuropeptides diffuse through the brain at about 1 mm/h, the spatial separation of secretion and binding of neuropeptide Y suggests that it may mediate slow-channel information transfer within the brain.

As discussed previously, axial flow of ions in the neuronal cytoplasm and propagation of the action potential require a corresponding ionic flow in the cerebrospinal fluid by virtue of Kirchoffs law. The potentials produced by these local currents propagate electronically, thereby altering the electrical environment of distant neurons by *volume conduction,* resulting in the *electroencephalogram* (EEG). This passive electrical consequence of the activity of individual neurons carrying out their hard-wired role in information transfer could, in principle, convey information to more distant neurons. As measured on the scalp, the EEG is a nonstationary, time-dependent voltage that can be observed between any two points; the spontaneous activity of the EEG exhibits characteristic changes during sensory processing (Fig. 19) and sleep, and in some clinical conditions, including brain tumors, epilepsy, and infection. Some of the processes thought to underlie these characteristic patterns include a predominance of electrical activity in a group of neurons functioning in a synchronized fashion, standing waves such as the normal modes of a vibrating membrane, and various metabolic alterations. For the special case of *n* point-current sources in a homogeneous conductor, the potential is

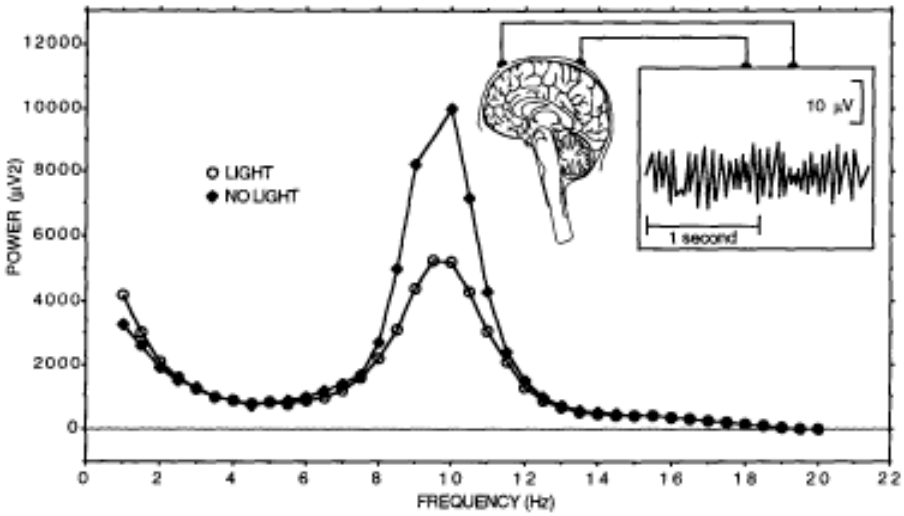

FIG. 19. Change in Fourier representation of the EEG during sensory processing. The EEG is a summation signal consisting of contributions from myriad action potentials and synaptic potentials occurring throughout the brain, particularly in the cerebellum. The signal is nonstationary in the sense that the amplitudes of its component frequencies vary with time, even under conditions of sensory deprivation. For analysis, an interval of EEG may be Fourier transformed, resulting in a representation of the EEG in terms of its component frequencies. The top curve depicts the results of Fourier transformations of 2-s epochs from an isolated subject (no sensory clues); the lower curve was obtained while the subject was presented with a weak light stimulus (in both cases, the results are averaged over 50 epochs). The data show that, as a direct consequence of detection of the light, an alteration occurred in the pattern of neuronal currents, resulting in a diminution in the amplitude of the EEG components in the frequency range 8—12 Hz.

$$V(\mathbf{r},t)=\frac{1}{4\pi\sigma}\sum_{i=1}^{n}\frac{I_{i(t)}}{R_i}, \tag{10}$$

where $I_i(t)$ is the total current flowing from the i^{th} point source into a medium of conductivity σ, and R_i is the distance of the i^{th} source from the field point **r**. Thus, in principle, ionic flow in each neuron makes a contribution to the electrical environment of the other approximately 10^{10} neurons in the human central nervous system. It seems plausible to expect that these interactions are physiologically significant, and there is evidence in the case of cells from some fishes that volume conduction has such consequences (Faber, 1991).

4.5 Learning and Memory

Memory (accessible storage of information) is a prerequisite for learning; the hippocampus is generally thought to be the most significant locus of memory in the vertebrate (Levitan and Kaczmarek, 1991). Memory involves an acquisition stage in which information is somehow encoded in neural networks in the hippocampus and stored as a consequence of a change in synaptic pattern (Shepherd, 1990). If the change is permanent, the information is essentially imprinted on the organism, resulting in *long-term* memory; if the *plasticity* of the synaptic patterns is more labile, then the result is *short-term* memory (Hall, 1992). Plasticity is hypothesized to be the basis of cognitive neuroscience (Kandel, *et al.*, 1991).

Associative learning involves the connection between two stimuli. The response of Pavlov's dog is an example of the *classical conditioning* form of associative behavior; another form involves *operant conditioning*, which is a paradigm in which an animal learns a task (such as pressing a lever), and is then studied to determine details regarding the nature of the learned behavior. The two forms of *nonassociative learning* are habituation and sensitization. *Habituation* is the decrease in the magnitude and duration of a response while the intensity of the stimulus remains constant over time. *Sensitization* involves the occurrence of an enhanced response to a stimulus. Historically, it was argued that associative learning was a systemic response distinct from specific physiological and biophysical processes occurring at the cellular and molecular levels and that, consequently, there was no necessary conceptual link between behavior and the underlying physical processes (Skinner, 1966). Subsequently, associative learning came to be perceived as a specific cellular process, but a diffuse property of the brain, not present in any specific cell or group of cells (Lashley, 1950). The modern view is that associative learning is a result of the activity of groups of cells assembled in neuronal networks, and that it occurs because of the formation of a time-dependent pattern of synaptic connections within particular circuits *(synaptic plasticity)* (Shepherd, 1988). The general hypothesis is that strengthening of synaptic connections, by either increased channel density or chemical modification of channel proteins, is the physical basis underlying learning and memory (Shepherd, 1988; Shepherd, 1990).

Mechanisms important in mediating some forms of nonassociative learning in some invertebrates have been identified. From studies of the gill-withdrawal reflex in *Aplysia*, for example, it appears that alterations in Ca^{2+} channel currents mediate both habituation and sensitization via an effect on neurotransmitter release. Because of the complexity of the mammalian nervous system, experimental efforts have concentrated largely on animals with relatively simple nervous systems and on *in vitro* preparations. In such systems, it is sometimes possible to locate specific neuronal pathways involved in particular behaviors, and to identify specific changes in synaptic connections or membrane conductances that are associated with learning or memory. But large gaps in knowledge remain, particularly with regard to criteria governing permissible extrapolation of results from lower to higher organisms. Presently, it remains unclear how the identified mechanisms could, even in principle, be synthesized into a deterministic explanation of behavior of the higher life forms.

GLOSSARY

Action Potential: A propagating electrical signal that may be initiated in a neuron when the membrane potential becomes depolarized beyond a threshold value.

Associative Learning: Learning involving the perception of a connection by the subject between two stimuli.

Back-Propagation Algorithm: An iterative mathematical procedure for training an artificial neural network.

Cell-Attached Configuration: A patch-clamp technique in which the micropipette is sealed onto an intact cell (also called on-cell configuration).

Classical Conditioning: A form of associative learning in which the subject is not required to act on its environment.

Delayed Rectifier: An axonal K^+ channel that opens with depolarization and is largely responsible for repolarizing the axon membrane following an action potential.

Dendritic Spines: Narrow projections from dendrites of many types of neurons; they can be the locus of synaptic inputs.

Driving Force: The total membrane potential minus the resting membrane potential.

Electroencephalogram (EEG): A nonstationary electrical signal consisting of the sum of the action potentials and synaptic potentials occurring throughout the brain.

Electrotonus: The passive spread of electrical potential in the neuron.

Equivalent Cylinder Model: A model of the dendritic tree, developed by Rail, used to model the passive spread of potential.

Gap Junctions: Electrical synapses.

Gating Current: The movement of electrical charge through a transmembrane protein that subserves the protein conformational change in voltage-gated channels that occurs during formation of a transmembrane ion pore.

Generator Potential: A change in membrane potential in a receptor cell that determines the specificity of a sensory response. Also known as a receptor potential.

Habituation: A form of nonassociative learning in which a constant stimulus produces a decrease in response over time.

Inside-Out Patch: A patch-clamp configuration in which, upon withdrawal of the micropipette, the cytoplasmic surface of the membrane comes to face the bath solution.

Ion Channel: The transmembrane protein that is the membrane-level effector in the nervous system.

Ligand-Gated Channels: Ion channels whose conductance depends directly or indirectly on the binding of a neurotransmitter to the channel.

Long-Term Memory: Information storage in the nervous system that causes a permanent change in synaptic patterns.

Memory: Accessible storage of information in the brain.

Myelin: An insulating material covering some axons.

Neuron: The electrically active cell of the nervous system.

Neurotransmitter: A chemical agent synthesized and secreted by a presynaptic cell which effects a communication with another cell.

Nodes of Ranvier: Periodic gaps in the myelin sheath of axons.

Nonassociative Learning: Learning not involving the perception of a connection by the subject between two stimuli; the principal types are habituation and sensitization.

On-Cell Configuration: A patch-clamp technique in which the micropipette is sealed onto an intact cell (also called cell-attached configuration).

Operant Conditioning: A form of learning behavior in which the subject is required to act on its environment.

Outside-Out Patch: A patch-clamp configuration in which, upon withdrawal of the micropipette, the cytoplasmic surface of the membrane comes to face the micropipette solution.

Patch-Clamping: An experimental technique for directly observing ion channels.

Plasticity: The tendency of synapses and neural circuits to change as a result of activity.

Receptor: In the case of chemical signals, the sensor portion of ion channels.

Receptor Cell: A cell that directly transduces a chemical, mechanical, or energetic stimulus to produce the receptor potential.

Receptor Potential: A change in membrane potential in a receptor cell that determines the specificity of a sensory response. Also known as a generator potential.

Resting Membrane Potential: The membrane potential under steady-state conditions; i.e., no net current, and no applied voltage.

Reversal Potential: For a channel with one permeant ion, the equilibrium potential determined from the Nernst equation.

Selectivity: The permeant ion species of a channel pore.

Sensitization: A form of nonassociative learning in which a response to a stimulus is enhanced over time.

Short-Term Memory: Information storage resulting in a temporary change in synaptic patterns.

Silent Synapse: Synapses that are not activated by entry of the action potential into the presynaptic axon terminal.

Synapse: A specialized junction between neighboring neurons.

Synaptic Plasticity: A time-dependent pattern of synaptic connections within particular circuits; used to explain learning.

Voltage-Clamp Technique: A technique for studying transmembrane ion kinetics in which the membrane potential is held at a predetermined value.

Voltage-Gated Channels: Ion channels whose conductance depends on the membrane potential.

Volume Conduction: A form of volume transmission consisting of the electrotonic propagation of postsynaptic and action potentials, resulting in an alteration of the electrical environment of distant neurons.

Volume Diffusion: A form of volume transmission in which neurotransmitters diffuse through the cerebrospinal fluid, thereby reaching distant receptors.

Volume Transmission: Information transfer within the nervous system by means other than a synapse.

Whole-Cell Configuration: A patch-clamp technique in which the cell membrane patch may be ruptured with the micropipette, thereby establishing a connection between the micropipette and the cell interior.

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