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Neural Science: A Century of Progress and the Mysteries that Remain

Review

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Part I. Introduction

The goal of neural science is to understand the biological mechanisms that account for mental activity. Neural science seeks to understand how the neural circuits that are assembled during development permit individuals to perceive the world around them, how they recall that perception from memory, and, once recalled, how they can act on the memory of that perception. Neural science also seeks to understand the biological underpinnings of our emotional life, how emotions color our thinking and how the regulation of emotion, thought, and action goes awry in diseases such as depression, mania, schizophrenia, and Alzheimer's disease. These are enormously complex problems, more complex than any we have confronted previously in other areas of biology.

Historically, neural scientists have taken one of two approaches to these complex problems: reductionist or holistic. Reductionist, or bottom–up, approaches attempt to analyze the nervous system in terms of its elementary components, by examining one molecule, one cell, or one circuit at a time. These approaches have converged on the signaling properties of nerve cells and used the nerve cell as a vantage point for examining how neurons communicate with one another, and for determining how their patterns of interconnections are assembled during development and how they are modified by experience. Holistic, or top–down approaches, focus on mental functions in alert behaving human beings and in intact experimentally accessible animals and attempt to relate these behaviors to the higher-order features of large systems of neurons. Both approaches have limitations but both have had important successes.

The holistic approach had its first success in the middle of the nineteenth century with the analysis of the behavioral consequences following selective lesions of the brain. Using this approach, clinical neurologists, led by the pioneering efforts of Paul Pierre Broca, discovered that different regions of the cerebral cortex of the human brain are not functionally equivalent (Schiller, 1992; Ryalls and Lecours, 1996). Lesions to different brain regions produce defects in distinctively different aspects of cognitive function. Some lesions interfere with comprehension of language, other with the expression of language; still other lesions interfere with the perception of visual motion or of shape, with the storage of long-term memories, or with voluntary action. In the largest sense, these studies revealed that all mental processes, no matter how complex, derive from the brain and that the key to understanding any given mental process resides in understanding how coordinated signaling in interconnected brain regions gives rise to behavior. Thus, one consequence of this top–down analysis has been initial demystification of aspects of mental function: of language perception, action, learning, and memory (Kandel et al., 2000).

A second consequence of the top–down approach came at the beginning of the twentieth century with the work of the Gestalt psychologists, the forerunners of cognitive psychologists. They made us realize that percepts, such as those which arise from viewing a visual scene, cannot simply be dissected into a set of independent sensory elements such as size, color, brightness, movement, and shape. Rather, the Gestaltists found that the whole of perception is more than the sum of its parts examined in *isolation*. How one perceives an aspect of an image, its shape or color, for example, is in part determined by the context in which that image is perceived. Thus, the Gestaltists made us appreciate that to understand perception we needed not only to understand the physical properties of the elements that are perceived, but more importantly, to understand how the brain reconstructs the external world in order to create a coherent and consistent internal representation of that world.

With the advent of brain imaging, the holistic methods available to the nineteenth century clinical neurologist, based mostly on the detailed study of neurological patients with defined brain lesions, were enhanced dramatically by the ability to examine cognitive functions in intact behaving normal human subjects (Posner and Raichle, 1994). By combining modern cognitive psychology with high-resolution brain imaging, we are now entering an era when it may be possible to address directly the higher-order functions of the brain in normal subjects and to study in detail the nature of internal representations.

The success of the reductionist approach became fully evident only in the twentieth century with the analysis of the signaling systems of the brain. Through this approach, we have learned the molecular mechanisms through which individual nerve cells generate their characteristic long-range signals as all-or-none action potentials and how nerve cells communicate through specific connections by means of synaptic transmission. From these cellular studies, we have learned of the remarkable conservation of both the long-range and the synaptic signaling properties of neurons in various parts of the vertebrate brain, indeed in the nervous systems of all animals. What distinguishes one brain region from another and the brain of one species from the next, is not so much the signaling molecules of their constituent nerve cells, but the number of nerve cells and the way

they are interconnected. We have also learned from studies of single cells how sensory stimuli are sorted out and transformed at various relays and how these relays contribute to perception. Much as predicted by the Gestalt psychologists, these cellular studies have shown us that the brain does not simply replicate the reality of the outside world, but begins at the very first stages of sensory transduction to abstract and restructure external reality.

In this review we outline the accomplishments and limitations of these two approaches in attempts to delineate the problems that still confront neural science. We first consider the major scientific insights that have helped delineate signaling in nerve cells and that have placed that signaling in the broader context of modern cell and molecular biology. We then go on to consider how nerve cells acquire their identity, how they send axons to specific targets, and how they form precise patterns of connectivity. We also examine the extension of reductionist approaches to the visual system in an attempt to understand how the neural circuitry of visual processing can account for elementary aspects of visual perception. Finally, we turn from reductionist to holistic approaches to mental function. In the process, we confront some of the enormous problems in the biology of mental functioning that remain elusive, problems in the biology of mental functioning that have remained completely mysterious. How does signaling activity in different regions of the visual system permit us to perceive discrete objects in the visual world? How do we recognize a face? How do we become aware of that perception? How do we reconstruct that face at will, in our imagination, at a later time and in the absence of ongoing visual input? What are the biological underpinnings of our acts of will?

As the discussions below attempt to make clear, the issue is no longer whether further progress can be made in understanding cognition in the twenty-first century. We clearly will be able to do so. Rather, the issue is whether we can succeed in developing new strategies for combining reductionist and holistic approaches in order to provide a meaningful bridge between molecular mechanism and mental processes: a true molecular biology of cognition. If this approach is successful in the twenty-first century, we may have a new, unified, and intellectually satisfying view of mental processes.

Part II. The Signaling Capabilities of Neurons

The Neuron Doctrine

Modern neural science, as we now know it, began at the turn of the century when Santiago Ramón y Cajal provided the critical evidence for the *neuron doctrine*, the idea that neurons serve as the functional signaling units of the nervous system and that neurons connect to one another in precise ways (Ramón y Cajal, 1894, 1906, 1911). Ramón y Cajal's neuron doctrine represented a major shift in emphasis to a cellular view of the brain. Most nineteenth century anatomists—Joseph von Gerlach, Otto Deiters, and Camillo Golgi, among them—were perplexed by the complex shape of neurons and by the seemingly endless extensions and interdigitations of their axons and dendrites (Shepherd, 1991). As a result, these anatomists believed that the elements of the nervous system *did not* conform to the *cell theory* of Schleiden and Schwann, the theory that the cell was the functional unit of all eukaryotic tissues.

The confusion that prevailed amongst nineteenth century anatomists took two forms. First, most were unclear as to whether the axon and the many dendrites of a neuron were in fact extensions that originated from a *single* cell. For a long time they failed to appreciate that the cell body of the neuron, which housed the nucleus, almost invariably gave rise to two types of extensions: to *dendrites* that serve as input elements for neurons and that receive information from other cells, and to an *axon* which serves as the output element of the neuron and conveys information to other cells, often over long distances. Appreciation of the full extent of the neuron and its processes came ultimately with the histological studies of Ramón y Cajal and from the studies of Ross Harrison, who observed directly the outgrowth of axons and dendrites from neurons grown in isolation in tissue culture.

A second confusion arose because anatomists could not visualize and resolve the cell membrane and therefore they were uncertain whether neurons were delimited by membranes throughout their extent. As a result many believed that the cytoplasm of two apposite cells was continuous at their points of contact and formed a syncytium or reticular net. Indeed, the neurofibrils of one cell were thought to extend into the cytoplasm of the neighboring cell, serving as a path for current flow from one cell to another. This confusion was solved intuitively and indirectly by Ramón y Cajal in the 1890s and definitively in the 1950s with the application of electron microscopy to the brain by Sanford Palay and George Palade.

Ramón y Cajal was able to address these two questions using two methodological strategies. First, he turned to studying the brain in newborn animals, where the density of neurons is low and the expansion of the dendritic tree is still modest. In addition, he used a specialized silver staining method developed by Camillo Golgi that labels only an occasional neuron, but labels these neurons in their entirety, thus permitting the visualization of their cell body, their entire dendritic tree, and their axon. With these methodological improvements, Ramón y Cajal observed that neurons, in fact, are discrete cells, bounded by membranes, and inferred that nerve cells communicate with one another only at specialized points of appositions, contacts that Charles Sherrington was later to call *synapses* (Sherrington, 1897).

As Ramón y Cajal continued to examine neurons in different parts of the brain, he showed an uncanny ability to infer from static images remarkable functional insights into the dynamic properties of neurons. One of his most profound insights, gained in this way, was the *principle of dynamic polarization*. According to this principle, electrical signaling within neurons is unidirectional: the signals propagate from the receiving pole of the neuron—the dendrites and the cell body—to the axon, and then, along the axon to the output pole of the neuron—the presynaptic axon terminal.

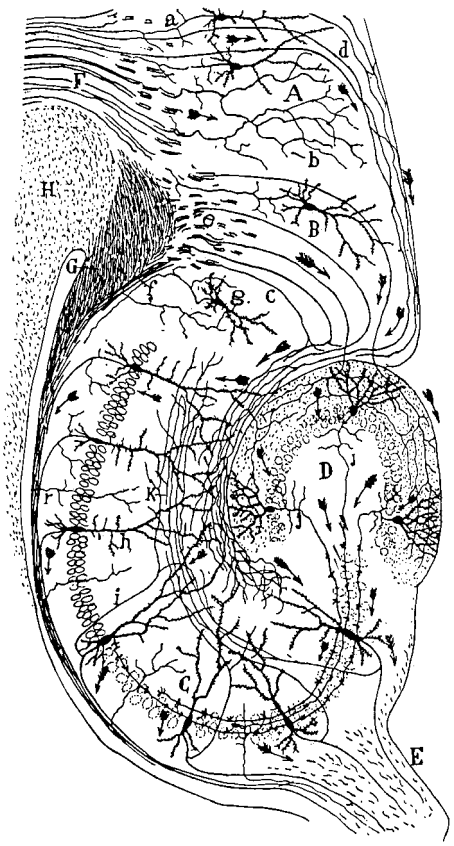

Figure 1. Ramón y Cajal's Illustration of Neural Circuitry of the Hippocampus

A drawing by Ramón y Cajal based on sections of the rodent hippocampus, processed with a Golgi and Weigert stain. The drawing depicts the flow of information from the entorhinal cortex to the dentate granule cells (by means of the perforant pathway) and from the granule cells to the CA3 region (by means of the mossy fiber pathway), and from there to the CA1 region of the hippocampus (by means of the Schaeffer collateral pathway). (Based on Ramón y Cajal, 1955.)

The principle of dynamic polarization proved enormously influential because it provided the first functionally coherent view of the various compartments of neurons. In addition, by identifying the directionality of information flow in the nervous system, dynamic polarization provided a logic and set of rules for mapping the individual components of pathways in the brain that constitute a coherent neural circuit (Figure 1). Thus, in contrast to the chaotic view of the brain that emerged from the work of Golgi, Gerlach, and Deiters who conceived of the brain as a *diffuse nerve* net in which every imaginable type of interaction appeared possible, Ramón y Cajal focused his experimental analysis on the brain's most important function: the processing of information.

Sherrington incorporated Ramón y Cajal's notions of the neuron doctrine, of dynamic polarization, and of the synapse into his book *The Integrative Action of the Nervous System* (1906). This monograph extended thinking about the function of nerve cells to the level of behavior. Sherrington pointed out that the key function of the nervous system was integration; the nervous system was uniquely capable of weighing the consequences of different types of information and then deciding on an appropriate course of action based upon that evaluation. Sherrington illustrated the integrative capability of the nervous system in three ways. First, he pointed out that reflex actions serve as prototypic examples of behavioral integration; they represent coordinated, purposeful behavior in response to a specific input. For example in the flexion withdrawal and cross-extension reflex, a stimulated limb will flex and withdraw rapidly in response to a painful stimulus while, as part of a postural adjustment, the opposite limb will extend (Sherrington, 1910). Second, since each spinal reflex—no matter how complex—used the motor neuron in the spinal cord for its output, Sherrington developed the idea that the motor neuron was the *final common pathway* for the integrative actions of the nervous system (Sherrington, 1906). Finally, Sherrington discovered—what Ramón y Cajal could not infer—that not all synaptic actions were excitatory; some could be inhibitory (Sherrington, 1932). Since motor neurons receive a convergence of both excitatory and inhibitory synaptic input, Sherrington argued that motor neurons represent an example—the prototypical example—of a cellular substrate for the integrative action of the brain. Each motor neuron must weigh the relative influence of two types of inputs, inhibitory and excitatory, before deciding whether or not to activate a final common pathway leading to behavior. Each neuron therefore recapitulates, in elementary form, the integrative action of the brain.

In the 1950s and 1960s, Sherrington's last and most influential student, John C. Eccles, used intracellular recordings from neurons to reveal the ionic mechanisms through which motor neurons generate the inhibitory and excitatory actions that permit them to serve as the final common pathway for neural integration (Eccles, 1953). In addition, Eccles, Karl Frank, and Michael Fuortes found that motor neurons had a specialized region, the initial segment of the axon, which served as a crucial integrative or decision-making component of the neuron (Fuortes et al., 1957; Eccles, 1964). This component summed the total excitatory and inhibitory input and discharged an action potential if, and only if, excitation of the motor neuron exceeded inhibition by a certain critical minimum.

The findings of Sherrington and Eccles implied that each neuron solves the competition between excitation and inhibition by using, at its initial segment, a *winner takes all* strategy. As a result, an elementary aspect of the integrative action of the brain could now be studied at the level of individual cells by determining how the summation of excitation and inhibition leads to an integrated, all-or-none, output at the initial segment. Indeed, it soon became evident that studies of the motor neuron had predictive value for all neurons in the brain. Thus, the initial task in understanding the integrative action of the brain could be reduced to understanding signal integration at the level of individual nerve cells.

The ability to extend the analysis of neuronal signaling to other regions of the brain was, in fact, already being advanced by two of Sherrington's contemporaries, Edgar Adrian and John Langley. Adrian developed methods of *single unit analysis* within the central nervous

system, making it possible to study signaling in any part of the nervous system at the level of single cells (Adrian, 1957). In the course of this work, Adrian found that virtually all neurons use a conserved mechanism for signaling *within* the cell: the *action potential*. In all cases, the action potential proved to be a large, all-or-none, regenerative electrical event that propagated without fail from the initial segment of the axon to the presynaptic terminal. Thus, Adrian showed that what made one cell a sensory cell carrying information of vision and another cell a motor cell carrying information about movement was not the nature of the action potential that each cell generated. What determined function was the neural circuit to which that cell belonged.

Sherrington's other contemporary, John Langley (1906), provided some of the initial evidence (later extended by Otto Loewi, Henry Dale, and Wilhelm Feldberg) that, at most synapses, signaling *between* neurons—*synaptic transmission*—was chemical in nature. Thus, the work of Ramón y Cajal, Sherrington, Adrian, and Langley set the stage for the delineation, in the second half of the twentieth century, of the mechanisms of neuronal signaling—first in biophysical (ionic), and then in molecular terms.

Long-Range Signaling within Neurons: The Action Potential

In 1937 Alan Hodgkin found that an action potential generates a local flow of current that is sufficient to depolarize the adjacent region of the axonal membrane, in turn triggering an action potential (Hodgkin, 1937). Through this spatially interactive process along the surface of the membrane, the action potential is propagated without failure along the axon to the nerve terminal (Figure 2A). In 1939 Kenneth Cole and Howard Curtis further found that when an all-or-none action potential is generated, the membrane of the axon undergoes a change in ionic conductance, suggesting that the action potential reflects the flow of ionic current (Figure 2B).

Hodgkin, Andrew Huxley, and Bernhard Katz extended these observations by examining which specific currents flow during the action potential. In a landmark series of papers in the early 1950s, they provided a quantitative account of the ionic currents in the squid giant axon (Hodgkin et al., 1952). This view, later called the *ionic hypothesis*, explained the resting membrane potential in terms of voltage-insensitive (nongated or leakage) channels permeable primarily to K^+ and the generation and propagation of the action potential in terms of two discrete, voltage-gated conductance pathways, one selective for Na^+ and the other selective for K^+ (Figure 2C).

The ionic hypothesis of Hodgkin, Huxley, and Katz remains one of the deepest insights in neural science. It accomplished for the cell biology of neurons what the structure of DNA did for the rest of biology. It unified the cellular study of the nervous system in general, and in fact, the study of ion channels in general. One of the strengths of the ionic hypothesis was its generality and predictive power. It provided a common framework for all electrically excitable membranes and thereby provided the first link between neurobiology and other fields of cell biology. Whereas action potential signaling is a relatively specific mechanism distinctive to nerve and muscle cells, the permeability of the cell membrane to small ions is a general feature shared by all cells. Moreover, the ionic hypothesis of the 1950s was so precise in its predictions that it paved the way for the molecular biological explosion that was to come in the 1980s.

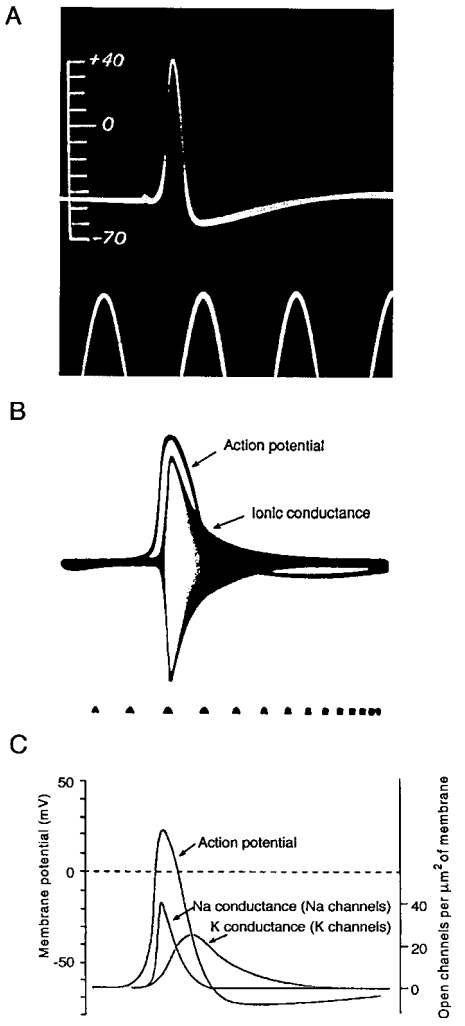

Figure 2. The Action Potential

(A) This historic recording of a membrane resting potential and an action potential was obtained by Alan Hodgkin and Andrew Huxley with a capillary pipette placed across the membrane of the squid giant axon in a bathing solution of sea water. Time markers (500 Hz) on the horizontal axis are separated by 2 ms. The vertical scale indicates the potential of the internal electrode in millivolts; the sea water outside is taken as zero potential. (From Hodgkin and Huxley, 1939.)

(B) A net increase in ionic conductance in the membrane of the axon accompanies the action potential. This historic recording from an experiment conducted in 1938 by Kenneth Cole and Howard Curtis shows the oscilloscope record of an action potential superimposed on a simultaneous record of the ionic conductance. (Modified from Kandel et al., 2000).

(C) The sequential opening of voltage-gated Na^+ and K^+ channels generates the action potential. One of Hodgkin and Huxley's great achievements was to separate the total conductance change during an action potential, first detected by Cole and Curtis (Figure 2B), into separate components that could be attributed to the opening of Na^+ and K^+ channels. The shape of the action potential and the underlying conductance changes can be calculated from the properties of the voltage-gated Na^+ and K^+ channels. (From Kandel et al., 2000.)

Despite its profound importance, however, the analysis of Hodgkin, Huxley, and Katz left something unspecified. In particular, it left unspecified the molecular nature of the pore through the lipid membrane bilayer and the mechanisms of ionic selectivity and gating. These aspects were first addressed by Bertil Hille and Clay Armstrong. In the late 1960s, Hille devised procedures for measuring Na^+ and K^+ currents in isolation (for review see Hille et al., 1999). Using pharmacological agents that selectively block one but not the other ionic conductance pathway, Hille was able to infer that the Na^+ and K^+ conductance pathways of Hodgkin and Huxley corresponded to independent ion channel proteins. In the 1970s Hille used different organic and inorganic ions of specified size to provide the first estimates of the size and shape of the pore of the Na^+ and the K^+ channels. These experiments led to the defining structural characteristic of each channel—the *selectivity filter*—the narrowest region of the pore, and outlined a set of physical–chemical mechanisms that could explain how Na^+ channels are able to exclude K^+ and conversely, how K^+ channels exclude Na^+.

In parallel, Armstrong addressed the issue of gating in response to a change in membrane voltage. How does an Na^+ channel open rapidly in response to voltage change? How, once opened, is it closed? Following initial experiments of Knox Chandler on excitation contraction coupling in muscle, Armstrong measured minute "gating" currents that accompanied the movement, within the transmembrane field, of the voltage sensor postulated to exist by Hodgkin and Huxley. This achievement led to structural predictions about the number of elementary charges associated with the voltage sensor. In addition, Armstrong discovered that mild intracellular proteolysis selectively suppresses Na^+ channel inactivation without affecting voltage-dependent activation, thereby establishing that activation and inactivation involve separate (albeit, as later shown, kinetically linked) molecular processes. Inactivation reflects the blocking action of a globular protein domain, a "ball," tethered by a flexible peptide chain to the intracellular side of the channel. Its entry into the mouth of the channel depends on the prior activation (opening) of the channel. This disarmingly simple "mechanical" model was dramatically confirmed by Richard Aldrich in the early 1990s. Aldrich showed that a cytoplasmic amino terminal peptide "ball" tethered by a flexible chain does indeed form part of the K^+ channel and underlies its inactivation, much as Armstrong predicted.

Until the 1970s, measurement of current flow was carried out with the voltage-clamp technique developed by Cole, Hodgkin, and Huxley, a technique that detected the flow of current that followed the opening of thousands of channels. The development of patch-clamp methods by Erwin Neher and Bert Sakmann revolutionized neurobiology by permitting the characterization of the elemental currents that flow when a single ion channel—a single membrane protein—undergoes a transition from a closed to an open conformation (Neher and Sakmann, 1976) (Figure 4A). This technical advance had two additional major consequences. First, patch clamping could be applied to cells as small as 2–5 μm in diameter whereas voltage clamping could only be carried out routinely on cells 50 μm or larger. Now, it became possible to study biophysical properties of the neurons of the mammalian brain and to study as well a large variety of nonneuronal cells. With these advances came the realization that virtually all cells harbor in their surface membrane (and even in their internal membranes) Ca^{2+} and K^+ channels similar to those found in nerve cells. Second, the introduction of patch clamping also set the stage for the analysis of channels at the molecular level, and not only voltage-gated channels of the sort we have so far considered but also of ligand-gated channels, to which we now turn.

Short-Range Signaling between Neurons: Synaptic Transmission

The first interesting evidence for the generality of the ionic hypothesis of Hodgkin, Huxley, and Katz was the realization in 1951 by Katz and Paul Fatt that, in its simplest form, chemical synaptic transmission represents an extension of the ionic hypothesis (Fatt and Katz 1951, 1952). Fatt and Katz found that the synaptic receptor for chemical transmitters was an ion channel. But, rather than being gated by voltage as were the Na^+ and K^+ channels, the synaptic receptor was gated chemically, by a ligand, as Langley, Dale, Feldberg, and Loewi had earlier argued. Fatt and Katz and Takeuchi and Takeuchi showed that the binding of acetylcholine, the transmitter released by the motor nerve terminal, to its receptors leads to the opening of a new type of ion channel, one that is permeable to *both* Na^+ and K^+ (Figure 3) (Takeuchi and Takeuchi, 1960). At inhibitory synapses, transmitters, typically γ-aminobutyric acid (GABA) or glycine, open channels permeable to Cl^- or K^+ (Boistel and Fatt, 1958; Eccles, 1964).

In the period 1930 to 1950, there was intense controversy within the neural science community about whether transmission between neurons in the central nervous system occurred by electrical or chemical means. In the early 1950s Eccles, one of the key proponents of electrical transmission, used intracellular recordings from motor neurons and discovered that synaptic excitation and inhibition in the spinal cord was mediated by chemical synaptic transmission. He further found that the principles of chemical transmission derived by Fatt and Katz from studies of peripheral synapses could be readily extended to synapses in the nervous system (Brock et al., 1952; Eccles, 1953, 1964). Thus, during the 1960s and 1970s the nature of the postsynaptic response at a number of readily accessible chemical synapses was analyzed, including those mediated by acetylcholine, glutamate, GABA, and glycine (see for example Watkins and Evans, 1981). In each case, the transmitter was found to bind to a receptor protein that directly regulated the opening of an ion channel. Even prior to the advent in the 1980s of molecular cloning, which we shall consider below, it had become clear, from the biochemical studies of Jean-Pierre Changeux and of Arthur Karlin that in ligand-gated channels the transmitter binding site and the ionic channel constitute different domains within a *single* multimeric protein (for reviews see Changeux et al., 1992; Karlin and Akabas, 1995; Cowan and Kandel, 2000).

As with voltage-gated channels, the single channel measurements of Neher and Sakmann brought new insights into ligand-gated channels (Neher and Sakmann, 1976). For example, in the presence of ligand, the acetylcholine (ACh) channel at the vertebrate neuromuscular

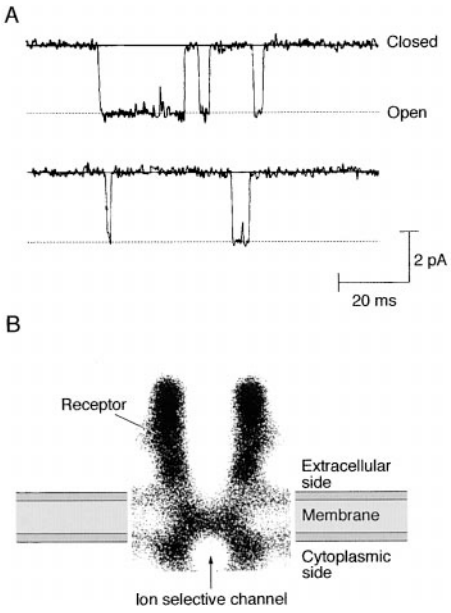

Figure 3. The Conductance of Single Ion Channels and a Preliminary View of Channel Structure

(A) Recording current flow in single ion channels. Patch-clamp record of the current flowing through a single ion channel as the channel switches between its closed and open states. (Courtesy of B. Sakmann.)

(B) Reconstructed electron microscope view of the ACh receptor-channel complex in the fish *Torpedo californica*. The image was obtained by computer processing of negatively stained images of ACh receptors. The resolution is 1.7 nm, fine enough to visualize overall structure but too coarse to resolve individual atoms. The overall diameter of the receptor and its channel is about 8.5 nm. The pore is wide at the external and internal surfaces of the membrane but narrows considerably within the lipid bilayer. The channel extends some distance into the extracellular space. (Adapted from studies by Toyoshima and Unwin.) (From Kandel et al., 2000.)

junction opens briefly (on average for 1 to 10 ms) and gives rise to a square pulse of inward current, roughly equivalent to 20,000 Na^+ ions per channel per ms. The extraordinary rate of ion translocation revealed by these single channel measurements confirmed directly the idea of the ionic hypothesis—that ions involved in signaling cross the membrane by passive electrochemical movement through aqueous transmembrane channels rather than through transport by membrane carriers (Figure 3A).

Following the demonstration of the chemical nature of transmission at central as well as peripheral synapses, neurobiologists began to suspect that communication at all synapses was mediated by chemical signals. In 1957, however, Edwin Furshpan and David Potter made the discovery that transmission at the giant fiber synapse in crayfish was electrical (Furshpan and Potter, 1957). Subsequently, Michael Bennett (1972) and others showed that electrical transmission was widespread and operated at a variety of vertebrate and invertebrate synapses. Thus, neurobiologists now accept the existence of two major modes of synaptic transmission: *electrical*, which depends on current through gap junctions that bridge the cytoplasm of pre- and postsynaptic cells; and *chemical*, in which pre- and postsynaptic cells have no direct continuity and are separated by a discrete extracellular space, the synaptic cleft (Bennett, 2000).

The Proteins Involved in Generating Action Potentials and Synaptic Potentials Share Features in Common

In the 1980s, Shosaku Numa, Lily Yeh Jan, Yuh Nung Jan, William Catterall, Steven Heineman, Peter Seeburg, Heinrich Betz, and others cloned and expressed functional voltage-gated Na^+, Ca^{2+}, and K^+ channels, as well as the ligand-gated receptor channels for ACh, GABA, glycine, and glutamate (Numa, 1989; Armstrong and Hille, 1998; Green et al., 1998). Prior biophysical studies already had taught us much about channels, and as a consequence molecular cloning was in a position rapidly to provide powerful new insights into the membrane topology and subunit composition of both voltage-gated and ligand-gated signaling channel proteins (Armstrong and Hille, 1998; Colquhoun and Sakmann, 1998). Molecular cloning revealed that all ligand-gated channels have a common overall design and that this design shares features with voltage-gated channels.

Based on sequence identity, ligand-gated channels can be divided into two superfamilies: (1) receptors for glutamate (of the NMDA [N-methyl-D-aspartic acid] and non-NMDA classes) and (2) receptors for other small molecule transmitters: nicotinic ACh, 5-hydroxytryptamine, GABA, glycine, and ATP (Green et al., 1998) (Figure 6). Of these, the most detailed information is again available on the nicotinic ACh receptors of skeletal muscle (Figure 3B). This receptor is made up of four distinct subunits, α, β, γ, and δ, with the α subunit represented twice in a five-subunit channel ($\alpha_2\beta\gamma\delta$). Three-dimensional images reveal a channel made up of the five subunits surrounding the water-filled channel pore (Figures 3B and 4). Much as predicted by Hille, the channel appears to be divided into three regions: a relatively large entrance region on the external surface; a narrow transmembrane pore, only a few atomic diameters wide, which selects for ions on the basis of their size and charge; and a large exit region on the internal plasma membrane surface.

The first of the voltage-sensitive channels to be cloned, the brain Na^+ channel, was found to consist of one large (α) and two smaller (β) subunits. The α subunit is widely distributed and is the major pore-forming subunit essential for transmembrane Na^+ flux, whereas the smaller subunits are regulatory and are expressed only by subsets of cells (where they participate in channel assembly and inactivation). The α subunit consists of a single peptide of about 2000 amino acids with four internally repeated domains of similar structures. Each domain contains six putative membrane-spanning segments, S1 to S6, which are thought to be α helical, and a reentrant P loop. The P loop connects the S5 and S6 segments and forms the outer mouth and selectivity filter of the channel.

The voltage-gated Ca^{2+} channels are similar to the Na^+ channel in their overall design. However, each of the cloned K^+ channels encodes only a single domain, of about 600 amino acids, containing the six putative transmembrane regions and the P loop. As might be predicted from this structure, four of these subunits are required to form a functional channel (either as homo- or as heterotetramers).

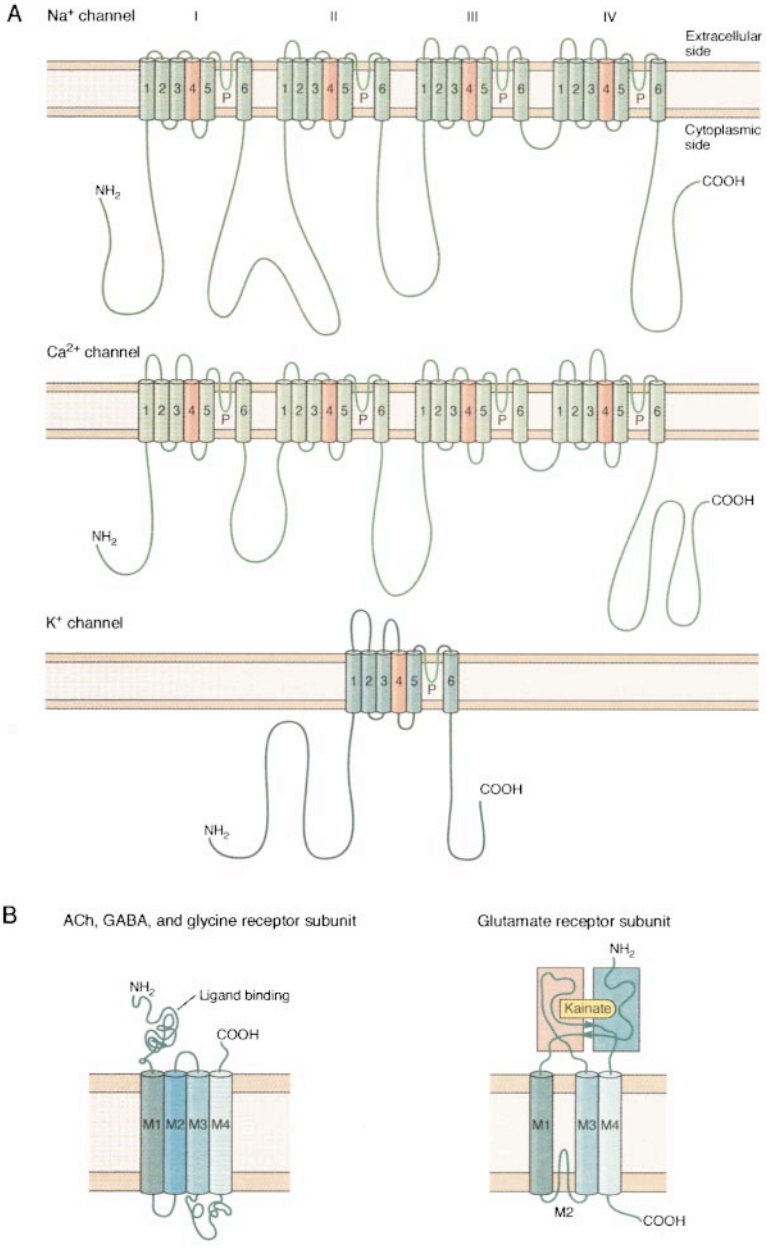

Figure 4. The Membrane Topology of Voltage- and Ligand-Gated Ion Channels

(A) The basic topology of the α subunit of the voltage-gated Na^+ channel, and the corresponding segments of the voltage-gated Ca^{2+} and K^+ channels. The α subunit of the Na^+ and Ca^{2+} channels consists of a single polypeptide chain with four repetitions of six membrane-spanning α-helical regions. The S4 region, the fourth membrane-spanning α-helical region, is thought to be the voltage sensor. A stretch of amino acids, the P region between the 5th and 6th α helices, dips into the membrane in the form of two strands. A 4-fold repetition of the P region is believed to line the pore. The shaker type K^+ channel, by contrast, has only a single copy of the six α helices and the P region. Four such subunits are assembled to form a complete channel. (Adapted from Catterall, 1988; Stevens, 1991.) (B) The membrane topology of channels gated by the neurotransmitters ACh, GABA glycine, and kainate (a class of glutamate receptor ligand). (From Kandel et al., 2000.)

The wealth of sequence information that emerged from molecular cloning illustrated the remarkable conservation of channel molecules, and in turn demanded information on the structure of these channels. One of the recent successes of ion channel biology has been the first steps in the elucidation of ion channel structure. The first ion channel structure to be revealed was that of a K^+ channel (called KcsA) from the bacterium, *Streptomyces lividans*. The amino acid sequence of KcsA shows it to be most similar to the inward rectifier type of K^+ channel that contributes to the regulation of the resting membrane potential. The amino acid sequence of these channels predicts only two transmembrane domains connected by a P loop, in contrast to the more familiar voltage-gated K^+ channels, which have six transmembrane domains. When reconstituted in lipid bilayers, KcsA forms a tetramer. The 3.2 Å resolution crystal structure reported by Roderick MacKinnon and his colleagues revealed that the tetramer has two transmembrane-spanning α helices connected by the P region (Doyle et al., 1998) (Figures 5A and 5B).

In retrospect it was remarkable how accurately this structure had been anticipated by the earlier biophysical studies of Hille and Armstrong. Hille and Armstrong had, for example, correctly predicted the selectivity filter to be a narrow region near the outer face of the membrane lined by polar residues. One surprise, however, is that the channel pore is not lined by hydrophilic amino acid side chains but by the carbonyl backbone of conserved amino acids, containing glycine-tyrosine-glycine residues that are characteristic of nearly all K^+-selective channels. The narrow channel in the selectivity filter

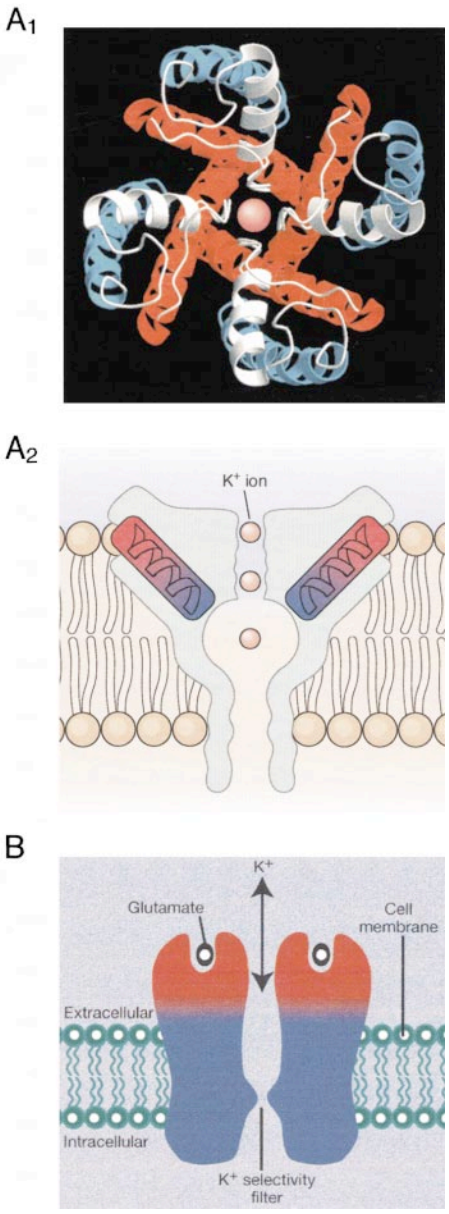

Figure 5. The Crystal Structure of a Bacterial Inward-Rectifying K^+ Channel and a Glutamate Receptor

(A_1) A view of the bacterial K^+ channel in cross section in the plane of the membrane. The four subunits are shown, with each subunit depicted in different color. The membrane-spanning helices are arranged as an inverted teepee.

(A_2) A side-view of the channel illustrating three K^+ ions within the channel. The pore helices contribute a negative dipole that helps stabilize the K^+ ion in the water-filled inner chamber. The two outer K^+ ions are loosely bound to the selectivity filter formed by the P region. (From Doyle et al., 1998.)

(B) Schematic depiction of a bacterial ligand-gated glutamate receptor channel with a K^+ channel pore. The extracellular regions of the channel show sequence similarity to the ligand-binding domains of glutamate receptors (red in the figure here). The pore region resembles an inverted potassium channel pore (blue). (Image courtesy of E. Gouaux; see Chen et al., 1999.)

rapidly broadens in hourglass fashion to form a "lake" roughly halfway through the membrane, in which 60–100 water molecules diffuse the charges of K^+ ions residing in this cavity. Four short α helices in the P loops have their helix dipole negative electrostatic fields focused on the cavity, further stabilizing the K^+ ion poised at the selectivity filter. Finally, a long water-filled hydrophobic channel tunnels to the cytoplasm.

MacKinnon's compelling images even visualized two K^+ ions within the selectivity filter. Thus, a total of three K^+ ions are positioned at distinct sites within the pore, each separated from the other by about 8 Å. This view of a single pore capable of accommodating three K^+ ions was precisely as predicted by Hodgkin some fifty years earlier. MacKinnon's structure thus provided explanations for K^+ channel selectivity and conduction. What we lack, however, is an insight into the mechanisms of voltage-dependent gating.

The membrane subunits of many voltage-dependent potassium channels associate with additional proteins known as the β subunits (Isom et al., 1994). One function of β subunits is to modify the gating of K^+ channels. MacKinnon and his colleagues have now gone on to provide the structure of the β subunit of a voltage-dependent K^+ channel from eukaryotic cells (Gulbis et al., 1999). Like the integral membrane components of the potassium channel, the β subunits have a 4-fold symmetrical structure. Surprisingly, each subunit appears similar to an oxido reductase enzyme, complete with a nicotinamide cofactor active site. Several structural features of the enzyme active site, including its location with respect to the 4-fold axis, implies that it may interact directly or indirectly with the K^+ channel's voltage sensor. Thus, the oxidative chemistry of the cell may be intrinsically linked to changes in membrane potential by the interaction of the α and β subunits of the voltage-dependent K^+ channels.

The expression of ligand-gated receptors also is not limited to multicellular organisms. For example, it has become evident recently that even prokaryotes have functional ligand-gated glutamate receptors. Eric Gouaux and his colleagues (Chen et al., 1999) have cloned and expressed a glutamate-gated channel from the cyanobacterium *Synechocystis* PCC 6803, and in so doing have provided a further surprise: the receptor has a transmembrane structure similar to that of KcsA and forms a K^+ selective pore. Thus, this receptor is related both to the inward rectifier K^+ channels and to eukaryotic glutamate receptors (Figure 5B). The extracellular region bears sequence homology to the ligand-binding domains of glutamate receptors whereas the pore region bears resemblance to an inverted K^+ channel. This finding has led Gouaux and his colleagues to propose a prokaryotic glutamate receptor as the precursor of eukaryotic receptors. In addition, this receptor provides a missing link between K^+ channels and glutamate receptors, and indicates that both ligand- and voltage-gated ion channels have a similar architecture, suggesting that they both derive from a common bacterial ancestor.

Synaptic Receptors Coupled to G Proteins Produce Slow Synaptic Signals

In the 1970s evidence began to emerge from Paul Greengard and others that the neurotransmitters that activate ligand-gated (*ionotropic*) channels to produce