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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.

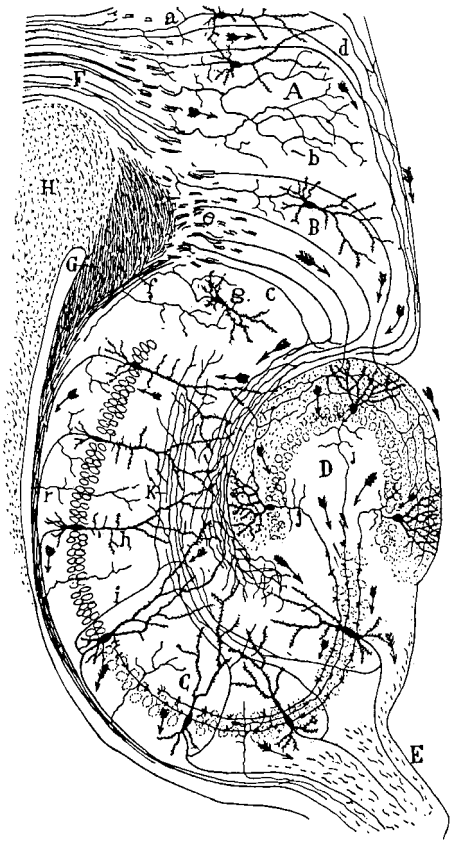

Figure 1. Ramón y Cajal's Illustration of Neural Circuitry of the Hippocampus

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.

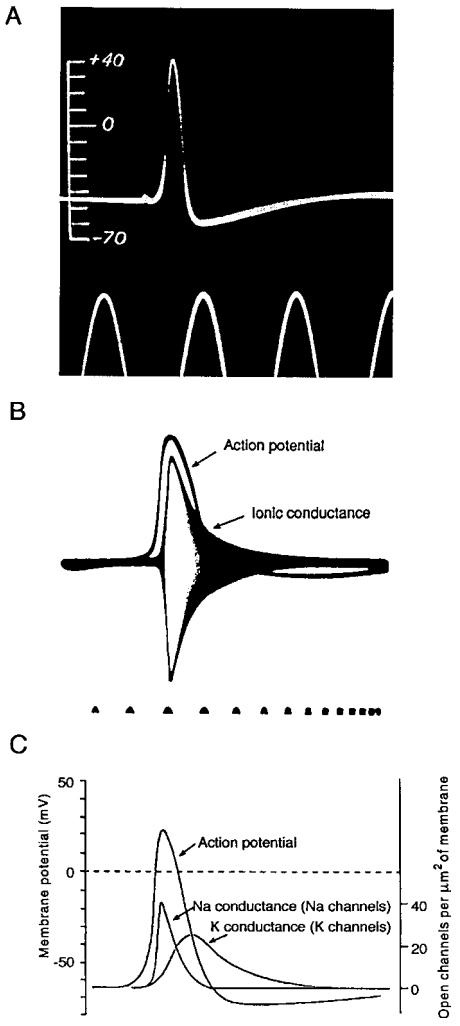

Figure 2. The Action Potential

(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

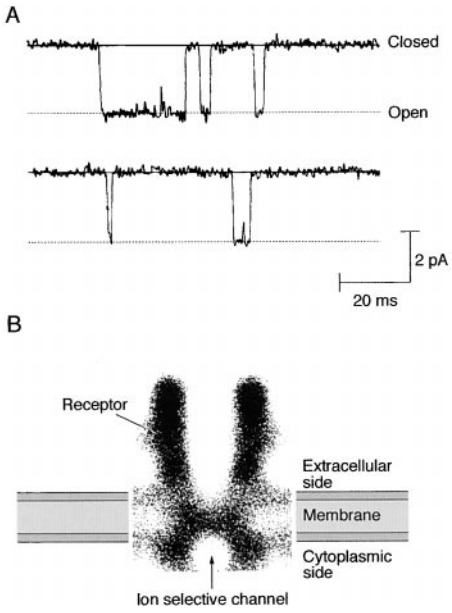

Figure 3. The Conductance of Single Ion Channels and a Preliminary View of Channel Structure

(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).

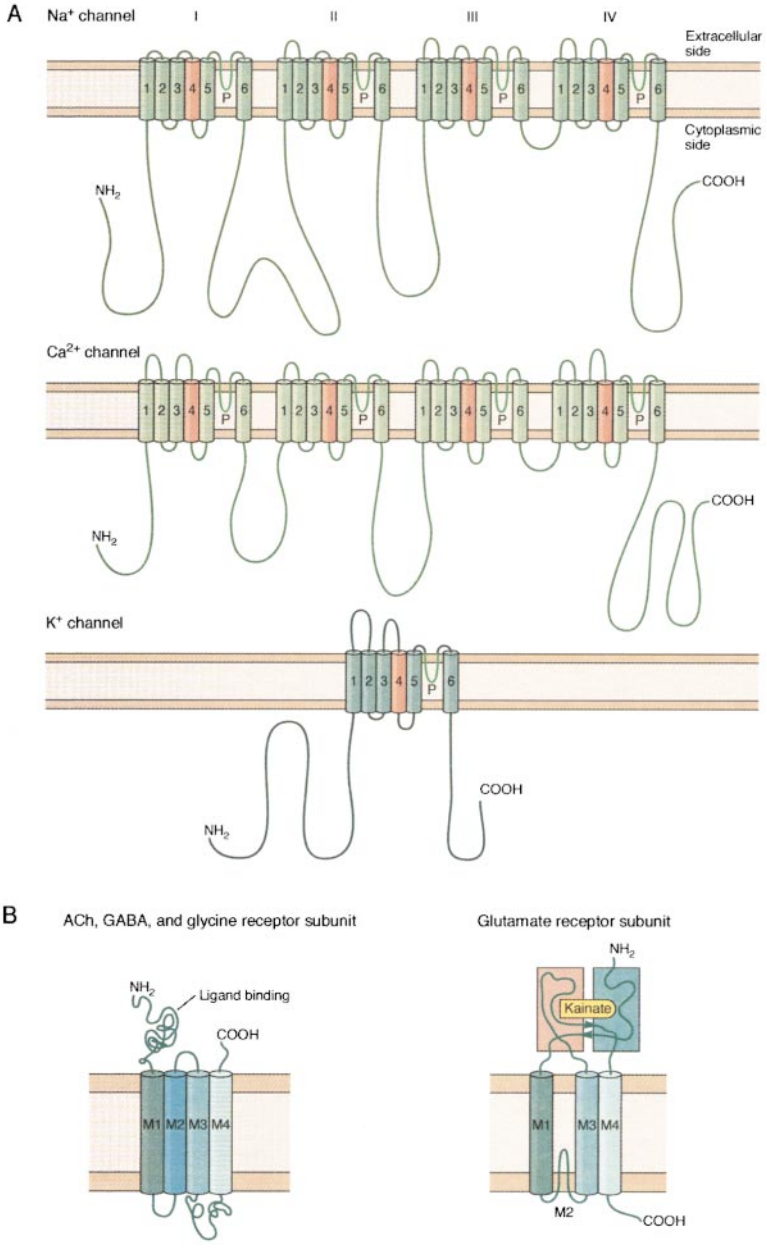

Figure 4. The Membrane Topology of Voltage- and Ligand-Gated Ion Channels

(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

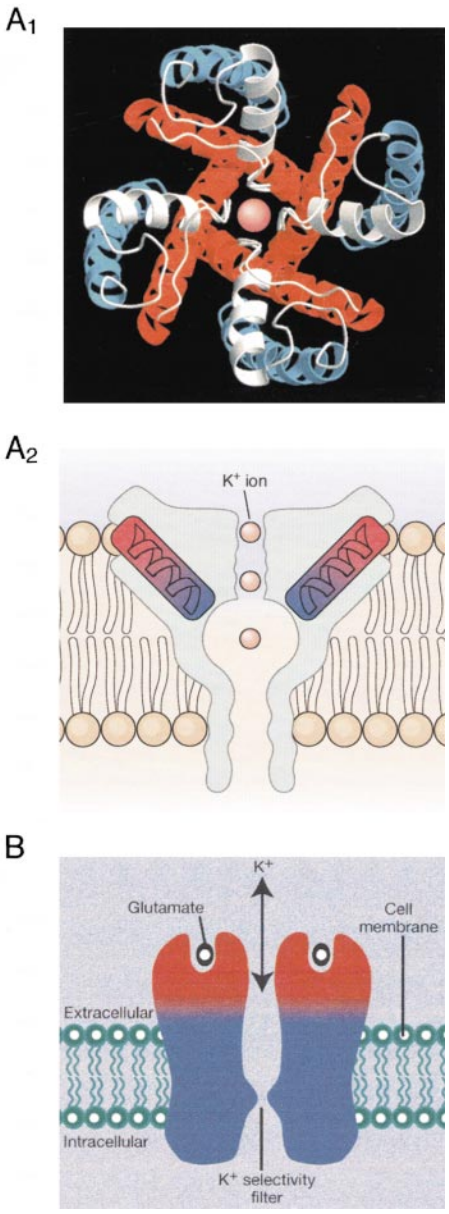

Figure 5. The Crystal Structure of a Bacterial Inward-Rectifying K^+ Channel and a Glutamate Receptor

(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

rapid synaptic potentials lasting only milliseconds—glutamate, ACh, GABA, serotonin—also interact with a second, even larger class of receptors (termed *metabotropic* receptors) that produce slow synaptic responses which persist for seconds or minutes (for review see Nestler and Greengard, 1984). Thus, a single presynaptic neuron releasing a single transmitter can produce a variety of actions on different target cells by activating distinct ionotropic or metabotropic receptors.

Molecular cloning revealed that these slow synaptic responses are transduced by members of a super-family of receptors with seven transmembrane-spanning domains, which do not couple to ion channels directly, but do so indirectly by means of their coupling to G proteins. G proteins couple this class of receptors to effector enzymes that give rise to second messengers such as cAMP, cGMP, diacylglycerol, and metabolites of arachidonic acid. G proteins and second messengers can activate some channels directly. More commonly, these messengers activate further downstream signaling molecules, often a protein kinase that regulates channel function by phosphorylating the channel protein or an associated regulatory protein (for review see Nestler and Greengard, 1984). The family of G protein–coupled seven transmembrane-spanning receptors is remarkably large, and its members serve not only as receptors for small molecule and peptide transmitters, but also as the sensory receptors for vision and olfaction.

The study of slow synaptic potentials mediated by second messengers has added several new features to our understanding of chemical transmission. Four of these are particularly important. First, second messenger systems regulate channel function by acting on cytoplasmic domains of channels. This type of channel regulation can be achieved in three different ways: (1) through the phosphorylation of the channel protein by a second messenger-activated protein kinase, (2) through the interaction between the channel protein and a G protein activated by the ligated receptor, or (3) by the direct binding to the channel protein of a cyclic nucleotide as the case with the ion channels of photoreceptor and olfactory receptor cells gated by cAMP or cGMP. Second, by acting through second messengers, transmitters can modify proteins other than the channels, thereby activating a coordinated molecular response within the postsynaptic cell. Third, second messengers can translocate to the nucleus and modify transcriptional regulatory protein, in this way controlling gene expression. Thus, second messengers can covalently modify preexisting proteins as well as regulate the synthesis of new proteins. This latter class of synaptic action can lead to long-lasting structural changes at synapses. Finally, we are beginning to appreciate functional differences in slow synaptic actions. Whereas fast synaptic actions are critical for routine behavior, slow synaptic actions are often *modulatory* and act upon neural circuits to regulate the intensity, form, and duration of a given behavior (Kandel et al., 2000).

Chemical Transmitter Is Released from the Presynaptic Terminal in Multimolecular Packets

In addition to providing initial insights into the structure and function of the ligand-gated postsynaptic receptors responsible for postsynaptic transmission, Katz and Fatt also provided the groundwork for a molecular analysis of transmitter release from the presynaptic terminals with the discovery of its *quantal* nature (reviewed in Katz, 1969). Katz, with Fatt and Jose del Castillo, discovered that chemical transmitters, such as ACh, are released not as single molecules but as multimolecular packets called *quanta*. At the neuromuscular junction each quantum comprises about 5,000 molecules of transmitter (Fatt and Katz 1952; del Castillo and Katz, 1954). Each quantum of ACh (and of other small molecule transmitters such as glutamate or GABA) is packaged in a single small organelle, the *synaptic vesicle*, and is released by exocytosis at specialized release sites within the presynaptic terminal called the *active zones*. In response to a presynaptic action potential, each active zone generally releases 0 or 1 quantum, in a probabilistic manner (Figure 6). Synapses that release large quantities of transmitter to evoke a large postsynaptic response, such as the synapse between nerve and muscle, contain several hundred active zones (Figures 8A and 8B) (Heuser, 1977). In the central nervous system, however, many presynaptic terminals contain only a single active zone.

Fatt and Katz discovered that synapses release quanta spontaneously, even in the absence of activity, giving rise to *spontaneous miniature synaptic potentials* (Fatt and Katz, 1952). For a single active zone the rate of spontaneous release is quite low, around 10^{-2} per second. In response to a presynaptic action potential, the rate of release is dramatically, but transiently, elevated to around 1000 per second. Within a few milliseconds, the quantal release rate then decays back to its low resting level. We know from the work of Katz and Ricardo Miledi as well as from the studies of Rodolfo Llinas that intracellular Ca^{2+} is the key signal that triggers the increase in release. When the action potential invades the terminal, it opens voltage-gated Ca^{2+} channels that are enriched near the active zone. The resultant influx of Ca^{2+} produces localized accumulations of Ca^{2+} (to $>100\ \mu M$) in microdomains of the presynaptic terminal near active zone release site. The local increase in Ca^{2+} concentration greatly enhances the probability of vesicle fusion and transmitter release. Many presynaptic terminals also have ionotropic and metabotropic receptors for transmitters, and these, in turn, modulate Ca^{2+} influx during an action potential and thus modify transmitter release.

Kinetic analyses suggest that the exocytotic release of neurotransmitter from synaptic vesicles involves a cycle composed of at least four distinct steps: (1) the transport (or mobilization) of synaptic vesicles from a reserve pool (tethered to the cytoskeleton) to a releasable pool at the active zone; (2) the docking of vesicles to their release sites at the active zone; (3) the fusion of the synaptic vesicle membrane with the plasma membrane during exocytosis, in response to a local increase in intracellular Ca^{2+}; and (4) the retrieval and recycling of vesicle membrane following exocytosis.

A major advance in the analysis of transmitter release was provided by the biochemical purification and molecular cloning of the proteins that participate in different aspects of the vesicle release cycle (Figure 7). Paul Greengard's work on the synapsins and their role in

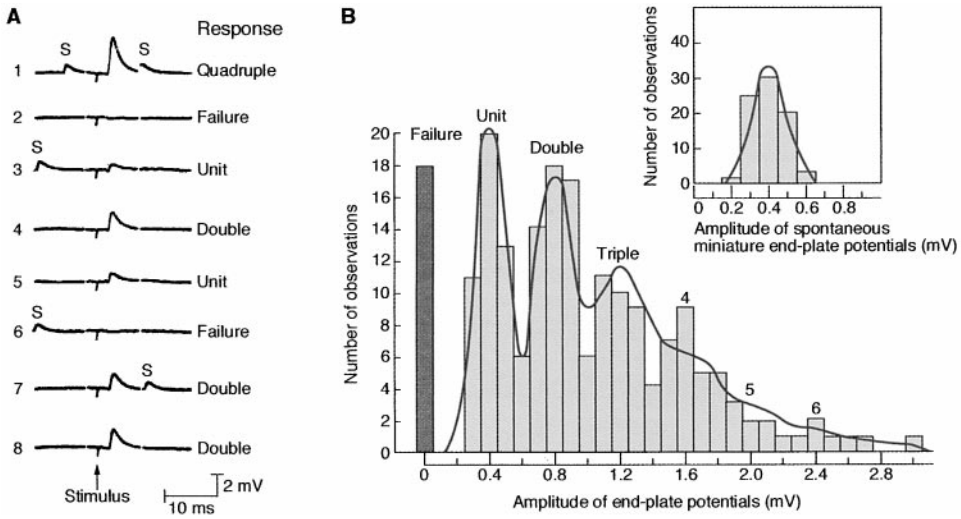

Figure 6. The Quantal Nature of Neurotransmitter Release

Neurotransmitters are released in fixed unitary increments, or quanta. Each quantum of transmitter produces a postsynaptic potential of fixed amplitude. The amplitude of the postsynaptic potential depends on the quantal unit amplitude multiplied by the number of quanta of transmitter. (A) Intracellular recordings show the change in potential when eight consecutive stimuli of the same size are applied to a motor nerve. To reduce transmitter output and to keep the end-plate potentials small, the tissue was bathed in a Ca^{2+}-deficient (and Mg^{2+}-rich) solution. The responses to the stimulus vary. Two impulses produce complete failures, two produce unit potentials, and the others produce responses that are approximately two to four times the amplitude of the unit potential. The spontaneous miniature end-plate potentials (S) are similar in size to the quantal unit potential. (Adapted from Liley, 1956.)

(B) The quantal nature of neurotransmitter release. After recording many end-plate potentials, the number of responses at each amplitude was counted and plotted. The distribution of responses falls into a number of peaks. The first peak, at 0 mV, represents release failures. The first peak at 0.4 mV, represents the unit potential, the smallest elicited response. This unit response is the same amplitude as the spontaneous miniature potentials (inset). The other peaks in the histogram occur at amplitudes that are integral multiples of amplitude of the unit potential. The solid line shows a theoretical Gaussian distribution fitted to the data of the histogram. Each peak is slightly spread out, reflecting the fact that the amount of transmitter in each quantum varies randomly about the peak. The distribution of amplitudes of the spontaneous miniature potentials, shown in the inset, also fits a Gaussian curve (solid line). (Adapted from Boyd and Martin, 1956.)

short-term synaptic plasticity, the work of Thomas Südhof and Richard Scheller on vesicle-associated proteins, and the work of Pietro De Camilli on membrane retrieval have each contributed seminally to our current view of the dynamics of synaptic vesicle mobilization, docking, and release (for review see Bock and Scheller, 1999; Fernandez-Chacon and Südhof, 1999). Although we now know most of the molecular participants, at present we still do not have a precise understanding of the molecular events that control any of the four kinetic stages of release. In some instances, however, we have a beginning.

By reconstituting the vesicle cycling system in a test tube, James Rothman and his colleagues have succeeded in identifying proteins that are essential for vesicle budding, targeting, recognition, and fusion (Sullener, 1993; Nickel et al., 1999; Parlati et al., 1999). Based on these studies, Rothman and colleagues have proposed an influential model, according to which vesicle fusion requires specialized *donor proteins* (*vesicle snares* or *v-snares*) intrinsic to the vesicle membrane that are recognized by and bind to specific *receptor proteins* in the target membrane (*target snares* or *t-snares*).

Rothman, Scheller, and their colleagues have found that two proteins located in the nerve terminal plasma membrane—syntaxin and SNAP-25—appear to have the properties of plasma membrane t-snares, whereas synaptobrevins/VAMP (vesicle-associated membrane protein), located on the membrane of the synaptic vesicles, have the properties of the donor proteins, or v-snares. The importance of the three snare proteins—VAMP, syntaxin, and SNAP-25—in synaptic transmission was immediately underscored by the findings that these three proteins are the targets of various clostridial neurotoxins, metalloproteases that irreversibly inhibit synaptic transmission. Subsequent reconstitution studies by Rothman and his colleagues showed that fusion could occur with liposomes containing v- and t-snares (Weber et al., 1998). Finally, structural studies by Reinhard Jahn and his colleagues based on quick-freeze/deep-etch electron microscopy and X-ray crystallography demonstrated that VAMP forms a helical coiled-coil structure with syntaxin and SNAP-25 that is thought to promote vesicle fusion by bringing the vesicle and plasma membrane into close apposition (Hanson et al., 1997; Sutton et al., 1998). From these studies it would appear that vesicle fusion uses a helical coiled-coil mechanism analogous to that used for viral fusion proteins (Söllner et al., 1993; Bock and Scheller, 1999; Nickel et al., 1999; Parlati et al., 1999). Indeed, VAMP resembles a viral fusion peptide.

One of the most important insights to emerge from research on synaptic vesicle associated proteins is that sets of molecules similar to those involved in mediating evoked transmitter release are also important for constitutive release. Indeed, homologs of the *v-* and *t-snares* participate in many aspects of membrane trafficking and constitutive vesicle fusion, including the trafficking of

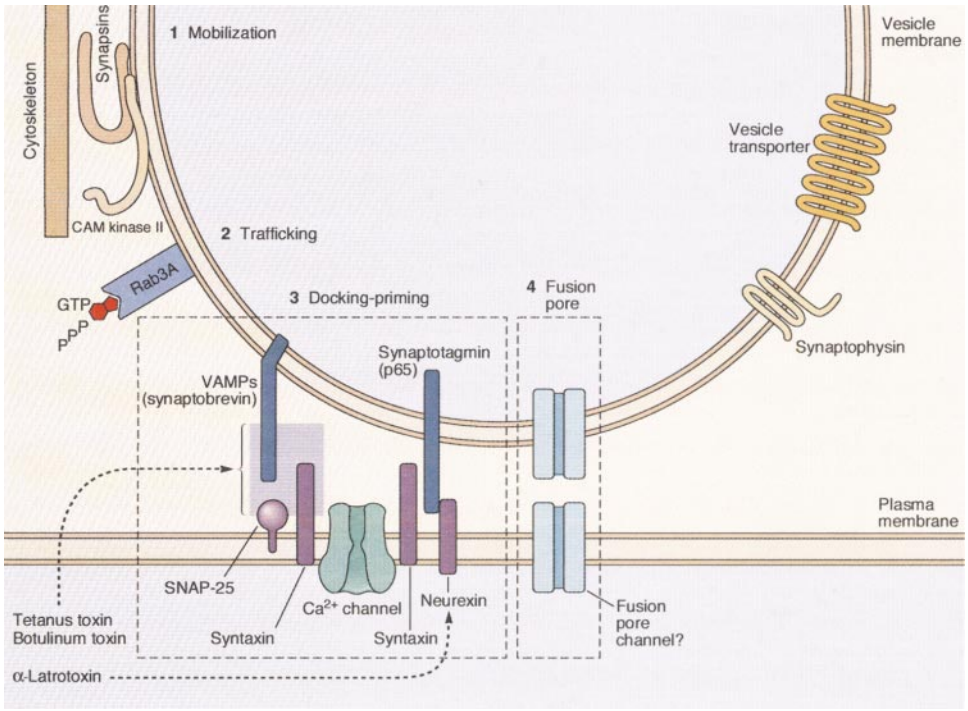

Figure 7. Some Vesicle Terminal Membrane–Associated Proteins

This diagram depicts characterized synaptic vesicle proteins and some of their postulated receptors and functions. Separate compartments are assumed for (1) storage (where vesicles are tethered to the cytoskeleton), (2) the trafficking and targeting of vesicles to active zones, (3) the docking of vesicles at active zones and their priming for release, and (4) release. Some of these proteins represent the targets for neurotoxins that act by modifying transmitter release. VAMP (synaptobrevin), SNAP-25, and syntaxin are the targets for tetanus and botulinum toxins, two zinc-dependent metalloproteases, and are cleaved by these enzymes. α-latrotoxin, a spider toxin that generates massive vesicle depletion and transmitter release, binds to the neurexins. (1) Synapsins are vesicle-associated proteins that are thought to mediate interactions between the synaptic vesicle and the cytoskeletal elements of the nerve terminal. (2) Rab GTPases appear to be involved in vesicle trafficking within the cell and also in the targeting of vesicles within the nerve terminal. (3) Vesicle docking, fusion, and release appear to involve distinct interactions between vesicle proteins and proteins of the nerve terminal plasma membrane: VAMP (synaptobrevin) and synaptotagmin (p65) are located on the vesicle membrane, and syntaxins and neurexins on the nerve terminal membrane. Arrows indicate potential interactions suggested on the basis of in vitro studies. (4) The identity of the vesicle and plasma membrane proteins that comprise the fusion pore remains unclear. Synaptophysin, an integral membrane protein in synaptic vesicles, is phosphorylated by tyrosine kinases and may regulate release. Vesicle transporters are involved in the concentration of neurotransmitter within the synaptic vesicle. (From Kandel, Schwartz, and Jessell, 2000.)

vesicles from the endoplasmic reticulum to the Golgi. Thus, the properties of *v- and t-snares* do not by themselves explain the specific tight Ca^{2+}-dependent regulation of vesicle fusion characteristic of evoked transmitter release from nerve terminal. Südhof has presented evidence that this calcium-dependent step in synaptic vesicle fusion is mediated by the synaptic vesicle proteins, the *synaptotagmins* (or p65). The synaptotagmins contain two domains (C2 domains) homologous to the Ca^{2+} and phospholipid-binding regulatory region of protein kinase C. This property suggested to Südhof that the synaptotagmins might insert into the presynaptic phospholipid bilayer in response to Ca^{2+} influx, thus serving as the *Ca^{2+} sensor* for exocytosis. Indeed, as shown by Charles Stevens, mice lacking the synaptotagmin-1 gene lack the fast synchronized Ca^{2+}-dependent phase of synaptic transmitter, although spontaneous release (which does not depend on Ca^{2+} influx) occurs normally (Fernandez-Chacon and Südhof, 1999).

Neurotransmitter Is Taken Up by Membrane Transporters

Acetylcholine was the first transmitter substance to be identified. In the course of studying its function, it soon became apparent that the action of ACh was terminated by the enzyme acetylcholinesterase. This enzyme is located in the basal membrane in close apposition to the acetylcholine receptor and regulates the amount of acetylcholine available for interaction with the receptor and the duration of its action. Thus, drugs that inhibit the acetylcholinesterase potentiate and prolong the synaptic effects of acetylcholine.

Based upon this set of findings in the cholinergic system, most neurobiologists in the 1950s assumed that all neurotransmitter systems would similarly be inactivated by enzymatic degradation. Thus, when norepinephrine was discovered to be an autonomic transmitter, it was expected that there would be enzymes with a dedicated degradative function. But in 1959, Julius Axelrod and his colleagues found that actions of norepinephrine were terminated not by enzymatic degradation but by a pump-like mechanism that transports norepinephrine back into the presynaptic nerve terminal (Hertting and Axelrod, 1961; Iversen, 1967). Similar uptake mechanisms were soon found for serotonin and for other amine and amino acid neurotransmitters. The mechanism of enzymatic degradation that inactivates acetylcholine, in

fact, turned out to be an exception rather than a rule. Reuptake pumps now have been shown to represent the standard way in which the nervous system inactivates the common amino acid and amine neurotransmitters after they have been released from the synapse. Many therapeutically important drugs, among them antidepressants, are powerful inhibitors of the uptake of norepinephrine and serotonin. Indeed effective antidepressants such as Prozac are selective inhibitors of the uptake of serotonin.

Peptide Transmitters

In addition to small molecules, it is now clear from the work of Thomas Hokfelt and his colleagues that neurons also release small peptides as transmitters. The number of peptides that act in this way exceeds several dozen and raised the question: how do their actions relate to classical neurotransmitters? Originally it was thought that the peptide-containing neurons represented a separate class of cells: neuroendocrine cells. However, Hokfelt and his colleagues showed that peptides and classical small molecule transmitters such as acetylcholine, norepinephrine, and serotonin coexist in individual neurons. Insight into the functional significance of cotransmission has emerged over the last two decades. In the salivary gland, for example, parasympathetic cholinergic neurons contain VIP-like peptides. In contrast, sympathetic norepinephrine neurons contain neuropeptide Y (NPY). In both cases these peptides act to augment the action of the classical transmitter. Thus, VIP induces a phase of vasodilatation and enhances the secretory effects of acetylcholine, while neuropeptide Y causes phasic vasoconstriction, like norepinephrine (Hokfelt, 1991). Gene targeting studies in mice are now beginning to reveal many additional functions for neuropeptide transmitters within the central nervous system.

The Plastic Properties of Synapses

Ramón y Cajal first introduced the principle of *connection specificity*: the idea that a given neuron will not connect randomly to another but that during development a given neuron will form specific connections only with some neurons and not with others. The precision of connections that characterizes the nervous system posed several deep questions: How are the intricate neural circuits that are embedded within the mature nervous system assembled during development? How does one reconcile the properties of a specifically and precisely wired brain with the known capability of animals and humans to acquire new knowledge in the form of learning? And how is knowledge, once learned, retained in the form of memory?

One solution to this problem was proposed by Ramón y Cajal in his 1894 Croonian Lecture to the Royal Society in which he suggested that: "... mental exercise facilitates a greater development of the protoplasmic apparatus and of the nervous collaterals in the part of the brain in use. In this way, preexisting connections between groups of cells could be reinforced by multiplication of the terminal branches of protoplasmic appendix and nervous collaterals. But the preexisting connections could also be reinforced by the formation of new collaterals and protoplasmic expansions."

An alternative solution for memory storage was formulated in 1922 by the physiologist Alexander Forbes. Forbes suggested that memory was sustained not by *plastic changes* in synaptic strength of the sort suggested by Ramón y Cajal but by dynamic reverberating activity within a closed, interconnected loop of self-reexciting neurons. This idea was elaborated by Ramón y Cajal's student, Rafael Lorente de Nó (1938), who found examples in his own analyses of neural circuitry and in those of Ramón y Cajal that neurons were often interconnected in the form of closed chains, circular pathways that could sustain reverberatory information.

This view of synaptic plasticity also was seriously challenged by B. Deslisle Burns in his influential book of 1958, *The Mammalian Cerebral Cortex.* Adopting a dynamic view, Burns wrote critically of plasticity mechanisms:

> The mechanisms of synaptic facilitation which have been offered as candidates for an explanation of memory ... have proven disappointing. Before any of them can be accepted as the cellular changes accompanying conditioned reflex formation, one would have to extend considerably the scale of time on which they have been observed to operate. The persistent failure of synaptic facilitation to explain memory makes one wonder whether neurophysiologists have not been looking for the wrong kind of mechanisms. (pp. 96–97)

The distinction between these two ideas—of *dynamic* as opposed to *plastic* changes for memory storage—was first tested experimentally in invertebrates where studies of nondeclarative memory storage in the marine snail *Aplysia* showed that memory is stored as a plastic change in synaptic strength, not as self-reexciting loops of neurons. These studies found that simple forms of learning—habituation, sensitization, and classical conditioning—lead to functional and structural changes in synaptic strength of specific sensory pathways that can persist for days and that these synaptic changes parallel the time course of the memory process (Kandel and Spencer, 1968; Castellucci et al., 1970). These findings reinforced the early ideas of Ramón y Cajal, which have now become one of the major themes of the molecular study of memory storage: Even though the anatomical connections between neurons develop according to a definite plan, the strength and effectiveness are not entirely predetermined and can be altered by experience (Squire and Kandel, 1999).

Modern cognitive psychological studies of memory have revealed that memory storage is not unitary but involves at least two major forms: declarative (or explicit) memory and nondeclarative (or implicit) memory. Declarative memory is what is commonly thought of as memory. It is the conscious recall of knowledge about facts and events: about people, places, and objects. This memory requires the medial temporal lobe and a structure that lies deep to it: the hippocampus. Nondeclarative memory such as habituation, sensitization, classical and operant conditioning, and various habits reflect the nonconscious recall of motor and perceptual skills and strategies (Squire and Zola-Morgan, 1991). In invertebrates these memories are often stored in specific sensory and motor pathways. In vertebrates these memories are stored, in addition, in three major subcortical structures: the amygdala, the cerebellum, and the basal ganglia (Milner et al., 1998).

Behavioral studies of both simple nondeclarative and more complex declarative memories had earlier shown

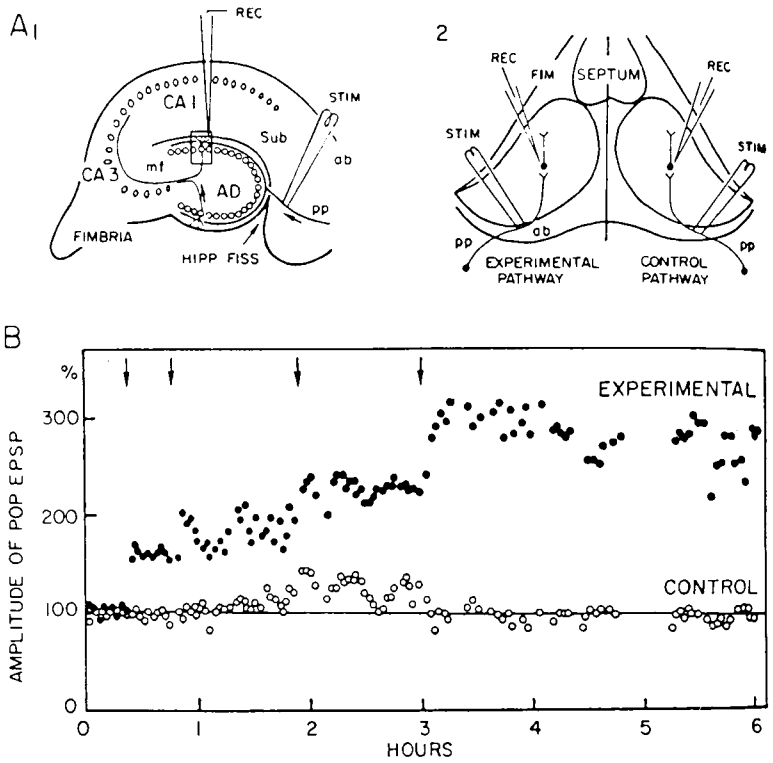

Figure 8. The Phenomenon of Long-Term Potentiation

Long-lasting posttetanic potentiation of the hippocampus.
(A) (1) A diagrammatic view of a parasagittal section of the hippocampus showing a stimulating electrode placed beneath the angular bundle (ab) to activate perforant pathway fibers (PP) and a recording microelectrode in the molecular layer of the dentate area (AD). Hipp Fiss, hippocampal fissure; Stim, stimulatory electrode; Rec, recording electrode; Fim, fimbrial. (2) Arrangement of electrodes for stimulation of the experimental pathway and the control pathway (in the contralateral hippocampus).
(B) Amplitude of the population of excitatory postsynaptic potential (EPSP) for the experimental pathway (filled dots) and ipsilateral control pathway (open dots) as a function of time and of conditioning impulse trains (15/s for 10 s) indicated by arrows. Each value is a computed average of 30 responses. Values are plotted as a percentage of the mean preconditioning value of the population (POP) EPSP. (From Bliss and Lømo, 1973.)

that for each of these forms of memory there are at least two temporally distinct phases: a short-term memory lasting minutes and a long-term memory lasting days or longer (Milner, 1965; Milner et al., 1998). These two phases differ not only in their time course, but also in their molecular mechanism: long-term but not short-term memory requires the synthesis of new proteins. Molecular studies in *Aplysia* and in mice have revealed that these distinct stages in behavioral memory are reflected in distinct molecular phases of synaptic plasticity (Montarolo et al., 1986; Bourtchouladze et al., 1994; Abel et al., 1997). In *Aplysia*, these stages have been particularly well studied in the context of sensitization, a form of learning in which an animal strengthens its reflex responses to previously neutral stimuli, following the presentation of an aversive stimulus (Carew et al., 1983; Byrne and Kandel, 1996; Squire and Kandel, 1999). The short- and long-term behavioral memory for sensitization is mirrored by the short- and long-term strengthening of the synaptic connections between the sensory neuron and the motor neuron that mediate this reflex. In this set of connections, serotonin, a neurotransmitter released in vivo by interneurons activated by sensitizing stimuli leads to a short-term synaptic enhancement, lasting minutes, which results from a covalent modification of preexisting proteins mediated by the cAMP-dependent protein kinase A (PKA) and by protein kinase C (PKC). By contrast, facilitation lasting several days results from the translocation of PKA and mitogen-activated protein kinase (MAPK) to the nucleus of the sensory neurons where these kinases activate CREB1 and derepress CREB2, leading to the induction of a set of immediate response genes and ultimately resulting in the growth of new synaptic connections (Bartsch et al., 1995, 1998).

A similar cascade of gene induction is recruited for nondeclarative memory storage in *Drosophila* (Yin et al., 1995; Yin and Tully, 1996; Dubnau and Tully, 1998) and for spatial and object recognition memory, forms of declarative (explicit) memory storage that can be studied in mice (Bourtchouladze et al., 1994; Impey et al., 1996, 1998, 1999; Abel et al., 1997; Silva et al., 1998) indicating that this set of mechanisms may prove to be quite general. In both *Aplysia* and mice, experimental manipulations that reduce the level of the repressor CREB2 or enhance the level of the activator CREB1 act to enhance synaptic facilitation and amplify memory storage (Bartsch et al., 1995; Yin et al., 1995). Thus, this set of mechanisms may prove to be quite general and to apply to instances of both declarative and nondeclarative memory in both vertebrates and invertebrates.

The requirement for transcription provided a provisional molecular explanation for the behavioral observation that long-term memory requires the synthesis of new proteins. This requirement, however, posed a cell biological problem: how can the activation of genes in the nucleus lead to long-lasting changes in the connectivity of those synapses that are active and not in inactive synapses? Recent studies have shown that this synapse-specific, spatially restricted plasticity requires both the activity of the activator CREB1 in the nucleus as well as local protein synthesis in those processes of the sensory cell exposed to serotonin (Martin et al., 1998; Casadio et al., 1999).

This synapse-specific facilitation can be captured by another synapse of the neuron. Once synapse-specific long-term facilitation has been initiated, stimuli which per se induce only transient facilitation are able to recruit long-term facilitation and the growth of new connections when applied to a second branch (Martin et al., 1998;

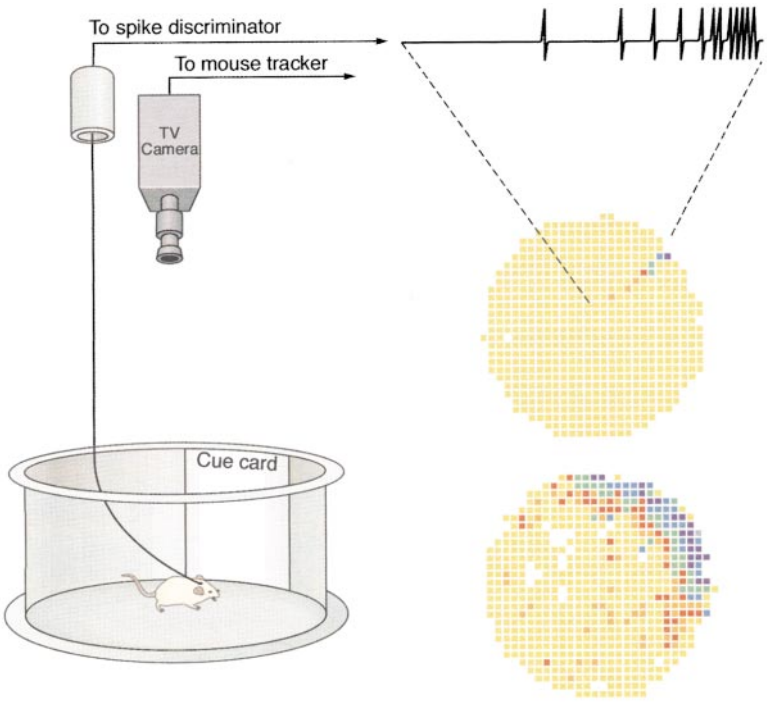

Figure 9. The Detection of Place Field Cells in the Mammalian Hippocampus

(A) A recording chamber used to record the firing patterns of place cells. The head of a mouse inside the chamber is attached to a recording cable that is attached to a device able to resolve the timing of action potentials ("spikes") from one or more CA1 pyramidal (place) cells. As the mouse explores the chamber, the location of a light attached to its head is recorded by an overhead TV camera. Its output goes to a tracking device that detects the position of the mouse. The occurrence of spikes as a function of position is extracted and used to form two-dimensional firing-rate patterns that can be analyzed quantitatively or visualized as color-coded firing-rate maps.

(B) The firing patterns from a recording session of a single CA1 hippocampal pyramidal place cell. Darker colors (violet or red) indicate high rates of firing and lighter color (yellow) indicates a low firing rate. Before the recording session the animal was moved and then reintroduced into the circular enclosure. During the recording session, the mouse explores all areas of the enclosure equally. However, each place cell fires only when the mouse is in a specific location. Each time the mouse is returned to the chamber, place cells fire when the animal occupies the same locations that fired those cells previously. The firing pattern for a given cell from a wild-type mouse is stable. (Courtesy of R. Muller.)

Casadio et al., 1999). A similar capture of long-term synaptic plasticity has been found in the hippocampus by Frey and Morris (1997). As we have seen, the hippocampus, a region essential for declarative memory, is involved in the storage of memory for objects and space (Milner et al., 1998). In 1973 Tim Bliss and Terje Lømo made the remarkable discovery that major synaptic pathways in the hippocampus, including the Schaeffer collateral pathway, undergo a long-term form of synaptic plasticity (long-term potentiation or LTP) in response to a burst of high-frequency stimulation (Figure 8). Subsequent studies by Graham Collingridge, Roger Nicoll, and others found that LTP in the Schaeffer collateral pathway depends on activation of an NMDA receptor to glutamate in the postsynaptic cell (the pyramidal cell of the CA1 region), resulting in an influx of Ca^{2+} and an activation of the Ca^{2+} calmodulin-dependent protein kinase IIα (CaMKIIα) (see Collingridge and Bliss, 1995 for review).

The correlation between LTP in the Schaeffer collateral pathway and spatial memory is not perfect (see for example Zamanillo et al., 1999 for an important dissociation). Nevertheless, a variety of experiments have found that interfering with LTP in this pathway (by means of gene knockouts of the NMDA receptor or by the expression of dominant-negative transgenes) commonly interferes both with the representation of space by the neurons of the hippocampus (place cells) and with memory for space in the intact animal (Figure 9) (Tsien et al., 1996; Mayford and Kandel, 1999). Moreover, enhancing LTP in the Schaeffer collateral pathway enhances memory storage for a variety of declarative tasks (Han and Stevens, 1999; Tang et al., 1999).

Despite these initial attempts to link LTP to behavioral memory storage, we still lack a satisfactory knowledge about most key facets of hippocampal synaptic plasticity in relationship to memory storage. For example, the facilitation used experimentally to induce LTP involves frequencies of firing that are unlikely to be used normally. The form of LTP used in most experiments therefore is best viewed as a marker for a general capability for synaptic plasticity. How the animal actually uses this capability is not yet known. In addition, although there is agreement that LTP is induced postsynaptically (by the activation of the NMDA receptor and consequent Ca^{2+} influx), there is no consensus on whether the mechanisms of expression are postsynaptic or presynaptic. The persistence of this lack of consensus suggests, as one possibility, that the mechanism for expression of LTP is complex and involves a coordinated pre- and postsynaptic mechanism. Finally, the hippocampus is only one component of a larger medial temporal cortical system. How the components of this system interact and how they relate to neocortical sites of storage is entirely unknown.

A Future for the Study of Neuronal Signaling

Molecular Structure, Molecular Machines, and the Integration of Signaling Pathways

During the last four decades, we have gained great insight from the reductionist approach to neuronal signaling and synaptic plasticity. The molecular characterization of voltage- and ligand-channels and of the many G protein–coupled receptors that we have gained has dramatically advanced the initial insights of Hodgkin,

Huxley, and Katz, and has revealed a structural unity among the various molecules involved in neural signaling. Elucidation of the primary sequence of these proteins also immediately revealed a commonality in the signaling functions of proteins in neurons and those of other cells. For example, many of the proteins involved in synaptic vesicle exocytosis are used for vesicle transport and for secretion in other cells including yeast. Conversely, bacteriorhodopsin, a bacterial membrane protein has proven to be the structural prototype for understanding G protein–coupled seven transmembrane-spanning receptors such as those that are activated by light, odorants, and chemical transmitters. Receptors of this class come into play during certain forms of learning and memory, and may even be important in primates for aspects of arousal and attention.

Although we are now only beginning to enter the era of the structural biology of voltage and of ligand-gated channels, we already appreciate that the existing molecular understanding of receptors and of ion channels is remarkably good. In retrospect, however, the obstacles confronted in the study of channels and receptors were comparatively straightforward. The essential properties of receptors and channels are contained within a single molecular entity, and these functions had been well characterized by earlier biophysical and protein chemical studies. Thus, the initial information about primary protein sequence was immediately informative in generating models of transmembrane protein topography and in defining domains that represent the voltage sensor, the ligand-binding domain, the pore, and the inactivation gate. Subsequent site-directed mutagenesis permitted rapid tests of these early predictions, tests that proved surprisingly informative because the structure of channels and receptors predicted the existence of distinct modular domains.

But we now know that many of these receptors, such as the NMDA and AMPA receptors for glutamate, do not function alone, but possess specialized cytoplasmic protein domains that serve as platforms for assembling protein machines important for signaling. Thus, in shifting the focus of analysis from the ion channel to cytoplasmic signaling, we are entering a more complex arena of protein–protein interaction and in the interaction between different intracellular signaling pathways where function depends less on the properties of single molecules and intramolecular rearrangement and more on the coordination of a series of molecular events.

Fortunately, in the search for some of the components of these multimolecular machines, such as the presynaptic proteins important for the targeting and docking of vesicles at release sites and the assembly of the molecular machinery for fusion and exocytosis, the study of synaptic transmission will be aided by parallel studies in other areas of cell biology, such as membrane trafficking and viral and cellular fusion events in nonneuronal systems. Thus, despite the new realities and complexities that confront the study of cytoplasmic signaling and transmitter release, it seems safe to predict that these problems will be solved in the near future and that the romantic phase of neuronal signaling, synaptic transmission, and synaptic plasticity will reach closure in the first decades of the twenty-first century.

The great challenge for a reductionist approach in the subsequent decades of the twenty-first century will be of two sorts, first in its application to disease states, and second in its ability to contribute to the analysis of brain systems important for cognition.

Molecular Biology of Disease

During the last two decades, we have made remarkable progress in analyzing genes important for neurological disorders, especially monogenic diseases. That this progress has been so dramatic encourages one to believe that within the next decade the corpus of neurology may be transformed (for review see Cowan et al., 1999). By contrast, progress in understanding the complex polygenic diseases that characterize psychiatry has been noticeably slower.

The analyses of monogenic diseases dates to the beginning of the twentieth century, but it accelerated markedly in 1989 when Louis Kunkel and his associates first succeeded in cloning the gene for Duchenne's muscular dystrophy and found that the protein that it encodes, *dystrophin*, is homologous to α-actinin and spectrin, two cytoskeletal proteins found on the inner surface of the plasma membrane of muscle (Hoffman et al., 1987; Hoffman and Kunkel, 1989). Kunkel and his associates were able to show that in severe forms of Duchenne's dystrophy the dystrophic protein (dystrophin) is lacking completely, whereas in a milder form, Becker dystrophy, functional protein is present but in much reduced amounts. Kevin Campbell and his colleagues extended this work importantly by showing that dystrophin is only one component of a larger complex of glycoproteins (the *dystroglycoprotein complex*) that links the cytoskeleton of the sarcoplasm to the extracellular matrix (Straub and Campbell, 1997).

A second major step in the analysis of monogenic diseases was taken in 1993 when James Gusella, Nancy Wexler, and their colleagues in the Huntington's Disease Collaborative Research Group isolated the gene responsible for Huntington's disease. In so doing they discovered that the gene contains an extended series of CAG repeats, thereby placing it together with a number of other important neurological diseases in a new class of disorders: the *trinucleotide repeat diseases*. These repeats were first encountered in the gene responsible for the Fragile X form of mental retardation (Kremer et al., 1991, Verkerk et al., 1991). Subsequently, other hereditary disorders of the nervous system were found to have similar repeats. Together, the trinucleotide repeat disorders now constitute the largest group of dominantly transmitted neurological diseases (for review see Pauls and Fischbeck, 1996; Reddy and Housman, 1997; Ross, 1997). Based on the nature of their repeats, the trinucleotide repeat disorders can be divided into two groups: type I and type II (Paulson and Fischbeck, 1996).

In *type I disorders*, which include Huntington's disease, the number of CAG repeats usually does not exceeding 90. The repeats lie within the coding region of the gene, are translated as polyglutamine runs, and seem to cause disease by a gain-of-function mechanism. The observation that the glutamine repeats form β sheets consisting of 6 to 8 residues per strand suggested to Max Perutz and his colleagues that the repeats could act as a polar zipper that binds and traps other copies of either the same protein or other proteins (Perutz et al., 1994). This trapping might not only prevent

the protein from functioning normally, but also could form large aggregates that may be toxic to the cells. In the case of Huntington's disease, Perutz postulated that the accumulation of huntingtin in neurons might lead to the formation of toxic protein aggregates, similar to those observed in Alzheimer's disease or certain prion disorders. Recent studies have indeed shown the existence of such nuclear aggregates, although whether such aggregates reflect the cause or the consequence of the disease remains an unsolved issue.

Type II repeat disorders, which include Fragile X, have repeats found in either the 5′ or 3′ untranslated regulatory regions of the gene that result in the mRNA and protein not being expressed. In Fragile X, for example, the FMR1 protein is not expressed. The wild-type protein contains RNA-binding motifs (Warren and Ashley, 1995) and in one severely retarded patient the mutation is not in the regulatory region but in the coding region. Here a single point mutation in one of the RNA-binding domains is sufficient to cause the disease. These disorders are manifest by attenuated or absent expression of the gene, and the disorder is not progressive but remains fixed from early development onward.

Even in the case of these monogenic neurodegenerative disorders, however, the problem of defining the molecular basis of the disease does not stop with the identification of mutant genes. For several familial forms of neurological diseases, notably Parkinson's disease and amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) the identification of mutant protein isoforms has not yet resulted in a clearer understanding of the cellular basis of the disease. For example, our appreciation of the fact that gain-of-function mutations in the superoxide dismutase 1 (SOD1) protein underlie certain familial forms of ALS has not revealed the nature of the alteration in the function of this protein. Similarly, the identification of mutated forms of synuclein and Parkin proteins responsible for certain familial cases of Parkinson's disease has left unresolved the issue of how altered forms of these proteins lead to the degeneration of mesencephalic dopaminergic neurons. In addition, for these two disorders and for Huntington's disease, the miscreant proteins are widely expressed by virtually all neurons in the central nervous system, yet in each disease quite distinct classes of neurons undergo degeneration. The advent of more refined methods for translating the information revealed through genomic sequencing to biochemical information about the function of specific proteins in individual classes of neurons, the so-called proteomic approaches, appears to offer considerable promise in resolving these critical issues.

Of all the monogenic diseases, perhaps the most spectacular progress has been made in elucidating the defects that underlie the hereditary myotonias, periodic paralysis, and certain forms of epilepsy. These defects have now been shown to reside in one or another voltage- or ligand-gated ion channels of muscle. These disorders therefore are now referred to as the *channelopathies*—disorders of ion channel function (for review see Brown 1993; Ptácek, 1997, 1998; Cowan et al., 1999). As can be inferred from our earlier discussions, the remarkable progress in understanding these diseases can be attributed directly to the extensive knowledge about ion channel function that was already available.

For example, hyperkalemic periodic paralysis and paramyotonia congenita, two channelopathies due to ion channel disorders that result from mutations in the α subunit of the Na^+ channel, are caused by a number of slightly different dominant mutations that make the Na^+ channel hyperactive by altering the inactivation mechanisms either by changing the voltage dependency of Na^+ activation or by slowing the coupling of activation and inaction (for review see Brown, 1993; Ptácek et al., 1997). As was already evident from earlier physiological studies, rapid and complete inactivation of the Na^+ channel is essential for normal physiological functioning of nerve and muscle cells (Catterall, 2000). These mutations do not occur randomly but in three specific regions of the channel: the inactivation gate, the inactivation gate receptor, and the voltage sensor regions that have been shown to be functionally important by the earlier biophysical and molecular studies.

In contrast to these particular monogenic diseases, the identification of the genetic basis of other degenerative neurological disorders has been slower. Nevertheless in some complex diseases such as Alzheimer's disease, appreciable progress has been made recently. This disease begins with a striking loss of memory and is characterized by a substantial loss of neurons in the cerebral cortex, the hippocampus, the amygdala, and the nucleus basalis (the major source of cholinergic input to the cortex). On the cellular level, the disease is distinguished by two lesions: (1) there is an extracellular deposition of neuritic plaques; these are composed largely of β-amyloid (Aβ), a 42/43–amino acid peptide; and (2) there is an intracellular deposition of neurofibrillary tangles; these are formed by bundles of paired helical filaments made up of the microtubule-associated protein Tau. Three genes associated with familial Alzheimer's disease have been identified: (1) the gene encoding the β-amyloid precursor protein (APP), (2) presenilin 1, and (3) presenilin 2.

The molecular genetic study of Alzheimer's disease has also provided us with the first insight into a gene that modifies the severity of a degenerative disease. The various alleles of the APO E gene serve as a bridge between monogenic disorders and the complexity we are likely to encounter in polygenic disorders. As first shown by Alan Roses and his colleagues, one allele of apolipoprotein E (APO E4) is a significant risk factor for late onset Alzheimer's disease, acting as a dose-dependent modifier of the age of onset (Strittmatter and Roses, 1996).

The findings with APO E4 stand as a beacon of hope for the prospect of understanding the much more difficult areas of psychiatric disorders. Here the general pace of progress has been disappointing for two reasons. First, the diseases that characterize psychiatry, diseases such as schizophrenia, depression, bipolar disorder, and anxiety states, tend to be complex, polygenic disorders. Second, even prior to the advent of molecular genetics, neurology had already succeeded in localizing the major neurological disorders to various regions of the brain. By contrast, we know frustratingly little about the anatomical substrata of most psychiatric diseases. A reliable neuropathology of mental disorders is therefore severely needed.

Systems Problems in the Study of Memory and Other Cognitive States

As these arguments about anatomical substrata of psychiatric illnesses make clear, neural science in the long run faces problems of understanding aspects of biology of normal function and of disease, the complexity of which transcends the individual cell and involves the computational power inherent in large systems of cells unique to the brain.

For example, in the case of memory, we have here only considered the cell and molecular mechanisms of memory storage, mechanisms that appear to be shared, at least in part, by both declarative and nondeclarative memory. But, at the moment, we know very little about the much more complex systems problems of memory: how different regions of the hippocampus and the medial temporal lobe—the subiculum, the entorrhinal, parahippocampal, and perirhinal cortices—participate in the storage of nondeclarative memory and how information within any one of these regions is transferred for ultimate consolidation in the neocortex. We also know nothing about the nature of recall of declarative memory, a recall that requires conscious effort. As these arguments and those of the next sections will make clear, the systems problems of the brain will require more than the bottom–up approach of molecular and developmental biology; it will also require the top–down approaches of cognitive psychology, neurology, and psychiatry. Finally, it will require a set of syntheses that bridge between the two.

Part III. The Assembly of Neuronal Circuits

The primary goal of studies in developmental neurobiology has been to clarify the cellular and molecular mechanisms that endow neurons with the ability to form precise and selective connections with their synaptic partners—a selectivity that underlies the appropriate function of these circuits in the mature brain. Attempts to explain how neuronal circuits are assembled have focused on four sequential developmental steps. Loosely defined, these are: the specification of distinct neuronal cell types; the directed outgrowth of developing axons; the selection of appropriate synaptic partners; and finally, the refinement of connections through the elimination of certain neurons, axons, and synapses. In recent years, the study of these processes has seen enormous progress (Cowan et al., 1997), and to some extent, each step has emerged as an experimental discipline in its own right.

In this section of the review, we begin by describing some of the major advances that have occurred in our understanding of the events that direct the development of neuronal connections, focusing primarily on the cellular and molecular discoveries of the past two decades. Despite remarkable progress, however, a formidable gap still separates studies of neuronal circuitry at the developmental and functional levels. Indeed, in the context of this review it is reasonable to question whether efforts to unravel mechanisms that control the development of neuronal connections have told us much about the functions of the mature brain. And similarly, it is worth considering whether developmental studies offer any prospect of providing such insight in the foreseeable future. In discussing the progress of studies on the development of the nervous system, we will attempt to indicate why such a gap exists and to describe how recent technical advances in the ability to manipulate gene expression in developing neurons may provide new experimental strategies for studying the function of intricate circuits embedded in the mature brain. In this way it should be possible to forge closer links between studies of development and systems-oriented approaches to the study of neural circuitry and function.

The Emergence of Current Views of the Formation of Neuronal Connections

Current perspectives on the nature of the complex steps required for the formation of neuronal circuits have their basis in many different experimental disciplines (Cowan, 1998). We begin by discussing separately, some of the conceptual advances in understanding how the diversity of neuronal cell types is generated, how the survival of neurons is controlled and how different classes of neurons establish selective pathways and connections.

Inductive Signaling, Gene Expression, and the Control of Neuronal Identity

The generation of neuronal diversity represents an extreme example of the more general problem of how the fates of embryonic cells are specified. Extreme in the sense that the diversity of neuronal cell types, estimated to be in the range of many hundreds (Stevens, 1998), far exceeds that for other tissues and organs. Nevertheless, as with other cell types, neural cell fate is now known to be specified through the interplay of two major classes of factors. The first class constitutes cell surface or secreted signaling molecules that, typically, are provided by localized embryonic cell groups that function as organizing centers. These secreted signals influence the pathway of differentiation of neighboring cells by activating the expression of cell-intrinsic determinants. In turn, these determinants direct the expression of downstream effector genes, which define the later functional properties of neurons, in essence their identity. Tracing the pathways that link the action of secreted factors to the expression and function of cell-intrinsic determinants thus lies at the core of attempts to discover how neuronal diversity is established.

The first contribution that had a profound and long-lasting influence on future studies of neural cell fate specification was the organizer grafting experiment of Hans Spemann and Hilde Mangold, performed in the early 1920s (Spemann and Mangold, 1924). Spemann and Mangold showed that naive ectodermal cells could be directed to generate neural cells in response to signals secreted by cells in a specialized region of the gastrula stage embryo, termed the organizer region. Transplanted organizer cells were shown to maintain their normal mesodermal fates but were able to produce a dramatic change in the fate of neighboring host cells, inducing the formation of a second body axis that included a well-developed and duplicated nervous system.

Spemann and Mangold's findings prompted an intense, protracted, and initially unsuccessful search for

the identity of relevant neural inducing factors. The principles of inductive signaling revealed by the organizer experiment were, however, extended to many other tissues, in part through the studies of Clifford Grobstein, Norman Wessells, and their colleagues in the 1950s and 1960s (see Wessells, 1977). These studies introduced the use of in vitro assays to pinpoint sources of inductive signals, but again failed to reveal the molecular nature of such signals.

Only within the past decade or so has any significant progress been made in defining the identity of such inductive factors. One of the first breakthroughs in assigning a molecular identity to a vertebrate embryonic inductive activity came in the late 1980s through the study of the differentiation of the mesoderm. An in vitro assay of mesodermal induction developed by Peter Nieuwkoop (see Nieuwkoop, 1997; Jones and Smith, 1999) was used by Jim Smith, Jonathan Cooke, and their colleagues to screen candidate factors and to purify conditioned tissue culture media with inductive activity. This search led eventually to the identification of members of the fibroblast growth factor and transforming growth factor β (TGFβ) families as mesoderm-inducing signals (Smith, 1989).

Over the past decade, many assays of similar basic design have been used to identify candidate inductive factors that direct the formation of neural tissue and specify the identity of distinct neural cell types. The prevailing view of the mechanism of neural induction currently centers on the ability of several factors secreted from the organizer region to inhibit a signaling pathway mediated by members of the TGFβ family of peptide growth factors (see Harland and Gerhart, 1977). The function of TGFβ proteins, when not constrained by organizer-derived signals, appears to be to promote epidermal fates at the expense of neural differentiation. The constraint on TGFβ-related protein signaling appears to be achieved in part by proteins produced by the organizer such as noggin and chordin that bind to and inhibit the function of secreted TGFβ-like proteins. Other candidate neural inducers may act instead by repressing the expression of TGFβ-like genes. However, even now, the identity of physiologically relevant neural inducing factors and the time at which neural differentiation is initiated remain matters of debate.

Some of the molecules involved in the specification of neuronal subtype identity, notably members of the TGFβ, Fibroblast growth factor, and Hedgehog gene families, have also been identified (Lumsden and Krumlauf, 1996; Tanabe and Jessell, 1996). These proteins have parallel functions in the specification of cell fate in many nonneural tissues. Thus, the mechanisms used to induce and pattern neuronal cell types appear to have been co-opted from those employed at earlier developmental stages to control the differentiation of other cells and tissues. Some of these inductive signals appear to be able to specify multiple distinct cell types through actions at different concentration thresholds—the concept of gradient morphogen signaling (Wolpert, 1969; Gurdon et al., 1998). In the nervous system, for example, signaling by Sonic hedgehog at different concentration thresholds appears sufficient to induce several distinct classes of neurons at specific positions along the dorsoventral axis of the neural tube (Briscoe and Ericson, 1999).

The realization that many different neuronal cell types can be generated in response to the actions of a single inductive factor has placed added emphasis on the idea that the specification of cell identity depends on distinct profiles of gene expression in target cells. Such specificity in gene expression may be achieved in part through differences in the initial signal transduction pathways activated by a given inductive signal. But the major contribution to specificity appears to be the selective expression of different target genes in cell types with diverse developmental histories and thus different responses to the same inductive factor.

The major class of proteins that possess cell-intrinsic functions in the determination of neuronal fate are transcription factors: proteins with the capacity to interact directly or indirectly with DNA and thus to regulate the expression of downstream effector genes. The emergence of the central role of transcription factors as determinants of neuronal identity has its origins in studies of cell patterning in nonneural tissues and in particular in the genetic analysis of pattern formation in the fruit fly *Drosophila*. The pioneering studies of Edward Lewis on the genetic control of the *Drosophila* body plan led to the identification of genes of the *HOM-C* complex, members of which control tissue pattern in individual domains of the overall body plan (see Lewis, 1985). Lewis further showed that the linear chromosomal arrangement of *HOM-C* genes correlates with the domains of expression and function of these genes during *Drosophila* development. Subsequently, Christine Nüsslein-Vollhard and Eric Weischaus performed a systematic series of screens for embryonic patterning defects and identified an impressive array of genes that control sequential steps in the construction of the early embryonic body plan (Nüsslein-Volhard and Wieschaus, 1980). The genes defined by these simple but informative screens could be ordered into hierarchical groups, with members of each gene group controlling embryonic pattern at a progressively finer level of resolution (see St. Johnston and Nüsslein-Volhard, 1992).

Advances in recombinant DNA methodology permitted the cloning and structural characterization of the *HOM-C* genes and of the genes controlling the embryonic body plan. The genes of the *HOM-C* complex were found to encode transcription factors that share a 60–amino acid DNA-binding cassette, termed the homeodomain (McGinnis et al., 1984; Scott and Weiner, 1984). Many of the genes that control the embryonic body plan of *Drosophila* were also found to encode homeodomain transcription factors and others encoded members of other classes of DNA-binding proteins. The product of many additional genetic screens for determinants of neuronal cell fate in *Drosophila* and *C. elegans* led notably to the identification of basic helix-loop-helix proteins as key determinants of neurogenesis (Chan and Jan, 1999). In the process, these screens reinforced the idea that cell-specific patterns of transcription factor expression provide a primary mechanism for generating neuronal diversity during animal development.

The cloning of *Drosophila* and *C. elegans* developmental control genes was soon followed by the identification of structural counterparts of these genes in vertebrate organisms, in the process revealing a remarkable and somewhat unanticipated degree of evolutionary conservation in developmental regulatory programs. The identification of over thirty different families of vertebrate transcriptional factors, each typically comprising tens of individual family members (see Bang and Goulding, 1996), has provided a critical molecular insight into the extent of neural cell diversity during vertebrate development. Prominent amongst these are the homeodomain protein counterparts of many *Drosophila* genes. Vertebrate homeodomain proteins have now been implicated in the control of regional neural pattern, neural identity, axon pathfinding, and the refinement of exuberant axonal projections. The individual or combinatorial profiles of expression of transcription factors may soon permit the distinction of hundreds of embryonic neuronal subsets.

Genetic studies in mice and zebrafish have demonstrated that a high proportion of these genes have critical functions in establishing the identity of the neural cells within which they are expressed. In many cases, the classes of embryonic neurons defined on the basis of differential transcription factor expressions have also been shown to be relevant to the later patterns of connectivity of these neurons. Because of these advances, the problem of defining the mechanisms of cell fate specification in the developing nervous system can now largely be reduced to the issue of tracing the pathway that links an early inductive signal to the profile of transcription factor expression in a specific class of postmitotic neuron—a still daunting, but no longer unthinkable task.

Control of Neuronal Survival

The tradition of experimental embryology that led to the identification of inductive signaling pathways has also had a profound impact on studies of a specialized, if unwelcome, fate of developing cells: their death.

Many cells in the nervous system and indeed throughout the entire embryo are normally eliminated by a process of cell death. The recognition of this remarkable feature of development has its origins in embryological studies of the influence of target cells on the control of the neuronal number. In the 1930s and 1940s, Samuel Detwiler, Viktor Hamburger, and others showed that the number of sensory neurons in the dorsal root ganglion of amphibian embryos was increased by transplantation of an additional limb bud and decreased by removing the limb target (Detwiler, 1936). The target-dependent regulation of neuronal number was initially thought to result from a change in the proliferation and differentiation of neuronal progenitors. A then-radical alternative view, proposed by Rita Levi-Montalcini and Viktor Hamburger in the 1940s, suggested that the change in neuronal number reflected instead an influence of the target on the survival of neurons (Hamburger and Levi-Montalcini, 1949). For example, about half of the motor neurons generated in the chick spinal cord are destined to die during embryonic development. The number that die can be increased by removing the target and reduced by adding an additional limb (Hamburger, 1975). The phenomenon of neuronal overproduction and its compensation through cell death is now known to occur in almost all neuronal populations within the central and peripheral nervous systems (Oppenheim, 1981).

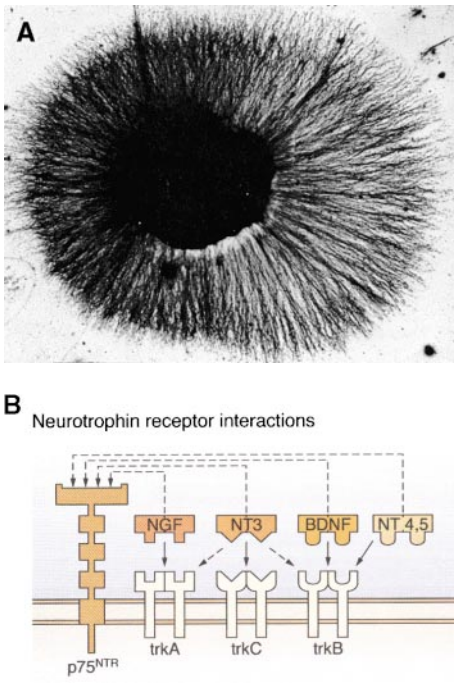

Figure 10. Growth Factors and Their Receptors

(A) The trophic actions of nerve growth factor on dorsal root ganglion neurons. Photomicrographs of a dorsal root ganglion of a 7-day chick embryo that had been cultured in medium supplemented with nerve growth factor for 24 hr. Silver impregnation. The extensive outgrowth of neurites is not observed in the absence of nerve growth factor. (From studies of R. Levi-Montalcini.) (Courtesy AAAS.)

(B) The actions of neurotrophins depend on interactions with Trk tyrosine kinase receptors. Neurotrophins interact with tyrosine kinase receptors of the trk class. The diagram illustrates the interactions of members of the neurotrophin family with distinct trk proteins. Strong interactions are depicted with solid arrows; weaker interactions with broken arrows. In addition, all neurotrophins bind to a low-affinity neurotrophin receptor $p75^{NTR}$. Abbreviations: NGF = nerve growth factor; NT = neurotrophin; BDNF = brain-derived neurotrophic factor. (Adapted from Reichardt and Fariñas, 1997 by Kandel et al., 2000.)

The findings of Levi-Montalcini and Hamburger led to the formulation of the *neurotrophic factor hypothesis*: the idea that the survival of neurons depends on essential nutrient or trophic factors that are supplied in limiting amounts by cells in the environment of the developing neuron, often its target cells (see Oppenheim, 1981). This hypothesis prompted Levi-Montalcini and Stanley Cohen to undertake the purification of a neurotrophic activity, an ambitious quest, but one that led eventually to the identification of nerve growth factor, the first peptide growth factor and a protein whose existence dramatically supported the neurotrophic factor hypothesis (Levi-Montalcini, 1966; Hamburger, 1993) (Figure 10A). The isolation of NGF was a milestone in the study of growth factors and in turn, motivated searches for additional neurotrophic factors. The efforts of Hans Thoenen, Yves Barde, and others revealed NGF is but the vanguard member of a large array of secreted factors that

possess the ability to promote the survival of neurons (Reichardt and Fariñas, 1997).

The best studied class of neurotrophic factors, which includes NGF itself, are the neurotrophins. Work by Mariano Barbacid, Luis Parada, Eric Shooter, and others subsequently showed that neurotrophin signaling is mediated by the interaction of these ligands with a class of membrane-spanning tyrosine kinase receptors, the trk proteins (see Reichardt and Fariñas, 1997) (Figure 10B). Nerve growth factor interacts selectively with trkA, and other neurotrophins interact with trkB and trkC. Other classes of proteins that promote neuronal survival include members of the TGFβ family, the interleukin 6–related cytokines, fibroblast growth factors, and hedgehogs (Pettmann and Henderson, 1998). Thus, classes of secreted proteins that have inductive activities at early stages of development can also act later to control neuronal survival. Neurotrophic factors were initially considered to promote the survival of neural cells through their ability to stimulate cell metabolism. Quite the contrary. Such factors are now appreciated to act predominantly by suppressing a latent cell suicide program. When unrestrained by neurotrophic factor signaling, this suicide pathway kills cells by *apoptosis*, a process characterized by cell shrinkage, the condensation of chromatin, and eventually cell disintegration (Jacobson et al., 1997; Pettmann and Henderson, 1998).

A key insight into the biochemical machinery driving this endogenous cell death program emerged from genetic studies of cell death in *C. elegans* by Robert Horvitz and his colleagues (Hengartner and Horvitz, 1994; Metzstein et al., 1998). Over a dozen cell death (*ced*) genes have now been ordered in a pathway that controls cell death in *C. elegans*. Of these genes two, *ced-3* and *ced-4,* have pivotal roles. The function of both genes is required for the death of all cells that are normally fated to die by apoptosis. A third key gene, *ced-9*, antagonizes the activities of *ced-3* and *ced-4*, thus protecting cells from death. Remarkably, this death pathway is highly conserved in vertebrate cells. The *ced-3* gene encodes a protein closely related to members of the vertebrate family of caspases, cysteine proteases that function as cell death effectors by degrading target proteins essential for cell viability. The *ced-4* gene encodes a protein structurally related to another vertebrate apoptosis-promoting factor, termed Apaf-1. The *ced-9* gene encodes a protein that is structurally and functionally related to the Bcl-2 like proteins, some of which also act to protect vertebrate cells from apoptotic death. Apaf-like proteins appear to promote the processing and activation of caspases, whereas, some Bcl-2 like proteins interact with Apaf-1/ced-4 and in so doing, inhibit the processing and activation of caspases.

These findings have revealed a core biochemical pathway that regulates the survival of cells and which is thought to serve as the intracellular target of neurotrophic factors. The practical significance of this core cell death pathway has not escaped attention. Pharmacological strategies to inhibit caspase activation are now widely sought after in attempts to prevent the apoptotic neuronal death that accompanies many neurodegenerative disorders.

Axonal Projections and the Formation of Selective Connections

Attempts to unravel how selective neuronal connections are formed in the developing brain have a somewhat different provenance. The electrophysiological studies of John Langley, Charles Sherrington, and others at the turn of the twentieth century, as discussed earlier, had revealed the exquisite selectivity with which mature neuronal circuits function (Langley, 1897; Sherrington, 1906) and in the process provided an early hint that their formation may also be a selective process. In parallel, histological studies of the developing brain, applied most decisively by Ramón y Cajal but also by many others, provided dramatic illustration of embryonic neurons captured in the process of extending dendrites and axons, apparently in a highly stereotyped manner (see Ramón y Cajal, 1911). These pioneering anatomical descriptions provided circumstantial but persuasive evidence that the assembly of neuronal connections is orchestrated in a highly selective manner. By the middle of the twentieth century, many elegant in vivo observations in simple vertebrate organisms had further shown that developing axons extend in a highly reproducible fashion (see Speidel, 1933). But even these findings did not result in general acceptance of the idea that the specificity evident in mature functional connections had its basis in selective axonal growth and in selective synapse formation.

An alternative view, advanced most forcefully by Paul Weiss in the 1930s and 1940s, and termed the *resonance hypothesis*, argued instead that axonal growth and synapse formation were largely random events, with little inherent predetermination (see Weiss, 1941). Advocates of the resonance view proposed instead that the specificity of mature circuits emerges largely through the elimination of functionally inappropriate connections, and only at a later developmental stage. This extreme view, however, became gradually less tenable in the light of experiments by Roger Sperry, notably on the formation of topographic projections in the retino-tectal system of lower vertebrates. Sperry's studies revealed a high degree of precision in the topographic order of retinal axon projections to the tectum during normal development and further established that this topographic specificity is maintained after experimental rotation of the target tectal tissue—a condition in which the maintenance of an anatomically appropriate connection results in a behaviorally defective neuronal circuit (Sperry, 1943; see Hunt and Cowan, 1990) (Figure 11). Over the subsequent two decades, the consolidation of these early findings led Sperry, in the 1960s, to formulate the chemoaffinity hypothesis (Sperry, 1963), a general statement to the effect that the most plausible explanation for the selectivity apparent in the formation of developing connections is a precise system of matching of chemical labels between pre- and postsynaptic neuronal partners.

Sperry's studies also emphasized the utility of combining embryological manipulation and neuroanatomical tracing methods to probe the specificity of neuronal connectivity. This tradition was extended in the 1970s by Lynn Landmesser and her colleagues to demonstrate the specificity of motor axon projections in vertebrate embryos (Lance-Jones and Landmesser, 1981) and by

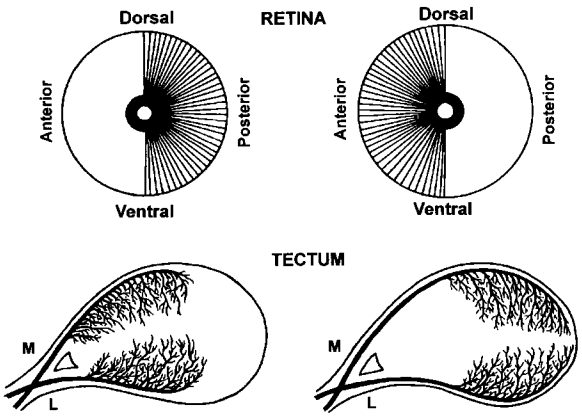

Figure 11. Sperry's Demonstration of Topographically Specific Retinotectal Projections

Anatomical evidence for retinal axon regeneration to original sites of termination in the optic tectum. Sperry's studies showed the pattern of regenerated fibers in the goldfish optic tract and tectum after removal of the anterior (left) or posterior (right) half-retina. The optic nerve was cut at the time of retinal extirpation. The course and termination of the regenerated axons was observed several weeks later, visualized by silver staining. Regenerating axons terminate in appropriate regions despite the availability of additional tectal tissue. M and L indicate medial and lateral optic tract bundles. (Adapted from Attardi and Sperry, 1963 as illustrated in Purves and Lichtman, 1985.)

Corey Goodman, Michael Bate, and their colleagues in analyses of the stereotyped nature of axonal pathfinding in insect embryos (Bate, 1976; Thomas et al., 1984). Thus by the late 1970s, the cellular evidence for a high degree of predetermination and selectivity in axonal growth and synapse formation was substantial, although still not universally accepted (see Easter et al., 1985).

In the 1980s and 1990s, attempts to clarify further the cellular mechanisms of axonal growth and guidance focused on reducing the apparent complexity inherent in the development of axonal projections to a few basic modes of environmental signaling and growth cone response (Goodman and Shatz, 1993). As a first approximation, the multitude of cues thought to exist in the environment of a growing axon was proposed to act in one of two ways: (1) at long range, through the secretion of diffusible factors, or (2) at short range through cell surface-tethered or extracellular matrix-associated factors. In addition, such long- and short-range cues were argued to act either as attractants or local factors permissive for axonal growth or, in a complementary manner, as repellants or factors that inhibit axon extension. What remained unclear after this phase of conceptional reductionism and simplification was the molecular basis of selective axon growth.

The Molecular Era of Axon Growth and Guidance

Today, there is no longer a paucity of molecules with convincing credentials as regulators of axonal growth and guidance (see Tessier-Lavigne and Goodman, 1996). This molecular cornucopia is the product of two main experimental approaches: in vertebrate tissues, the biochemical purification of proteins that promote cell adhesion and axonal growth and in *Drosophila* and *C. elegans*, the application of genetic screens to identify and characterize mutations that perturb axonal projection patterns. Over the past decade, these two complementary approaches have often supplied convergent information and have resulted in the compilation of a rich catalog of molecules with conserved functions in the control of axonal growth in insects, worms, and vertebrates.

An early advance in the molecular characterization of proteins that control axonal growth came with the biochemical dissection of two major adhesive forces that bind neural cells, one calcium independent and the other calcium dependent (Brackenbury et al., 1981). The design of assays to identify neural adhesion molecules based on antibody-mediated perturbation of cell adhesion by Gerald Edelman, Urs Rutishauser, and their colleagues led to the purification of NCAM, a major calcium-independent homophilic cell adhesion molecule (Rutishauser et al., 1982). The widespread expression of NCAM initially argued against a role for this protein in specific aspects of neuronal recognition. The discovery that NCAM is expressed in many different molecular isoforms, however, preserves the possibility that it has more specific functions in neural cell recognition and circuit assembly (Edelman, 1983). Although the precise contribution of NCAM to the growth of axons and the formation of neuronal connections remains uncertain, its isolation provided important credibility for the view that cell-adhesive interactions in the nervous system can be dissected in molecular terms. In addition, the realization that NCAM constitutes a divergent member of the immunoglobulin (Ig) domain superfamily (Barthels et al., 1987) brought the study of neural cell adhesion and recognition into the well-worked framework of cell and antigen recognition in the immune system. Since the discovery of NCAM, over a hundred Ig domain–containing neural adhesion and recognition proteins have been identified, although the function of most of these proteins in vivo remains unclear (Brummendorf and Rathjen, 1996).

In parallel, studies by Masatoshi Takeichi and his colleagues isolated the major calcium-dependent adhesive force binding vertebrate cells, the cadherin proteins (Takeichi, 1990). Cadherins have been shown to have major roles in the calcium-dependent adhesive interaction of virtually all cells in the vertebrate embryo, and cadherins have also been identified in *Drosophila* and *C. elegans*. The calcium dependence of cadherin function can be mapped to a critical calcium-binding domain required for protein stability. As we discuss below, cadherins, like Ig domain proteins, are now known to represent a very large family.

A third general adhesive system characterized in the 1980s was that involved in the interaction of cells with glycoproteins of the extracellular matrix. At this time biochemical studies by many groups had identified collagens, fibronectrins, and laminins as key adhesive glycoprotein components of the extracellular matrix. The search for cellular receptors for these structurally distinct glycoproteins converged with the identification of integrins, a large family of heterodimeric integral membrane proteins (Hynes, 1987; Ruoslahti, 1996). Integrins have prominent roles in cell–matrix adhesion within the

nervous system and in virtually all other tissue types. Thus, three main classes of neuronal surface membrane proteins—Ig domain proteins, cadherins, and integrins—appear to provide neural cells with the major adhesive systems necessary for the growth of axons, and these proteins may also contribute to more selective forms of neuronal recognition.

Many additional proteins that are expressed more selectively and appear to have selective roles in axonal growth have now been identified. For example, genetic screens in *C. elegans* and biochemical assays of axon growth regulatory factors in vertebrates collided with the characterization of netrins, a small class of secreted proteins with cell context–dependent axonal attractant and repellant activities (see Culotti and Merz, 1998). A similar convergence of biochemical and genetic assays led to the isolation of the semaphorin/collapsin class of growth cone collapse–inducing factors (Kolodkin, 1998) and to the characterization of a slit signaling pathway that appear to function both to repel axons and to promote axon branching (Guthrie, 1999). Independently, in vitro assays to examine the molecular basis of the topographic mapping of retinotectal projections culminated in the identification and functional characterization of ephrins: surface proteins that function as ligands for receptor tyrosine kinases of the Eph class (Drescher et al., 1997). Ephrin-Eph kinase signaling is now thought to have a dominant role in the establishment of the molecular gradients used to form projection maps in the retinotectal system and in other regions of the CNS (Figure 12)—perhaps corresponding to some of the matching chemical labels postulated earlier by Sperry.

With each of these discoveries, the veils that had previously shrouded the molecular analysis of axon guidance have been progressively stripped away. As a consequence, it is now realistic to begin to consider, at a molecular level, how the guidance of axons is directed by dynamic sets of molecular cues that either entice or deter the growth of axons at successive stages on their path to a final target. Despite these indisputable advances, many aspects of the logic of axon guidance remain unclear. With the multitude of candidate cues now shown to possess repellent or attractant functions, we still need to understand why individual sets of molecules are used in particular cellular contexts. Are there unique and as yet unappreciated functions provided by one but not another class of guidance cue? Or is there simply molecular opportunism? That is, can similar steps in selective axon pathfinding be achieved by any one of a large and structurally unrelated group of guidance molecules?

One route to resolving such issues will be through the dissection of the signal transduction pathways triggered in growth cones by activation of receptors for guidance cues. Already, such studies have begun to lead to the molecular classification of biochemical signaling pathways and their modulators within the growth cone (Mueller, 1999). They have also provided dramatic evidence in vitro that the ability of a growth cone to perceive an extrinsic signal as attractant or repellent can be modified by changing the ambient level of cyclic nucleotide activity. Further dissection of transduction mechanisms in the growth cone may thus help to clarify the logic that underlies the apparent selectivity of action of certain

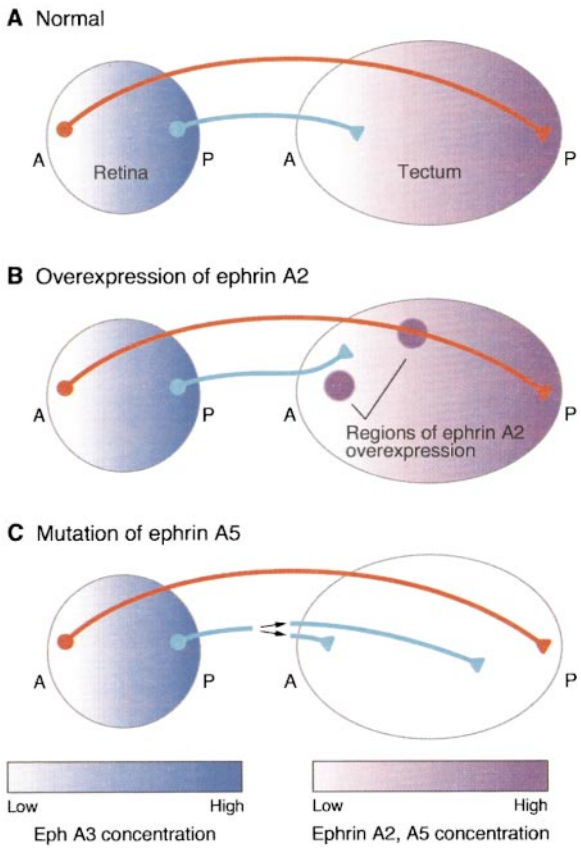

Figure 12. A Role for Ephrins and Eph Kinases in the Formation of the Retinotectal Map

(A) Members of the Eph kinase class of tyrosine kinase receptors are distributed in gradients in the retina, and some of their ligands, the ephrins, are distributed in gradients in the optic tectum. These two molecular gradients have been proposed to regulate retinotectal topograph through the binding of ephrins to kinases and the consequent inhibition of axon growth. The levels of ephrin A2 and ephrine A5 are higher in the posterior tectum than in the anterior tectum, and thus may contribute to the inhibition of extension of posterior retinal axons, which are rich in the kinase ephA3.

(B) Diagram showing the consequences of ephrin A2 expression in portions of the chick optic tectum that normally have low levels of this ligand. Posterior retinal axons avoid sites of ephrin A2 overexpression and terminate in abnormal positions. In contrast, anterior retinal axons, which normally grow into the ephrin-rich posterior tectum, behave normally when they encounter excess ephrin A2.

(C) In mice lacking ephrin A5 function, some posterior retinal axons terminate in inappropriate regions of the tectum.

(From the studies of O'Leary, Flanagan, Frisen, Barbacid and others, as summarized in Kandel et al., 2000.)

axonal growth and guidance factors. Another critical but poorly resolved issue is that of determining which guidance factors genuinely have instructive roles in directing axon growth and which merely provide permissive signals that enable growth cones to respond to other, more critical, signals.

The Selection and Refinement of Neuronal Connections

With the arrival of developing axons in the vicinity of their final position, growth cones are required to select specific target cells with which to form and maintain

functional connections. Although this process is critical in establishing the later functional properties of neural circuits, insight into the molecular basis of neuronal target cell selection remains fragmentary. As discussed above, one recurring issue has been the attempt to determine whether the formation of selective connections is the product of genetically determined factors that specify rules of connectivity in a precise manner, or whether the initial pattern of connections can tolerate a degree of inaccuracy that is subsequently resolved through the elimination of some connections and the consolidation of others (Cowan et al., 1984; Shatz, 1997). This latter view then represents the reemergence, albeit in a more restricted and comprehensible form, of the ideas originally articulated by Weiss in the 1940s.

A modern consensus view holds that both genetic predetermination and use-dependent refinement of connections are important contributors to the organization of mature circuits. The relative contribution of these two sets of factors are, however, likely to vary considerably with the particular neural circuit under study. One possibility is that circuits constructed early in evolution or at early stages in the development of an organism, as for example the spinal monosynaptic stretch reflex circuit, are established in a predominantly activity-independent manner (Frank and Wenner, 1993). In contrast, the more sophisticated cortical circuits associated with the processing of cognitive information, which emerge later in evolution and development, may require functional validation for the establishment of final patterns of connectivity (Shatz, 1997).

The pioneering studies of David Hubel and Torsten Wiesel in the 1960s provided the first evidence for a role for visually driven neural activity in the functional organization of the primary visual cortex (Hubel and Wiesel, 1998). Hubel and Wiesel deprived one eye of vision for several weeks during an early critical period of postnatal life. After this procedure, they observed that most neurons in layer 4 of the primary visual cortex could be activated only by input from the eye that had remained open, thus revealing a marked shift in the pattern of ocular dominance columns in the cortex. At an anatomical level, the terminal arbors of the axons of lateral geniculate neurons supplied by the intact eye were found by Simon Levay, Michael Stryker and their colleagues to be considerably more extensive than those supplied by the deprived eye (Hubel et al., 1977; Antonini and Stryker, 1993a, 1993b). Many subsequent studies have confirmed the essential role of activity in the formation of visual connections and have shown further that the temporal pattern of activity provided by the two eyes is an important parameter in the establishment of ocular dominance columns (Shatz, 1997). Under conditions in which visual input is provided to both eyes in a synchronous manner, the formation of ocular dominance columns is again perturbed (Stryker and Harris, 1986). Additional studies have shown that the level of activity in postsynaptic cortical neurons is necessary for ocular dominance column formation (Hata and Stryker, 1994). Collectively, these findings have begun to focus attention on the possible mechanisms by which the state of activity of postsynaptic cortical neurons could influence the pattern of arborization of presynaptic afferent fibers as they enter the cortex.

One advance in addressing this problem came with the proposal that the activation of the NMDA subclass of glutamate receptors on postsynaptic neurons might be involved in the normal segregation of afferent input to visual centers (Hofer and Constantine-Paton, 1994). An extension of this idea is that the NMDA receptor-mediated activation of cortical neurons results in the release of an activity-dependent retrograde signal that influences the growth and maintenance of presynaptic branches and nerve terminals. Several candidate mediators of such a retrograde signal have now been advanced, including nitric oxide and certain peptide growth factors. Much attention has also been directed at testing the possibility that the activity-dependent release of neurotrophins by cortical neurons is a critical step in the establishment of eye-specific projections into the visual cortex. Some support for this idea has been provided with the demonstration by Carla Shatz and colleagues that local infusion of the neurotrophins NT4 or BDNF into the developing cortex prevents the segregation of ocular dominance columns (Cabelli et al., 1995). Similar developmental defects are observed if the ligand-binding domains of neurotrophin receptors are introduced into the cortex, presumably the consequence of sequestering endogenous neurotrophins (Cabelli et al., 1997). Thus, an attractive if still speculative idea is that neurotrophic factors—classes of proteins identified initially on the basis of their critical roles in promoting the survival of neurons—have later and more subtle roles in shaping neuronal connections in the mammalian CNS.

Although the critical role of activity in the formation of neuronal circuits in the visual system and in many other regions of the CNS is well established, the precise nature of its contribution is less well defined. Information encoded by patterns of activity could be sufficient to direct certain connections. It remains possible, however, that for many neuronal circuits, a basal but unpatterned level of activity is all that is required. In this view, activity may simply permit neurons to respond to other signals that have more direct roles in the control of selective connections or may permit the maintenance of connections formed at earlier stages and through separate mechanisms. Evidence supportive of this latter view has come from studies by Michael Stryker and his colleagues on the role of visually driven activity in the formation of orientation and ocular dominance columns in the developing visual cortex (Crair et al., 1998). Neural activity may therefore exert its influence in large part by consolidating connections that have been established earlier through mechanisms which have their basis in molecular recognition between afferent neurons and their cortical target cells (see Crowley and Katz, 1999; Weliky and Katz, 1999).

Defining the relative contributions of sensory-evoked activity and genetically determined factors remains difficult first because the molecular basis of target recognition in any circuit is still unknown and second because the pathways by which activity modifies connectivity are poorly understood. Progress in resolving these issues will therefore require additional insight into the molecules that control synaptic specificity. One anticipated feature of molecules that contribute to the selection of neural connections is that of molecular diversity

(Serafini, 1999). Several classes of proteins that exhibit inordinate molecular variation have recently been identified, and not surprisingly, have been implicated in the formation of selective connections.

The cadherins as discussed above represent one class of cell surface recognition protein that exists in large numbers. Diversity in cadherin structure can be enhanced dramatically through a process in which one of a chromosomally arrayed cluster of variable cadherin domain gene sequences is appended to a nearby constant region sequence (Wu and Maniatis, 1999). The molecular mechanism used to assemble such modularly constructed cadherin proteins remains unclear, but the number of these variable domains is high, bringing the total number of predicted cadherins to well over 100. The vast majority of cadherins are known to be expressed by neural cells and studies of the patterns of expression of the classical cadherins have revealed a striking segregation of individual cadherins within functionally interconnected regions of the brain (Takeichi et al., 1997). In addition, cadherins are concentrated at apposing pre- and postsynaptic membranes at central synapses (Shapiro and Colman, 1999). Although intriguing, the link between selective cadherin expression and the specificity of synaptic connections remains to be demonstrated functionally.

A second class of proteins with the potential for considerable structural variation is the neurexins. Neurexins are surface proteins identified originally by virtue of their interaction with the neurotoxin α-latrotoxin (Missler and Südhof, 1998; Rudenko et al., 1999). Analysis of the potential for alternative splicing of the *neurexin* genes suggests, in principle, that $\sim$1000 protein isoforms can be generated and at least some of these potential isoforms are known to be expressed by central neurons. In addition, a class of neurexin receptors termed neuroligins have been identified (Song et al., 1999). Again, though, a functional role for neurexin–neuroligin interactions in the formation of synapses remains to be established.

A third highly diverse class of neuronal surface proteins are the seven-pass odorant receptors expressed on primary sensory neurons in the olfactory epithelium. Several major classes of odorant or pheromone receptors have now been identified in vertebrates, and in total this class of receptors is thought to be encoded by over 1000 distinct genes (Buck and Axel, 1991; Axel, 1995). This genetic diversity is likely to underlie the remarkable discriminatory capacity of the mammalian olfactory sensory system. The creative manipulation of odorant receptor gene regulatory sequences to map the central projections of olfactory sensory axons through reporter gene expression in transgenic mice has also revealed a precise anatomical convergence of sensory axons linked by common receptor gene expression to individual target glomeruli in the olfactory bulb (Mombaerts et al., 1996). This finding poses the additional question of the mechanisms directing sensory axon targeting to individual glomeruli. Strikingly, manipulation of the pattern of expression of individual odorant receptor genes in transgenic mice results in a predictable change in the central projection pattern of olfactory sensory axons (Wang et al., 1998). An intriguing implication of these findings is that olfactory sensory receptors function not only in peripheral odor discrimination but also in axon targeting, potentially providing a direct link between the sensory receptive properties of a neuron and its central pattern of connectivity.

Determining whether each or any of these classes of proteins have roles in selective synapse formation in the developing central nervous system is an important goal in itself and may also provide the entry point for a more rigorous examination of the relationship between neuronal activity, gene expression, and synaptic connectivity.

The events that initiate the formation of selective contacts between pre- and postsynaptic partners are, however, unlikely to provide sufficient information to establish the functional properties of synapses necessary for effective neuronal communication. A separate set of molecules and mechanisms appears to promote the maturation of early neuron–target contacts into specialized synaptic structures. Current views of this aspect of neuronal development derive largely from studies of one peripheral synapse, the neuromuscular junction (Sanes and Lichtman, 1999). These studies have their origins in many classical physiological studies of synaptic transmission at the neuromuscular junction. In particular, the ability to measure dynamic changes in the pattern of expression of acetylcholine receptors on the surface of muscle fibers as they become innervated (Fischbach et al., 1978) provided many early insights into the cellular mechanisms by which the motor axon organizes the elaborate program of postsynaptic differentiation necessary for efficient synaptic transmission. By the 1980s, powerful in vivo and in vitro assays to examine synaptic organization under conditions of muscle denervation and reinnervation had been developed, and these assays facilitated biochemical efforts to purify neuronally derived factors with synaptic organizing capacities (McMahan, 1990; Sanes and Lichtman, 1999).

These efforts culminated in the identification of two major pre- to postsynaptic signaling pathways that appear to coordinate many aspects of the synaptic machinery in the postsynaptic muscle membrane. Signals mediated by agrin, a nerve- and muscle-derived proteoglycan, through its tyrosine kinase receptor musk have an essential role in the clustering of acetylcholine receptors and also of other synaptically localized proteins at postsynaptic sites located in precise register with the presynaptic zones specialized for transmitter release (see McMahan, 1990; Kleiman and Reichardt, 1996). A second set of nerve- and muscle-derived factors, the neuregulins which signal through ErbB class tyrosine kinase receptors, appear instead to control the local synthesis of acetycholine receptor genes in muscle cells (see Sandrock et al., 1997), and perhaps also to direct the local insertion of newly synthesized receptors at synaptic sites.

These dramatic molecular successes have provided the foundations of a comprehensive understanding of the steps involved in the formation and organization of nerve–muscle synapses. The extent to which the principles that have emerged from the study of this synapse peripheral extend also to the organization of central synapses remains uncertain. There has, however, been considerable progress in recent years in defining the structural components of the presynaptic release apparatus at central synapses (Bock and Scheller, 1999) and

the proteins that concentrate postsynaptic receptors (Sheng and Pak, 1999). From the information now emerging, it seems likely that the identity of molecular signals that orchestrate the maturation of central synapses will soon be known, and in the process we will come to recognize principles of central synaptic organization similar to those that operate at the neuromuscular junction.

A Future for Studies of Neural Development

Despite the dramatic advances of the two past decades, several important but unresolved issues cloud our view of the assembly of synaptic connections. These problems will need to be addressed before any satisfying understanding of neural circuit assembly can be claimed.

One issue stems from the pursuit of mechanisms of neuronal cell fate determination and of the control of axonal pathfinding and connectivity as largely separate disciplines. With the many available details of cell fate specification and of the regulation of axonal growth and guidance, it is still not clear if and how the transcriptional codes that control neuronal identity intersect with the expression of the effector molecules that direct axonal connectivity. For example, in only a few cases have relevant genetic targets of the transcription factors that control early steps in neuronal identity been identified. Indeed, a superficial survey of patterns of expression of transcription factors and axonal receptors for guidance cues reveals little obvious coincidence at the cellular level. Thus, the extent to which the regulated expression of genes that encode receptors for axon guidance cues depends on the sets of determinant factors implicated in earlier aspects of neuronal subtype identity remains unclear. Defining the full complement of transcription factors that specify the identity of an individual neuronal subtype and the molecular sequence of cell–cell interactions that guide the axon of the same neuron to its target is one obvious but laborious route to resolving this issue.

Similarly, the relationship between transcription factor expression and other later aspects of neuronal phenotype, for example neurotransmitter synthesis and chemosensitivity, also remain unclear. In a few instances, cell-specific transcription factors have been linked to the expression of genes that control neurotransmitter synthesis (see Goridis and Brunet, 1999). Nevertheless, the general logic linking transcriptional identity and the expression of the neuronal traits that confer specialized synaptic signaling properties and connectivity remains obscure.

Assuming, as seems likely, that these issues can be solved in a relatively rapid fashion, what does the future hold for studies of neural development? Clearly, there will be interesting variation in the strategies used to establish selective connections in different regions of the developing brain and in different circuits. The documentation of these variations will provide a richer and more profound appreciation of the core principles of neuronal circuit assembly. But the reiteration of a few basic themes in different brain regions can sustain excitement in the field only briefly, and in any event will not provide an obvious intellectual bridge between studies of development and of the function of mature neuronal circuits.

Application of Neural Development to the Study of Neurological Disease

One future area in which studies of neural development are likely to have significant impact is in the application of fundamental information on the specification of cell fate and the guidance of axons to problems posed by neurodegenerative diseases and traumatic injury to the nervous system.

As discussed above, we are beginning to obtain a rather detailed outline of the relationship between inductive signaling and the expression of cell-specific transcription factors that define cell fate. In some cases, details of these pathways have progressed to the point that certain transcription factors expressed by single classes of CNS neurons have been shown to be sufficient to direct neuronal subtype fate in a manner that is largely independent of the prior developmental history of the progenitor cell (Tanabe et al., 1998). If this is the case for the few classes of neurons in which inductive signaling pathways have been particularly well studied, it seems likely that similar dedicated determinant factors will exist for many other classes of neurons in the CNS. The identification of such factors may be of significance in the context of the many ongoing attempts to identify neural progenitor cells and then to drive them along specific pathways of neurogenesis (Panchision et al., 1998; Doetsch et al., 1999; Johansson et al., 1999; Morrison et al., 1999). One outcome of such developmental studies may therefore be to rationalize strategies for reintroduction of fate-restricted neural progenitor cells into the CNS in vivo. In principle, these advances could offer the potential of more efficient cell replacement therapies in a wide variety of neurological degenerative disorders.

Similarly, the wealth of information on molecules that promote or inhibit axonal growth is likely to be of relevance for studies of axonal regeneration and repair. The pioneering studies of Albert Aguayo and colleagues of the regenerative capacity of central neurons in a cellular environment composed of peripheral rather than CNS nerve cells revealed the potential of central neurons to regenerate (Richardson et al., 1997; see Goldberg and Barres, 2000). These studies prompted the search for molecules expressed by cells of the mature central nervous system that inhibit the growth of axons (see Tatagiba et al., 1997) and for molecules expressed in early development that have the capacity to promote the growth of axons of CNS neurons (Tessier-Lavigne and Goodman, 1996). The progress in identification of axon growth–promoting and inhibitory factors may therefore eventually permit rational changes to be made in the environment through which regenerating axons in the mature CNS are required to project. Of equal promise are studies to clarify the signal transduction pathways by which axons respond to these environmental cues. The elucidation of these pathways may permit a more general manipulation of axonal responses, for example rendering axons insensitive to broad classes of inhibitory factors, or supersensitive to many distinct axonal growth–promoting factors. It may also be worth considering whether there is a common molecular basis for the marked differences in the regenerative capacity of different vertebrate species evident in studies of both nerve and limb regeneration (see for example Brockes, 1997).

Establishing a Link between the Development and Function of Neuronal Circuits

An additional, and potentially a more far-reaching contribution of neural development, may emerge by taking advantage of the compendium of information now available on cell-specific gene expression in developing neurons and of the ease of genetic manipulation in mammals, notably the mouse. With these methods in hand, it may be possible to modify the function of highly restricted classes of neurons in the adult animal and to assay resultant changes in the function of specific neuronal circuits.

One initial limitation in the application of information about neuronal subtype–specific gene expression during development is that the majority of such genes are transiently expressed. Thus, the normal temporal profile of gene expression does not permit direct tracing of the relationship between embryonic neuronal subtype identity and the physiological properties of the same neuronal subsets in the adult. This problem can now be overcome through the use of genetically based lineage tracing methods. For example, genes encoding yeast- or bacterially derived recombinase enzymes can be introduced into specific genetic loci by targeted recombination (Dymecki, 1996; Schwenk et al., 1998), to generate mouse strains which can then be crossed with other genetically modified mice in which recombinase-driven DNA rearrangement results in the irreversible activation of reporter gene expression at all subsequent stages in the life of a neuron (Zinyk et al., 1998; Lee et al., 2000). This relatively simple methodology offers the immediate promise of providing a direct link between subsets of neurons defined at embryonic stages and the location, and functional identity of these neurons within the mature CNS.

With the compilation of such lineage information, variants of this same basic genetic strategy can be used to modify the function of neuronal subsets at predefined times. One drastic method for eliminating neuronal function involves the activation of toxins in a neuron-specific manner, under precise temporal control (see for example Grieshammer et al., 1998; Watanabe et al., 1998), thus permitting the physical ablation of predefined populations of CNS neurons with a specificity unattainable by conventional lesioning methods. More subtly, specific populations of neurons could, in principle, be activated or inactivated reversibly in the adult animal through temporally regulated expression of ion channels that change the threshold for neuronal excitability (Johns et al., 1999). In addition, the development of transgenic mice methods for anterograde or retrograde transynaptic transport of foreign marker proteins (Coen et al., 1997; Yoshihara et al., 1999) may be helpful in providing novel information on neuronal connectivity in the CNS that cannot easily be extracted by other anatomical tracing methods.

In this way, the increasingly detailed molecular information that derives from attempts to examine the principles of neural circuit assembly during development should have clear application to the major problems of systems neuroscience discussed in the following sections of this review. At present, the routine application of these genetic methodologies is feasible only in the mouse, and thus the issue of linking studies obtained in lower mammals with information obtained in primates and ideally in man still needs to be addressed. Nevertheless, with advances in the resolution of functional imaging methods that are outlined later in this review, and in the application of these methods to small mammals, the link between studies in mouse and primates can be strengthened. When this is achieved, the information that emerges from studies of the development of neural circuits may assume a more prominent place in the repertoire of experimental strategies that aim to decipher how such circuits function in the adult brain.

Part IV: Neural Systems: From Neurons to Perception

The individual neurons that make up the brain work together in specialized groups, or systems, each of which serves a distinct function. *Systems neuroscience* is the study of these neural systems, which include those involved in vision, memory, and language. Neural systems possess a number of common properties, not the least of which is the fact that they all process higher-order information about an organism's environment and biological needs. In humans, this information often gains access to consciousness. Systems neuroscience thus places great emphasis on uncovering the neural structures and events associated with the steps in an information processing hierarchy. How is information encoded (sensation), how is it interpreted to confer meaning (perception), how is it stored or modified (learning and memory), how is it used to predict the future state of the environment and the consequences of action (decision making/emotion), and how is it used to guide behavior (motor control) and to communicate (language)? The twentieth century has seen remarkable progress in understanding these processes. This ascendance of modern systems neuroscience is attributable, in part, to the convergence of five key subdisciplines, each of which contributed major technical or conceptual advances.

Neuropsychology: Localization of the Biological Source of Mental Function

The first question one might ask about an information-processing device concerns its gross structure and the relationship between structural elements and their functions. The simplest approach to this question—and the approach that has best withstood the test of time—is to observe the behavioral or psychological consequences of localized lesions of brain tissue. The modern discipline of neuropsychology was founded on this approach and draws both from human clinical case studies—often provided during the early decades of the twentieth century by brain injuries sustained in battle—and from experimental studies of the effects of targeted destruction of brain tissue in animals. Through these means the functions of specific brain regions, such as those involved in sensation, perception, memory, and language, have been inferred.

Neuroanatomy: Patterns of Connectivity Identify Information Processing Stages

The discipline of neuroanatomy, which blossomed at the turn of the century following the adoption of the neuron doctrine and which has benefited from many

subsequent technical advances, has revealed much about the fine structure of the brain's components and the manner in which they are connected to one another. As we have seen, one of the earliest and most influential technical developments was the discovery by Camillo Golgi of a method for selective staining of individual neurons, which permitted their visualization by light microscopy. By such methods, it became possible to use differences in the morphology of cells in different brain regions as markers for functional diversity. This procedure, known as cytoarchitectonics, was promoted vigorously in the early decades of the twentieth century by the anatomists Korbinian Brodmann, and Oscar and Cecile Vogt. Brodmann's cytoarchitectonic map of the human cerebral cortex, which was published in 1909 and charted the positions of some 50 distinct cortical zones, has served as a guidebook for generations of scientists and clinicians, and as a catalyst for innumerable studies of cortical functional organization.

Arguably the most important outcome of the means to label neurons, however, was the ability it provided to trace connections between different brain regions. To this end, cell labeling techniques have undergone enormous refinement over the past three decades. Small quantities of fluorescent or radioactive substances, for example, can now be injected with precision into one brain region and subsequently detected in other regions, which provides evidence for connectivity. The products of anatomical tract tracing are wiring diagrams of major brain systems, which are continuously evolving in their precision and completeness, and have been indispensable to the analysis of information flow through the brain and for understanding the hierarchy of processing stages.

Neurophysiology: Uncovering Cellular Representations of the World

Adoption of the neuron doctrine and recognition of the electrical nature of nervous tissue paved the way to an understanding of the information represented by neurons via their electrical properties. Techniques for amplification and recording of small electrical potentials were developed in the 1920s by Edgar Adrian. This new technology enabled neurobiologists to relate a neuronal signal directly to a specific event, such as the presentation of a sensory stimulus, and became a cornerstone of systems neuroscience. By the 1930s, electrophysiological methods were sufficiently refined to enable recordings to be made from individual neurons. Sensory processing and motor control emerged as natural targets for study. The great successes of single-neuron electrophysiology are most evident from the work of Vernon Mountcastle in the somatosensory system, and David Hubel and Torsten Wiesel in the visual cortex, whose investigations, beginning in the late 1950s, profoundly shaped our understanding of the relationship between neuronal and sensory events.

Psychophysics: The Objective Study of Behavior

Historically, quantitation of behavior has been the province of experimental psychology, which emerged in the nineteenth century from deep-rooted philosophical traditions to become a distinct scientific discipline and a key component of modern systems neuroscience. Among the most notable steps in this emergence was the development by the German physicist and philosopher, Gustav Fechner, of a systematic scientific methodology for assessing the relationship between behavior and internal states. Fechner's Elements of Psychophysics, published in 1860, founded an "exact science of the functional relationship . . . between body and mind," based on the assumption that the relationship between brain and perception could be measured experimentally as the relationship between a stimulus and the sensation it gives rise to (Fechner, 1860). In practice, Fechner's psychophysics is applied by varying a sensory stimulus along some physical dimension—such as the intensity or wavelength of light—and obtaining reports from an observer regarding the sensations experienced. In this manner, one can identify the function that relates the physical dimension of the stimulus to an internal sensory dimension, and from that relationship infer the rules by which the sensory information is processed.

Throughout the twentieth century, the tools of psychophysics have been extremely useful in identifying the information processing strategies of sensory, perceptual, and motor systems of the brain. Beginning with the work of Mountcastle in the 1960s (Mountcastle et al., 1969), psychophysics has frequently been paired directly with electrophysiological methods to extraordinary effect in identifying the neuronal events that give rise to specific sensory and perceptual processes.

Computation: Divining the Mechanisms of Information Processing

Large neural systems such as those involved in vision, combine and analyze incoming signals to "interpret" their causes and generate appropriate outputs. The logical steps in these neuronal mechanisms have become accessible to quantitative and theoretical treatment. The goal has been to extract generic computational principles that can account for existing data and have predictive value. Some of the earliest work along these lines was directed at sensory and motor processing and was founded on engineering techniques and principles designed for the study of simple linear systems. One of the most successful examples of this approach is Georg von Bekesy's (1960) investigation of the cochlea and its relation to the frequency encoding of sound. von Bekesy began by investigating the patterns of vibration of the various components of the inner ear, and the relationship of these patterns to the characteristics of sound waves. From these observations he concluded that this system analyzes sound by a linear frequency decomposition, that is, the mechanical properties of the cochlea allow specific frequency components of sound to be independently isolated and detected by the sensory epithelium. Considerable gains have also been made using similar theoretical approaches to understand early stages of visual processing and the control of movements of the eyes.

Many levels of processing in neural systems deviate from linear forms of computation. The search for alternative computational principles, which was fueled in part by the rise of cognitive science in the 1980s and an unprecedented richness of physiological and anatomical data, has led to a number of novel and sophisticated

theoretical approaches, such as those embodied by neural networks (Rumelhart et al., 1987). These networks operate on the biologically plausible principle that information can be represented in a distributed fashion across a large population of "units," or modeled neurons. Moreover, this information may be combined in many different ways to yield complex cellular representations, simply by changing the strength of "synaptic" connections between modeled neurons.

Vision as a Model System

Collectively these five areas of neuroscience—neuropsychology, neuroanatomy, neurophysiology, psychophysics, and computation—constitute an experimental arsenal, which has already revealed in outline the structure, operational mechanisms, and functions of large neural systems, such as those involved in vision, memory, and language. Although the range of successes is broad, and many general principles of system organization and function have been discovered, the visual system has emerged as the model for experimental investigation and is consequently the area in which we have the greatest understanding.

Setting the Stage: Early Explorations of Visual Perception and Brain

Two early developments presaged the extraordinary progress in understanding visual function that is now a legacy of the twentieth century. The first of these occurred within the field of experimental psychology. Hermann von Helmholtz (1924) and Wilhelm Wundt (1902), two of Fechner's nineteenth century contemporaries, attempted to identify how different visual stimuli lead to different subjective experiences. Their method was initially observational and introspective, but later they exploited the objective methodology of psychophysics. The lasting outcome of these efforts was a quantitative appreciation of the elements of visual experience—color, brightness, motion, distance—and an initial set of ideas about how they might be represented by the brain. A second early development occurred within the field of neuropsychology. With mounting experimental evidence for localization of function within specific brain regions, Hermann Munk (1881) and Edward Schafer (1888) each used the method of focal ablation of brain tissue at the end of the nineteenth century to identify brain regions that serve visual function. They found that the occipital lobe of the cerebral cortex plays an essential role in the processing of visual information.

The Golden Era of Single-Neuron Electrophysiology

Perhaps the single greatest technical advance in vision science was the application of the electrophysiological methods that had emerged in the late 1920s and 1930s. In a pioneering series of studies begun in the early 1930s, Keefer Hartline recorded from single cells in the eye of the horseshoe crab (*Limulus*) and examined the relationship between the properties of the incoming sensory stimulus—which in this case happened to be a small spot of light projected onto the eye—and the neuronal response (i.e., the frequency of action potentials). Through these (Hartline and Graham, 1932) and subsequent experiments of a similar nature that involved recordings from single axons in the frog optic nerve (Hartline, 1938), Hartline made two important discoveries. First, he found that individual neurons respond to light only within a well-defined region of visual space, which Hartline termed the visual *receptive field*. Operationally defined, the receptive field is the portion of the sensory epithelium (the sheet of photoreceptors, in the case of vision) that when stimulated elicits a change in the frequency of action potentials for a given neuron. In anatomical terms, the receptive field describes all of the receptor and subsequent cells that converge upon and influence the firing pattern of the neuron under study. The concept of the receptive field has proven to be an extremely useful and general concept in systems neuroscience.

Hartline's second major discovery was that the visual responses to light were dependent upon contrast. Specifically, the amplitude of the neuronal response to a light in one region of visual space was greatest when there was no light in an adjacent region of space. Thus, rather than simply conveying the presence or absence of light, neurons in the visual system communicate information about the spatial structure or pattern of the incoming stimulus. Because these observations paralleled the well-known perceptual enhancement of brightness at contrast boundaries—exploited for centuries by artists wishing to enhance the range of light intensities perceived in their paintings (Leonardo da Vinci, 1956)—they were seized upon as a potential physiological substrate for the perceptual experience of brightness.

The tradition of single-neuron studies of visual processing continued through the 1940s and 1950s with a shift of emphasis to mammals (affording closer ties to human visual perception and made possible by advances in electrophysiological techniques). In 1953, Stephen Kuffler, a student of Eccles and Katz, examined the behavior of neurons in the cat retina. Kuffler (1953) focused on the retinal ganglion cells, the output cells of the retina, which carry visual information from the photoreceptors through the optic nerve to the lateral geniculate nucleus of the thalamus and other central processing regions. Following Hartline's model, Kuffler described the response characteristics of ganglion cells in terms of their receptive field properties. He discovered that the receptive fields of retinal ganglion cells were round in shape and had distinct concentric excitatory and inhibitory zones, which made them maximally sensitive to spatial contrast. On the basis of the architecture of their receptive field properties, Kuffler divided these cells into two groups. One class of cells had a central excitatory zone and a surrounding inhibitory region ("on-center" cells) whereas the other class of cells had an inhibitory central region and an excitatory surround region ("off-center" cells).

Beyond the Retina: Visual Contours Are Detected by Neurons in Primary Visual Cortex

Anatomical tracing experiments conducted over the past several decades have shown that the outputs of the retina terminate in several distinct brain regions. One of the largest projections extends from the retina to the lateral geniculate nucleus (LGN) of the thalamus, and continues on via the *geniculostriate pathway* to *primary visual cortex*. Otherwise known as *striate cortex* or *area V1*, this latter visual processing stage lies on the occipital pole of the cerebral cortex, and is known from the early

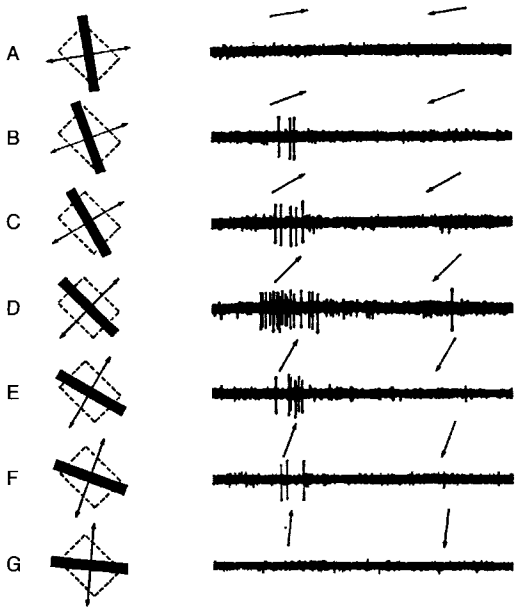

Figure 13. Neuronal Orientation Selectivity, as First Observed by Hubel and Wiesel (1968) in Primary Visual Cortex (Area V1) of a Rhesus Monkey

The receptive field of the cell that was recorded is indicated by broken rectangles in the left column. The visual stimulus that was viewed by the monkey consisted of a bar of light that was moved back and forth through the receptive field of the cell in each of seven different orientations (rows A–G). The different directions of motion used for each orientation are indicated by the small arrows. Recorded traces of cellular activity are shown at right, in which the horizontal axis represents time (2 s/trace) and each vertical line represents an action potential. This neuron responded most strongly to a bar of light oriented along the diagonal (stimulus D), particularly when the bar was moved through the receptive field from lower left to upper right. Neurons bearing such properties are common in the visual cortex, and their discovery revolutionized views on the neuronal bases of visual perception. (From Hubel and Wiesel, 1968.)

neuropsychological studies of Munk, Schafer, and Gordon Holmes (1927), and others to be critical for normal visual function.

The electrophysiological approach pioneered by Kuffler in studies of mammalian retinal ganglion cells was carried to these higher processing stages by two of Kuffler's young colleagues, David Hubel and Torsten Wiesel. In the late 1950s, Hubel and Wiesel began to examine the response properties of neurons in the cat and monkey lateral geniculate nucleus. These neurons were found to possess center-surround receptive field properties not unlike those of retinal ganglion cells. By contrast, Hubel and Wiesel (1959) found that the response properties of cells in the primary visual cortex of both cats and monkeys were very much more complicated. Cortical cells could not be effectively stimulated by the simple spots of light that proved so effective in the retina and in the lateral geniculate nucleus. To be effective a stimulus had to have linear properties; the best stimuli were lines, bars, rectangles, or squares (Figure 16). Hubel and Wiesel divided the cortical cells into simple and complex (for review see Hubel and Wiesel, 1977). We will use simple cells to illustrate in greater detail the types of stimulus selectivities observed (Figure 13).

A typical receptive field for a simple cell in primary visual cortex might have a central rectangular excitatory area with its long axis running from twelve to six o'clock, flanked on each side by similarly shaped inhibitory areas. For this type of cortical cell, the most effective excitatory stimulus is a bar of light with a specific axis of orientation—in this case from twelve o'clock to six o'clock—projected on the central excitatory area of the receptive field. Since this rectangular zone is framed by two rectangular inhibitory areas, the most effective stimulus for inhibition is one that stimulated one or both of the two flanking inhibitory zones. A horizontal or oblique bar of light would stimulate both excitatory and inhibitory areas and would therefore be relatively ineffective. Thus, a stimulus that is highly effective if projected vertically onto a given area of retina so as to be on target for the excitatory zone would become ineffective if held horizontally or obliquely. Other cells had similar receptive field shapes but different axes of orientation (vertical or oblique). For example, the most effective stimulus for a cell with an oblique field would be a bar of light running from ten o'clock to four o'clock or from two o'clock to eight o'clock (Figure 13).

The most interesting feature of the simple cortical cells is that they are much more particular in their stimulus requirement than the retinal ganglion cells or those in the lateral geniculate nucleus in requiring a proper axis of orientation. For a stimulus to be effective for a retinal ganglion or a geniculate cell, it only has to have the proper shape, in general circular, and the proper retinal position so as to activate appropriate receptors in the retina. Simple cortical cells not only have to represent all retinal positions and several shapes (lines, bars, rectangles), but also for each shape they have to represent all axes of orientation. These finding provided an initial insight as to why the visual cortex (or any cortex) needs so many cells for its normal functions. Cells are required to represent every retinal area in all axes of orientation so as to abstract the information presented to the cortex. Hubel and Wiesel suggested that the simplest explanation for the response properties of a cortical cell with a simple receptive field was that they received innervation from a set of geniculate cells that had appropriate on-center and off-center properties and appropriate retinal positions.

Another feature distinguishes cells in primary visual cortex from those in the lateral geniculate nucleus. Neurons of the lateral geniculate nucleus only respond to stimulation of one or the other eye. In primary visual cortex, one begins to find cells that are activated by stimulation of either eye (Figure 14). These cells provide a neural substrate for the integration of information from the two eyes. Binocular properties of this type are essential to the use of stereoscopic cues for depth vision in animals, such as primates with frontally placed eyes.

Beyond V1: Specialized Functions of Higher Cortical Visual Areas

Neuropsychological studies carried out over much of the past century have shown that deficits in visual function follow from damage anywhere within a vast expanse—over 30% in humans—of the cerebral cortex. Moreover, the type of deficit depends upon the site of damage:

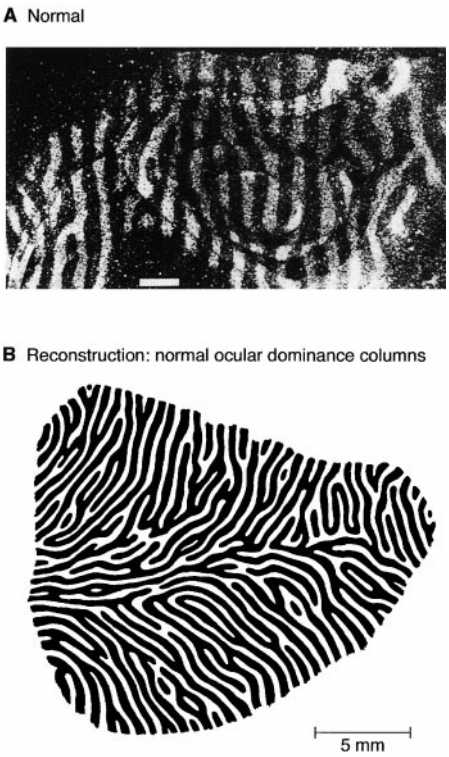

Figure 14. Anatomical Representation of Ocular Dominance Columns in Primate Visual Cortex

(A) The right eye of a normal adult was injected with the radiolabeled *proline* and *fucose*. This dark-field autoradiograph obtained after 10 days shows a tangential section of area 17 of the right hemisphere. Radioactivity can be seen in the form of white stripes, which correspond to thalamic axon terminals in layer 4 that relay input from the injected eye. The alternating dark stripes depict the position to geniculate afferents from the uninjected eye. (From Hubel et al., 1977.)
(B) Reconstruction of ocular dominance columns in area 17 of the right hemisphere showing the intricate organization of the map. (From LeVay et al., 1980.)

temporal lobe lesions cause impairments in object recognition (termed "agnosias" by Sigmund Freud) (for review see Farah, 1995), whereas parietal lobe lesions interfere with use of visual cues for spatially directed actions (for review see Mesulam, 1999). These early findings suggested that the complex cellular properties discovered in area V1 by Hubel and Wiesel were only the tip of the iceberg.

Motivated by this prospect, in the 1970s there was a dramatic increase in electrophysiological and anatomical studies designed to explore the organization of the *extrastriate visual cortex*, which lies beyond area V1. Two groups—Semir Zeki, and John Allman and John Kaas—noted that not only were extrastriate neurons vastly heterogeneous in their response properties, but that the extrastriate visual cortex could be neatly subdivided into a large number of distinct modules on the basis of these properties (for review see Van Essen, 1985). At present, the visual cortex of monkeys is thought to be composed of over 30 such modules (Figure 16).

These efforts to reveal order in the heterogeneity of visual cortex were a natural extension of the nineteenth century concept of localization of function. They were, moreover, reinforced by the computational view, advanced by the theorist David Marr (1982), that large system operations (such as seeing) can be subdivided and assigned to task-specific modules. Although little is yet known of the specific tasks "assigned" to the vast majority of extrastriate visual areas, there are some noteworthy exceptions. Of particular interest is the middle temporal visual area (MT), which appears to be specialized for motion.

Area MT lies near the junction of the occipital, parietal, and temporal lobes and is known to possess a high proportion of neurons that represent the trajectory of a moving visual stimulus, suggesting an important role in visual motion processing (for review see Albright, 1993). This idea has been supported by three related findings by William Newsome and his colleagues that imply a causal link between the activity of MT neurons and perceived motion. In the first experiment, Newsome and his colleagues found that focal destruction of area MT in monkeys results in motion blindness, demonstrating that MT is *necessary* for motion perception (Newsome and Paré, 1988) (Figure 15). In a second experiment, Newsome and Anthony Movshon obtained psychophysical measurements of a monkey's ability to discriminate direction of motion, simultaneously with electrophysiological measurements of the motion sensitivity of MT neurons in the monkey's brain. They found that the sensitivity of individual neurons correlated extremely well with performance on the behavioral task, demonstrating that the direction information encoded by neurons of MT is *sufficient* to account for the monkey's judgment of motion (Newsome et al., 1989). Newsome and colleagues reasoned that if this logic were correct, then it should be possible to alter the monkey's perception of motion by artificially modifying the firing rate of MT neurons. In a third experiment, these investigators did exactly that. Small electrical currents were used to stimulate clusters of neurons sensitive to a common direction of motion. Remarkably, doing so was found to bias the monkey's judgment toward that direction of motion (Salzman et al., 1990). Electrical stimulation of this sort thus has the effect of adding a fixed motion signal to the signal received by MT from the retina. Not only do these results strongly support the hypothesized role of area MT in motion processing, but also they imply that perceptual decisions can be based on the activity of relatively small populations of neurons.

Detection of Behaviorally Significant Visual Features

The initial discovery that visual neurons integrate complex spatial information led to speculation about the role of such neurons in detection of behaviorally significant visual features. Experiments conducted in the 1950s by Horace Barlow (1953), and by Jerome Lettvin and Humberto Maturana, reinforced this view with the finding that ganglion cells in the frog retina respond optimally to patterns of light that resemble the silhouette and flight of an insect (Lettvin et al., 1959), which is, of course, the primary food source for a frog. This finding led naturally to the concept that single visual cells were *feature detectors*. According to this view, receptive fields may operate as highly specialized templates for detection of significant features ("trigger features") in the visual image. The concept was expanded upon in

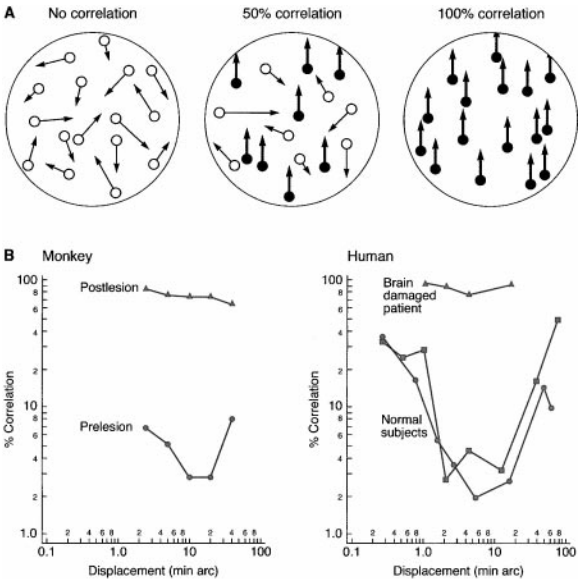

Figure 15. The Involvement of Cortical Area MT in the Perception of Visual Motion

A monkey with an MT lesion and a human patient with damage to extrastriate visual cortex have similar deficits in motion perception. (A) Displays used to study the perception of motion. In the display on the left there is no correlation between the directions of movement of several dots, and thus no net motion in the display. In the display on the right, all the dots move in the same direction (100% correlation). An intermediate case is in the center; 50% of the dots move in the same direction while the other 50% move in random directions (and are perceived as noise added to the signal).
(B) The perception of visual motion by a monkey before and after a lesion of MT (left). The performance of a human subject with bilateral brain damage is compared to two normal subjects (right). The ordinate of the graph shows the percent correlation in the directions of all moving dots (as in part [A]) required for the monkey to select the common direction. The abscissa indicates the size of the displacement of the dot and thus the degree of apparent motion. Note the general similarity between the performance of the humans and that of the monkey and the devastation to this performance after the cortical lesions (Pare, 1998; Baker et al., 1991).
(From experiments of Newsome and others as illustrated in Kandel, Schwartz and Jessell, 2000.)

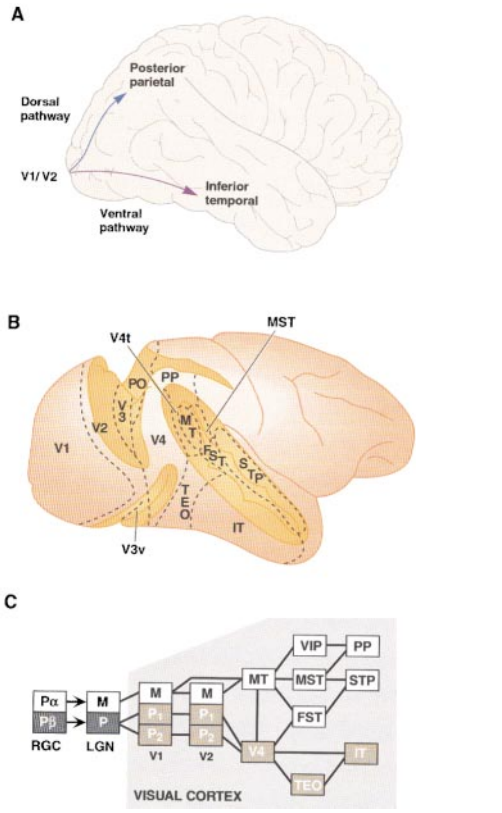

Figure 16. Visual Information Is Processed by Divergent Cortical Pathways

One of the most important discoveries of the past century in the field of sensory biology is the multiplicity of areas in the cerebral cortex that are involved in visual perception and visually guided behavior. These areas are thought to be functionally specialized and hierarchically organized into two parallel processing streams.
(A) The image is a lateral view of the rhesus monkey brain, illustrating these two major pathways, both originating from V1, the striate cortex. There is a dorsal ("where") cortical stream, which takes a dorsal route to the parietal cortex, and a ventral ("what") cortical stream, which takes a ventral route to the temporal cortex.
(B) The image illustrates the primary visual cortex (area V1) located on the occipital pole (left); the "extrastriate" cortical visual areas extend anteriorally (rightward) and are labeled by their commonly used abbreviations (see below). Indicated borders of visual areas (dashed lines) are approximate. Some sulci have been partially opened (shaded regions) to show visual areas that lie buried within these sulci.
(C) The image illustrates some of the anatomical connections known to exist from the retina through visual cortex. Except where indicated by arrows, anatomical connections are known to be reciprocal. (RGC, retinal ganglion cell layer; LGN, lateral geniculate nucleus of the thalamus; M, magnocellular subdivisions; P1 and P2, parvocellular subdivisions; MT, middle temporal; MST, medial superior temporal; FST, fundus superior temporal; PP, posterior parietal cortex; VIP, ventral intraparietal; STP, superior temporal polysensory.) (From Albright, 1993.)

the 1960s by the Polish psychologist Jerzy Konorski (1967), who proposed the existence of "gnostic units"—cellular representations of visual features, such as faces, that convey highly meaningful information to the observer. Horace Barlow (1972) expressed similar views in his "cardinal cell" hypothesis. The possibility that cells specialized for detection of faces might exist at higher levels of processing was a logical extension of the findings of Hubel and Wiesel (1977), which documented increasingly abstract cellular representations as one ascended the hierarchy of processing stages. In addition, the feature detector/cardinal cell hypothesis followed naturally from several decades of neuropsychological research demonstrating that damage to the inferior temporal lobe of the cerebral cortex compromised a human or monkey's ability to identify complex objects (Gross, 1973; Farah, 1995). Most importantly, damage to a small subregion of this cortex in humans results in an inability to recognize faces, a syndrome called *prosopagnosia*. Patients with prosopagnosia can identify a face as a face, its parts, and even specific emotions expressed by the face, but they are unable to identify a particular face as belonging to a specific person (Farah, 1995).

Strong electrophysiological evidence in support of the feature detector/cardinal cell hypothesis came from the work of Charles Gross beginning in the late 1960s. Using the same methods established by Kuffler, Hubel and

Wiesel, and others, Gross discovered cells in the inferior temporal cortex of monkeys that respond to specific types of complex stimuli such as hands and faces (Gross et al., 1969). For cells that respond to a hand, individual fingers are particularly critical. For cells that respond to faces, the frontal view of the face is the most effective stimulus for some cells, while for others it is the side view. Whereas some neurons respond preferentially to faces, others respond to facial expressions. It seems likely that such cells contribute directly to the perceptually meaningful experience of face recognition.

General Principles of Visual System Organization and Function

We have here covered only a few highlights of twentieth century research on the visual system. The complete legacy has led to a number of general principles of visual system organization and function to which we now turn.

The Visual System Is Hierarchically Organized

A consistent feature of the visual system is the presence of multiple hierarchically organized processing stages (Figure 16), through which information is represented in increasingly complex and abstract forms (for review see Van Essen, 1985). As first suggested by Hubel and Wiesel (1962), properties present at each stage result, in part, from selective convergence of information from the previous stage. The hierarchy begins with the photoreceptors, which detect the presence of a spot of light shining upon a particular part of the retina. Each retinal ganglion cell—the output cells of the retina—surveys the activity of retinal bipolar cells, which in turn survey activity in a group of receptors. The product of this convergence of information in the retina is a simple abstraction of light intensity, namely a representation of luminance contrast. A neuron in the lateral geniculate nucleus surveys a group of retinal ganglion cells, and activity in the geniculate cell also signals luminance contrast. A simple cell in primary visual cortex surveys a population of geniculate neurons and the firing of that simple cell reflects a still higher level of abstraction: the presence of an oriented contour in the visual image. At successively higher stages of processing, information is combined to form representations of even greater complexity, such that, for example, individual neurons within the pathway for visual pattern processing encode complex behaviorally significant objects, such as faces.

How far does this hierarchy go? Is there a group of cells that observes the hierarchies of simple cells and makes one aware of the total pattern? And if so, is there a still higher group in the hierarchy that looks at combinations of complex patterns as these enter our awareness? These are important questions for the future of systems neuroscience, which we address below in our discussions of visual feature "binding" and consciousness.

The Visual System Is Organized in Parallel Processing Streams

In addition to a hierarchy of processing stages, the visual system is organized in parallel streams (Figure 16). Incoming information of different types is channeled through these different streams, such that the output of each stream serves a unique function. This type of channeling occurs on several scales and at different hierarchical levels (for review see Van Essen, 1985).

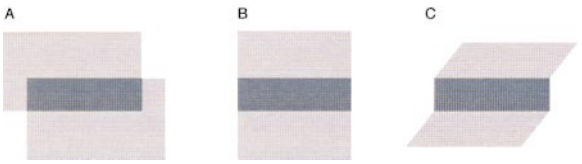

Figure 17. The Influence of Local Sensory Context on Visual Perception

Each of the three images displayed here contains a horizontal dark gray rectangle. Although the rectangles are physically identical, the surrounding features (the contexts) differ in the three images. As a result, the rectangle is attributed perceptually to different environmental causes in the three instances: In the image shown in (A), the rectangle appears to result from the overlap of two surfaces, one of which is transparent (e.g., a piece of tinted glass). In the image shown in (B), the rectangle appears to result from a variation in surface reflectance (e.g., a stripe painted across a large flat canvas). In the image shown in (C), the rectangle appears to result from partial shading of a three-dimensional surface (i.e., variation in the angle of the surface with respect to the source of illumination). These markedly different perceptual interpretations argue for the existence of different neuronal representations of the rectangle in each of the three instances. These representations can only be identified in neurophysiological experiments if the appropriate contextual cues are used for visual stimulation. See the text for details. (Courtesy of T. Albright and colleagues.)

One of the most pronounced examples of channeling occurs within the projection from retina to the lateral geniculate nucleus of the thalamus, and beyond. Different types of retinal ganglion cells project selectively to three different laminar subdivisions of the lateral geniculate nucleus, known as parvocellular, magnocellular, and koniocellular laminae. Each of these subdivisions is known to convey a unique spectrum of retinal image information and to maintain that information in a largely segregated form at least as far into the system as primary visual cortex.

Beyond V1, the ascending anatomical projections give rise to two visual pathways, each of which is organized hierarchically (Figure 16). One pathway extends dorsally to terminate within the parietal lobe, and includes area MT and visual areas of the posterior parietal cortex (Figure 19). A second pathway extends ventrally to terminate within the temporal lobe, and includes areas V4 and inferior temporal cortex (for review see Felleman and Van Essen, 1991). On the basis of a large body of neuropsychological data, Leslie Ungerleider and Mortimer Mishkin concluded in the early 1980s that these two cortical pathways serve different functions (Ungerleider and Mishkin, 1982). Accordingly, cortical areas of the dorsal pathway are concerned with "where" an object is in visual space. These areas represent motion, distance, and the spatial relations between surfaces in the visual world, and provide a crucial source of information for initiating and guiding movements. By contrast, areas of the ventral pathway are concerned with "what" an object is. These areas represent information about form and the properties of visual surfaces, such as color or texture, and play important roles in object recognition (for review see Milner and Goodale, 1995). Electrophysiological studies of the response properties of neurons in areas of the proposed "where" and "what" pathways have provided support for this functional dichotomy (Figure 17).

The "what" versus "where" distinction continues at still higher levels, where visual information is stored in memory for later retrieval. The dorsal stream projects to a subdivision of frontal cortex that is known to be critical for visual spatial memory. The ventral stream projects to a different subdivision of frontal cortex, which serves object recognition memory.

Many Visual Processing Stages Are Topographically Organized

Near the turn of the century, Hermann Munk (1881) suggested, in part on the basis of observed effects of cortical lesions, that the visual cortex may contain a spatial map of the retinal surface. This hypothesis was confirmed in the 1910s by Gordon Holmes (1927), who discovered a precise relationship between the site of damage in human visual cortex and the location in visual space where visual sensitivity was lost. Even better evidence came in 1941 from the work of Wade Marshall and Samuel Talbot, who used gross electrophysiological techniques to demonstrate an orderly topographic representation of visual space across the cortical surface, such that neurons with spatially adjacent receptive fields lie adjacent to one another in the brain (Talbot and Marshall, 1941). It has since been discovered that neuronal maps of visual space are characteristic of many visual processing stages (for review see Van Essen, 1985). Such maps may facilitate computations based on comparisons of visual information at adjacent regions of visual space. These maps are commonly distorted relative to the visual field, such that the numbers of neurons representing the center of gaze, which is particularly important for visual object recognition, greatly exceed those representing the visual periphery. These variations in "magnification factor" are thought to underlie variations in the observer's resolving power and sensitivity.

The Visual Cortex Is Organized in Vertical Columns

The existence of a column-like anatomical substructure in the cerebral cortex has been known since the beginning of the twentieth century, following the work of Ramón y Cajal, Constantin von Economo, and Rafael Lorente de Nó. Although Lorente de Nó (1938) first suggested that this characteristic structure might have some functional significance, it was the physiologist Vernon Mountcastle who developed the concept fully and proposed in the 1950s that this may be a general principle of cortical organization. Using single-cell electrophysiological techniques, Mountcastle (1957) obtained the first evidence in support of this proposal through his investigations of the primate somatosensory system.

The best studied example of columnar organization, however, is that discovered in the 1960s by Hubel and Wiesel. These investigators found that primary visual cortex is arranged in a series of narrow vertical columns, about 100–200 μm in width, running from the surface of the cortex to the white matter (Hubel and Wiesel, 1962, 1968). In a given column, cells have similar receptive field positions and generally similar receptive field properties, including preferred orientation. Consistent with Mountcastle's hypothesis, the columns seem to serve as elementary units of cortical organization designed both to bring cells together so that they can be appropriately interconnected and to generate from their interconnections the properties needed for cells with higher-order receptive fields. Additional evidence for functional columns, and for the validity of Mountcastle's proposal, has come from studies of higher visual areas. In the early 1980s, Thomas Albright and colleagues identified a system of functional columns in area MT (Albright et al., 1984). Interestingly, the spatial scale of this columnar system, which represents direction of stimulus motion, is virtually identical to that for orientation in primary visual cortex—consistent with the expression of a general organizational principle. Columnar systems have also been observed in inferior temporal cortex (Fujita et al., 1992).

Why are columns a preferred form of cortical architecture? Mountcastle's original proposal assumed the need for adequate "coverage," such that, for example, the machinery for detecting all contour orientations is available for all parts of the visual field represented in the cortex. There are also computational advantages (Schwartz, 1980) afforded by representing similar features adjacent to one another—such as the ability readily to compare and contrast similar orientations. Finally, it may be that columnar structure is derived simply from developmental constraints, such that it is easier and more economical to wire together a cortex that has similar properties in close proximity (Swindale, 1980; Miller, 1994; Goodhill, 1997).

The Visual System Is Modifiable by Experience during Early Postnatal Development

The mammalian brain develops through a complex multistaged process that extends from embryogenesis through early postnatal life. The end product of this developmental sequence is a set of patterned anatomical connections that give rise to the mature system properties of the brain. Once the principal features of mature visual system organization and function became known through the work of Hubel and Wiesel in the 1960s, it was natural to question whether those features reflect a genetically predetermined plan that is implemented during development, or whether they are influenced by the amount and type of visual stimulation that occurs before the developmental process is complete.

These questions have been addressed through experiments in which (1) the properties of the system are assessed at birth or shortly thereafter (precluding the possibility of any significant contribution of experience), or (2) animals are subjected to abnormal visual experience during postnatal development. The earliest experiments of the former type were conducted by Hubel and Wiesel (1963), who reported the visual sensitivities of neurons in the primary visual cortex of newborn kittens to be similar in most respects to those of mature animals. Although these findings suggested a large degree of genetic control, Wiesel and Hubel (1963, 1965) also discovered that abnormal visual experience, such as extended closing of the eyelids or induction of strabismus (both achieved surgically), dramatically altered the visual sensitivities of cortical neurons, provided that the intervention occurred during a "critical period," which extended for several weeks postnatally. Other studies have demonstrated close relationships between such critical periods and their presumed causes and effects,

i.e., the formation of appropriate anatomical connections (for review see Katz and Shatz, 1996) and the development of visual perceptual abilities (for review see Teller, 1997).

The general view that has emerged from all of these experiments is that the newborn visual system possesses a considerable degree of order, but that visual experience is essential during critical periods to maintain that order and to fine-tune it to achieve optimal performance in adulthood. Hubel and Wiesel (1965) summed up the implications of this view with characteristic prescience and breadth: "All of this makes one wonder whether more subtle types of deprivation may not likewise exert their ill effects through the deterioration of complex central pathways that either were not used or else were used inappropriately." The degree to which this may be true throughout the life of an organism is an issue we address below in the context of perceptual learning.

A Future for the Study of Neural Systems

Despite unprecedented progress, our understanding of visual system organization and function is far from complete. On the contrary, developments and discoveries of the past century have raised many new and often unanticipated questions regarding the visual system and other large brain systems. Here we focus on a few of the bigger issues at stake, with some predictions about where this field of research may be headed in the new millennium.

How Do Sensory Representations Lead to Perception?

The physiological studies of Hubel and Wiesel and many others over the past 50 years have revealed much about how basic features of the visual image, such as oriented lines and patches of color, are detected and represented by cortical neurons. But how do these cellular representations account for our perceptual experience of the world? The underlying assumption has been that perception of complex scenes would result from the collective activities of neurons whose properties we so far have considered and which were characterized under reduced stimulus conditions. It is, however, increasingly apparent that this assumption is flawed because it posits that individual neuronal representations of sensory features are independent of one another, and the field of sensory physiology is consequently at a turning point in its evolution.

One can fully appreciate the problem and begin to chart a new course for the future by tracing the origins of current views on the cellular bases of perception. One popular nineteenth century view was known as "elementism." According to this influential doctrine, any percept can be explained as a collection of independent internal states (sensations) elicited by individual sensory elements, such as brightness, color, and distance. The undeniable appeal of elementism rests on the power of reductionism, whereby it should be possible in principle to dissect out the elemental causes of perceptual experience—a red patch here, a yellow contour there, some motion in the center, etc.—much in the way one might dismantle a pump. There were, nonetheless, many early critics of this view, including the physicist Ernst Mach (1886), and its foundations crumbled at the turn of the century with the emergence of a Gestalt theory of visual function, which maintained that the perceptual whole is indeed far greater than the sum of the sensory parts.

This Gestalt perspective was promoted vigorously by the psychologists Max Wertheimer (1924), Wolfgang Kohler (1929), and Kurt Koffka (1935), and its legitimacy was most effectively communicated by simple and compelling visual demonstrations. Through such demonstrations it could easily be seen that the percept elicited by one stimulus element (e.g., brightness) is heavily dependent upon other stimulus attributes (e.g., three-dimensional form) in the same image. A key feature of Gestalt theory is thus *contextual interaction*: the perceptual interpretation of *each* visual image feature is a function of the context offered by *other* features in the image. *Why* contextual interaction occurs is in itself an interesting question. The answer can be found in the fact that visual images are only ambiguously related to the visual scenes that give rise to them (i.e., there are, in principle, an infinite number of scenes that can lead to the same visual image on the retina). The context in which an image feature appears provides a rich source of information that can be used to resolve its ambiguity—to, in other words, assist the viewer in identifying the "meaning" of the feature, as defined by the content of the scene that led to its appearance.

Although the holistic and eminently functional perspective of the Gestalt tradition bears great validity, the tradition has long lacked momentum, owing in part to its failure to develop mechanistic or neuronal foundations, and it has had surprisingly little influence throughout much of the twentieth century. Indeed, with the rise of single-cell studies of the mammalian visual system, we have witnessed an unwitting return to the principles of elementism, largely as a matter of investigative convenience. And therein lies the problem. When Hubel and Wiesel stimulated cortical neurons with single oriented lines, they purchased the power to reduce the response to a simple code for oriented image features, which has been of enormous benefit, but at the cost of a lack of generality. From the orientation tuning of a V1 neuron obtained by such means, one learns how the cell encodes the physical properties of the retinal stimulus. If, however, the *meaning* of the stimulus—i.e., its environmental cause, *the thing that is perceived*—is only revealed by context, as shown by the Gestalt theorists, then it is frankly impossible to learn what role the cell plays in perception using this experimental approach.

In the search for an alternative approach to carry us into a new millennium, it is useful to return to the principles of Gestalt theory and to develop an operational distinction between candidate neuronal substrates for sensation and for perception. Accordingly, candidate neuronal substrates for sensation—which have been the primary subjects of study over the past 50 years—encode the physical properties of the proximal stimulus (the visual image), such as orientation or direction of motion. Perceptual representations, on the other hand, reflect the world (the visual scene) that likely gave rise to the sensory stimulus. Contextual manipulations make it possible to dissociate local sensory properties from perception, and thereby offer a means to identify neuronal responses that are correlated with perception rather than the proximal sensory stimulus. Francis Crick

and Christof Koch (1998) have recently equated perceptual representations of the sort defined here with "neural correlates of consciousness," owing to their belief that perceptual awareness is a legitimate operational definition of consciousness. We take this issue up below in the section on vision and consciousness.

There are many ways in which this research strategy for studying candidate neuronal substrates for perception can be applied. These fall into two complementary categories. First, one can investigate whether neuronal responses to identical receptive field stimuli co-vary with the different percepts—determined by different contexts—that those stimuli elicit. The set of stimuli illustrated in Figure 17 are of this class, and a valid experimental goal would be to identify neuronal responses that vary with the percept elicited, even though the receptive field stimulus (the gray rectangle in Figure 17) remains physically unchanged. Second, one can investigate the neuronal responses to different receptive field stimuli—"sensory synonyms"—that elicit the same percept, owing to context. Both of these situations, which are prevalent in normal experience, afford opportunities to experimentally decouple sensation and perception.

The *first* of these two approaches—whether neuronal responses co-vary with different contexts—was used by Thomas Albright, Gene Stoner, and colleagues to understand the role of cortical visual area MT in motion perception (for review see Albright and Stoner, 1995). Moving objects in a typical visual scene commonly generate a complex array of moving visual image features. One objective of the visual system is to integrate these moving features to recover the coherent motions of the objects that gave rise to them. That integration process is heavily and necessarily context dependent, such that, for example, the *object* motions that are perceived from two identical collections of *image* motions can vary greatly as a function of the context in which the motions appear. A simple example of this phenomenon can be found in the appearance of two overlapping stripes that move in different directions. This type of visual stimulus, which is known as a "plaid pattern," is a simple laboratory incarnation of a common real-world occurrence, namely two objects moving past one another.

Albright and Stoner proposed that if contextual cues present in the image indicated that the two stripes lay at different distances from the observer, then the two stripes would appear to move in two different directions. On the other hand, if the very same motions were viewed in the presence of contextual cues that indicated no such depth ordering, then the two striped components of the plaid would appear to move coherently in one direction. There are a number of different contextual cues that can be used to elicit a percept in which the stripes are ordered in depth. In their initial experiment, Stoner, Albright, and V. S. Ramachandran (1990) used luminance cues for perceptual transparency to achieve this goal. Simply by adjusting the intensities of light coming from different regions of the plaid pattern, it was possible to make the plaids appear as either a single surface or as two overlapping surfaces. Moreover, as predicted, these contextual manipulations dramatically altered perceived motion, even though the image motion was unchanged. Once this dissociation between image and perceived motion was discovered, the dissociation became a useful tool to investigate whether the responses of cortical motion-sensitive neurons encode image motion or perceived motion.

In a second experiment, Stoner and Albright (1992) pursued this idea and found that when directionally selective neurons in cortical visual area MT were presented with identical image motions that were perceived differently as a function of context, the responses of many neurons co-varied with the motion that was perceived. These findings support the view that an important step in the visual processing hierarchy is the use of context to construct cellular representations of visual scene attributes—the stuff of perceptual experience—out of cellular representations of visual image features, such as oriented contours.

The *second* approach to decoupling sensation and perception is the reciprocal of the first and is based upon the phenomenon of perceptual constancy, in which multiple sensory stimuli give rise to the same percept, owing to appropriate contextual cues. Perceptual constancies reflect efforts by the visual system to recover behaviorally significant attributes of the visual scene, in the face of variation along behaviorally irrelevant sensory dimensions. Size constancy—the invariance of perceived size of an object across different retinal sizes—and brightness/color constancy—the invariance of perceived reflectance or color of a surface in the face of illumination changes—are classic examples. Generally speaking, the physiological approach advocated is one in which neuronal responses are evaluated to determine whether they co-vary with the changing receptive field stimulus, or whether they exhibit an invariance that mirrors the percept. Physiologists have only begun to employ this approach. Several studies, for example, have recently explored a phenomenon termed "form-cue invariance," in which a percept of motion or shape is invariant across different "form cues," such as luminance, chrominance, or texture, that enable the stimulus to be seen. In one such study, Albright (1992) discovered a population of motion-sensitive neurons in area MT that appear to encode the direction of motion of a stimulus independently of the fact that the form cue—an aspect of the retinal stimulus that is, in principle, irrelevant to motion detection—is varied. To paraphrase from Horace Barlow's (1972) Neuron Doctrine for Perceptual Psychology, the main function of such cells appears not to be the encoding of specific characteristics of retinal illumination, "but to continue responding invariantly to the same external patterns"—i.e., to the *meaningful* attributes of the input.

From the outset, the physiological approach to the operations of the visual system has seen itself as being the service of perceptual psychology. If the "exploration of psychological territory" is to continue, however, physiologists must advance beyond the acontextual approach that has been the standard of twentieth century research in this field. New experimental approaches in which contextual influences are exploited as tools for the study of neural substrates of perception are thus likely to be an important feature of future research in this area.

Binding It All Together

As we have seen, the representational strategy that the visual system has adopted is one in which the properties of the incoming signal are distributed across many neurons, such that each neuron only conveys a small piece of the larger picture. At the level of the retina, for example, the information represented by single cells is limited to a small circular region of space. At the level of primary visual cortex, cells integrate information from earlier stages in order to convey information about contour orientation, but they remain highly specialized. At still higher levels, visual information is further combined and abstracted to yield even more complex properties and greater specialization of function, as evidenced by the multiplicity of extrastriate visual areas. In view of this strategy, one cannot help but wonder how all of the specialized representations are bound together to render a neuronal signal that conforms to the complex patterns that we perceive. How is it, for example, that the cells representing the edges, the varying orientations, the colors and textures, and the different distances associated with the tree outside my corner window, are linked together to produce my percept of that tree? How are other properties of the same visual image, such as the attributes of a different but nearby tree, "segmented" and bound together as a separate entity from the first? Even more puzzling is the fact that only some of these complex patterns enter my awareness at any point in time. How are those patterns selected, and how are objects that we are simultaneously aware of linked together? What role does visual attention play in this process? Is there a distinguishing feature of the collections of neurons that happen to represent objects or collections of objects that we have become conscious of? Is there, as contemplated by Sherrington (1941) a half-century ago, one "pontifical cell" that represents the final outcome of this integration process? The representational problem addressed by these questions has become known as the "binding problem." In a more general form, the problem has preoccupied philosophers and cognitive scientists for decades, and it now stands among the most formidable challenges in modern neuroscience (see for example October 1999 issue of *Neuron*).

At its most basic level, the binding problem is simply that of representing conjunctions of attributes. There are, in principle, two mechanistic strategies that could accomplish this task, one based on neural space and the other based on time. On the one hand, attributes that must be conjoined—such as the color and the direction of motion of an object—could be represented in that form by selective convergence of information onto single neurons, yielding a neuron, for example, that selectively encodes rightward moving red objects. The appeal of such a strategy is that it follows naturally from what is already known of the hierarchical properties of the visual system. The neuronal representation of an oriented contour is, after all, nothing more than a product of selective convergence of information from the previous stage. Moreover, long-standing evidence indicates that neurons well up in the hierarchy encode very complex conjunctions of visual attributes, such as those associated with faces (Gross et al., 1969). The problem with this form of binding, however, is one of generality: the variety of unique perceivable conjunctions of visual attributes vastly exceeds the number of available neurons. So while this may be a strategy that the visual system has adopted for early levels of integration and for highly specialized and vital functions like facial recognition, it is simply untenable as a general mechanism for binding.

The alternative strategy is one in which visual attributes are bound in time, rather than by static spatial convergence. The obvious advantage of a dynamic binding mechanism is that, unlike the static design, it places no serious combinatorial limits on the pieces of information that can be conjoined. A form of this mechanism was suggested as early as 1949 by the psychologist Donald Hebb (1949), who hypothesized the existence of "cell assemblies." Each such assembly was conceived as a collection of neurons that are dynamically associated with one another as needed to link the features they independently represent. A key feature of this concept is the ability of each cell to hold membership in multiple overlapping assemblies—such that, for example, a cell that represents upward motion may be assembled with a cell representing the color red on one occasion, but assembled with a cell representing the color green on a different occasion. This view of binding was subsequently elaborated upon by Horace Barlow (1972), who noted it to be a particularly efficient form of representation because perceptions commonly "overlap with one another, sharing parts which continue unchanged from one moment to another."

The trick, of course, is identifying a *dynamic binding code* that can be used to transiently link cells into an assembly. One idea, which was implicit in Hebb's original proposal, developed significantly in the early 1980s by the theorist Christof von der Marlsberg (1981), and which has subsequently drawn a great deal of attention (see, for example, reviews in October 1999 issue of *Neuron*), is that temporal synchrony of neuronal firing patterns may underlie binding. As suggested in 1989 by Charles Gray, Wolf Singer, and colleagues, "synchrony of oscillatory responses in spatially separate regions of the cortex may be used to establish a transient relationship between common but spatially distributed features of a pattern" (Gray et al., 1989). This solution is in effect a dynamic switchboard that binds collections of complex features "on demand" via synchronized firing of the neurons that represent the individual features. Gray and Singer presented provocative data in support of this hypothesis. They found that the temporal spiking patterns of pairs of simultaneously recorded neurons in visual cortex were likely to be correlated if the separate visual stimuli that elicited those patterns appeared (to human observers) to be part of a common object. Other studies, however, have failed to find support for this synchrony hypothesis (e.g., Lamme and Spekreijse, 1998; for review see Shadlen and Movshon, 1999) and the matter remains unsettled.

If we accept the concept of dynamic cell assemblies, and the related proposal for temporal binding by synchronous firing (if only for the sake of argument), we face many critical questions. How, for example, are the transient patterns of synchrony "read out"? Does synchronous firing lead to transient synaptic facilitation of converging inputs onto a multipurpose pontifical cell

(or, perhaps more appropriately, given the democratic and ephemeral nature of the hypothesized convergence, a "presidential cell")? Or is the perceptual binding simply implicit in the activity of the synchronized neurons, which constitute flexible cell assemblies for specific percepts? What elicits synchrony to begin with? Is there a top–down supervisory module that identifies attributes that experience tells us are likely to be parts of the same object? Or is the process bottom–up, using a variety of "image segmentation cues" to parse out attributes that belong to the same versus different objects? And what happens when—as is often the case—there are multiple objects perceived simultaneously? Is spike timing sufficiently precise to allow multiple synchrony events to occur simultaneously?

While we await the answers to these and other questions, it nevertheless appears likely that visual integration rests upon a combination of static and dynamic binding mechanisms. Indeed, except for a few clever hypotheses and controversial details, our understanding of these processes has advanced little beyond the view advocated by Barlow in the early 1970s (as a counterpoint to Sherrington's pontifical cell metaphor), according to which a series of "cardinal cells" reside at the top of static convergence hierarchies, but "among the many cardinals only a few speak at once" in the form of dynamic cell assemblies (Barlow, 1972). But research now moves rapidly on these fronts and, with vast improvements in technology for monitoring the firing patterns of many neurons simultaneously, the existence and operations of cell assemblies and their role in binding should come into sharper focus in the coming years.

Vision and Consciousness

Interestingly, the binding problem and its proposed solution by a dynamic code have also been coupled with the phenomena of visual awareness and consciousness. In the case of visual awareness, the argument is quite natural (if not tautological), as there are good reasons to believe that the perceptual binding of visual attributes is tantamount to their reaching the perceiver's awareness. Indeed, Singer and colleagues have argued that "appropriate synchronization among cortical neurons may be one of the necessary conditions for the . . . awareness of sensory stimuli" (Engel et al., 1999). Developing the concept of neuronal "metarepresentations," Singer (1998) has furthermore suggested that this code may underlie all of the complex patterns that enter our awareness at any time. The extension of this line of argument to consciousness depends, of course, on how one defines the term. Although there is a long history of confusion about what consciousness actually means, and a plethora of colloquial uses of the term, Francis Crick and Christof Koch (1998) have recently attempted to facilitate scientific progress by arguing for a specific and limited definition that is relevant to vision. (We consider the issue more broadly in Part V below). Roughly speaking, that definition can be equated with perceptual awareness; it is "enriched" by visual attention, and may fill a window of time, which Gerald Edelman (1989) has termed "the remembered present" (see also William James, 1890). If we accept that operational and not-unreasonable definition, then dynamic representations of the sort proposed by Singer and others may be relevant to consciousness. But this is slick and unstable terrain, newly trodden by neuroscience and lacking the guideposts of established experimental paradigms (deflecting, to paraphrase Bertrand Russell, nearly all but fools and Nobel laureates). It is, nonetheless, one of the most compelling issues facing the future of neuroscience, and is certain to be a focal point of research in the next century.

What Are the Local Cellular Mechanisms of Vision?

As we have seen, physiological studies have revealed much about the types of visual information carried by neurons at different processing stages. In parallel, anatomical studies have told us a great deal about the gross pattern of connections within and between different stages of processing. Until recently, however, comparatively little has been known about the local circuits that confer neuronal properties and mediate the computations required for perception. This knowledge is an essential starting point for understanding how neurons integrate and store different sources of visual information, as well as alter their sensitivity to compensate for environmental and behavioral changes.

Recent progress in this area has been fueled by new technologies that allow finer resolution tracing of anatomical connections, in conjunction with methods that allow assessment of the contributions of these connections in their functional state. For example, optical imaging of neuronal activity, combined with cell labeling, is enabling us to determine the relationships between functional architecture and cortical circuits (Malach et al., 1993). Investigation of correlated firing patterns between pairs of neurons, in conjunction with precise measures of receptive field properties, has provided an approach complementary to anatomical tracing of local circuitry (for review see Usrey and Reid, 1999).

Another promising technique of this sort, known as photostimulation, was applied recently by Edward Callaway and Lawrence Katz. This technique, which enables one to assess the pattern and strength of synaptic connections between neurons in local cortical circuits, exploits a form of the excitatory neurotransmitter glutamate that is inactive ("caged") until illuminated (Katz and Dalva, 1994). Callaway and colleagues have revealed that the pattern of functional connections between different laminae in primary visual cortex provides far more opportunities for cross-talk between different visual processing streams than was evident from traditional anatomical studies (Callaway, 1998).

Another approach to understanding the relationship between circuitry and function might involve deactivation of individual circuit components, such as specific classes of cells, and assessment of the ensuing loss of function. At a gross level, this approach is recognizable as the lesion method that has been used in neuropsychology for over a century to reveal the functions of large neuronal systems. But is it realistic to expect that these methods can be extended to identify the fine details of circuit organization and function? The fact that circuit components are both anatomically and functionally intermingled—particularly in the cerebral cortex—would seem to preclude this possibility. A resolution, however, can be found in new molecular techniques that enable one to manipulate gene expression and to exploit

the genetic distinctiveness of cells that serve different functions.

These new techniques incorporate three key features: (1) the ability to introduce novel genes into neurons, the expressed products of which will alter neuronal function, (2) the ability to regulate expression of these transgenes in a time-dependent manner, and (3) the ability to regulate expression of these transgenes selectively in specific classes of cells. The first of these techniques has been standard fare for some time now in the form of germline transgenic manipulations in mice (for review see Picciotto, 1999). The same end point is now possible in other species, including primates, using viral vector transfection (Takahashi et al., 1999). In principle, as discussed earlier, it should be possible by these means to introduce novel genes that block neuronal cell firing when expressed, which would effectively remove affected cells from the circuit. Recent evidence suggests that overexpression of K^+ channels may be an effective means to transiently inhibit conduction of action potentials or their propagation into dendrites (Johns et al., 1999). The second technique is also becoming routine using one of a number of inducible systems that promote gene expression only in the presence of exogenous factors, which can be delivered by the experimenter (e.g., No et al., 1996). This temporal control permits before and after measures of the contributions of affected cells. The third technique—cell-specific expression—is absolutely critical, of course, if these tools are to provide any greater resolution than standard cell-ablation techniques. As discussed earlier in this review, this technique taps into gene "promoters" that are known to regulate expression of specific genes only in specific cell types. By replacing the genes that are normally regulated with novel genes, one can restrict expression of these transgenes to cells that recognize the promoter. Mark Mayford, Eric Kandel, and colleagues have demonstrated the feasibility of these three basic techniques using germline transgenic manipulations in mice to explore the functions of hippocampal neurons in relation to memory storage (Mayford et al., 1996).

Related techniques might also be used to facilitate anatomical analysis of local circuits. For example, instead of introducing and expressing a gene that disrupts cell firing, one might simply transfect neurons with genes that encode visible proteins, such as GFP. The end result in this case would be selective labeling of a specific class of cells, which could be used, for example, to identify those cells in a brain slice preparation for physiological recording, or simply for analysis of cell morphology and connections using light microscopy. Recent experiments document the feasibility of this general approach using germline transgenic manipulations in mice (Yoshihara et al., 1999).

There are many technical details that will need to be worked out—not the least of which is the identification of additional cell-specific promoters perhaps through the strategies outlined in our earlier discussion of the assembly of neural connections—before these fantasy experiments become a practical means to investigate the organization and function of local circuits in the primate visual system. Nonetheless, the potential benefit afforded by this unprecedented merger of molecular tools and systems approaches to brain function is clearly enormous. They are certain to become a staple of future experiments aimed at understanding the entire realm of brain systems.

How Do Cellular Representations Change with Visual Experience?

As we have seen, a central tenet of modern neuroscience is that stages in brain development correspond to specific stages in the development of perceptual abilities. These stages are known as critical periods, and they are characterized by an extraordinary degree of neuronal and perceptual plasticity. Only recently has it been recognized that the plasticity of the visual system is not restricted to these critical periods early in development, but is modifiable throughout the adult life of the organism. The forms of this adult plasticity are many and varied, but all can be viewed as recalibration of incoming signals to compensate for changes in the environment, the fidelity of signal detection (such as that associated with normal aging or trauma to the sensory periphery), or behavioral goals.

One of the most striking and revealing forms of adult neuronal plasticity is that associated with *perceptual learning*, which is an improvement with practice in the ability to discriminate sensory attributes. In humans, these learning phenomena are ubiquitous in everyday life and generally self-evident, and they have been a subject of scientific investigation for decades (for review see Karni and Bertini, 1997). Consider, for example, the copy editor who over time becomes particularly sensitive to graceless word pairings, or the assembly line worker who can instantly recognize the miswired transistor. Until recently, however, little effort had been made to investigate their neuronal bases. Indeed, the critical period concept had become so widespread and deeply rooted that there seemed little ground for believing that visual representations might be modifiable throughout life.

Thus, it was well before the modern neuroscience community was prepared to embrace the concept of plastic representations in mature animals, that Michael Merzenich began to address the degree to which sensory maps could change in response to a variety of manipulations (for review see Buonomano and Merzenich, 1998). This work began to have a broad impact in the mid-1980s with the demonstration of marked and systematic reorganization of somatosensory cortex in response to a change in the peripheral sensory field (e.g., selective deafferentation). Even more exciting and provocative was the subsequent demonstration that cortical maps reorganize in response to selective use of components of a sensory modality, in a manner that mirrors perceptual learning.

Following in the footsteps of Merzenich, Charles Gilbert has recently begun to investigate the relationship between adult visual perceptual learning and the receptive field properties of cortical neurons. In one set of experiments, Gilbert and colleagues have found that increases in perceptual sensitivity fail to generalize to spatial locations or stimulus configurations beyond those in the set of training stimuli (Crist et al., 1997). This high degree of spatial specificity suggests that the underlying neuronal changes may occur at a very early

stage of processing—perhaps V1—where the spatial resolving power of cortical neurons is greatest. Other behavioral observations support this conclusion (e.g., Karni and Sagi, 1993). Using a complementary approach, Gilbert and others have demonstrated plasticity of cortical representations more directly (Gilbert and Wiesel, 1992; Chino et al., 1992). In this case, the receptive field properties of V1 neurons were found to change following localized interruption of retinal input (caused by small retinal lesions). Similar to previous findings from the somatosensory system, these changes took the form of shifts in the spatial profile of receptive fields, such that cells normally responding to light in the area covered by the retinal lesion become sensitive to stimulation of adjacent regions of visual space. These changes began to occur in a matter of minutes following deafferentation. Although in this case, unlike perceptual learning, the plasticity was not induced by repeated exposure to a sensory stimulus, but was rather a response to a marked loss of stimulation, both can be viewed as forms of renormalization and the underlying cellular mechanisms may be similar.

This is among the most exciting areas of systems neuroscience today, bridging as it does the topics of sensory processing and learning. Early contributions to this field have been particularly inspiring and influential because the prevailing wisdom held that sensory representations were largely immutable following critical periods of developmental reorganization. As we have seen, recent observations prove that this is not the case. On the contrary, representational changes occur throughout life as part of a normalization process to compensate for damage or deterioration of the sensory periphery or to meet novel behavioral and perceptual requirements. But these findings naturally raise many new questions that will occupy neuroscientists for years to come. Little is yet known, for example, of the specific neuronal events that give rise to plasticity of the adult visual system, although such processes are sure to include changes in synaptic efficacy, changes in neuronal cell structure, and possibly neurogenesis. Future research is also likely to address the following questions: How are these experience-dependent changes in visual processing mediated? Evidence indicates that higher cortical areas, such as regions of the frontal cortex, contain neurons that represent attributes of memorized stimuli. What role, if any, do these mnemonic representations play in the formation of experience-dependent changes in visual cortex? What are the control signals that initiate such changes? Does representational plasticity occur at all stages of visual processing? Does it occur in the retina? Do such changes constitute the neuronal repository of long-term visual memories?

Beyond Vision: Exploring Links with Other Brain Systems

It is a pedagogical convenience to treat the visual system—as we have done here—as functionally independent and separable from other brain systems. The fact of the matter is that vision is but one cog in the wheel and is in many ways integrated with other major systems, including those responsible for memory, emotion, and motor control. Although we know less—much less, in some cases—about these other systems, it is now clear that the areas of interface between vision and those systems that serve storage, evaluation, and action are among the most important targets for future research in systems neuroscience. Here we consider one of these areas of interface: that associated with motor control.

Visual Guidance of Behavior: From Retina to Muscle

A major function of the visual system is to provide sensory input to guide actions, such as moving through the environment. Visual and motor control systems have in common the fact that they both represent space. But the relevant frames of reference—retinal space, in the case of vision, and ultimately muscle space, in the case of action—are radically different. How then does light falling on a particular location on my retina lead to a reaching arm movement (or an eye movement, or a leg movement, etc.) to the source of the light? The problem becomes even more puzzling if we consider that exactly the same arm movement will be executed regardless of what direction I am looking, implying that vastly different retinal signals can lead to the same motor output. In principle, there are a number of different means by which this coordinate transformation could be accomplished.

Perhaps in large part because of the compelling subjective sense that space is stable regardless of the orientations of our sense organs and our muscles, it has often been proposed that the brain contains a unified representation of space. This unified map might represent space in a three-dimensional "world-centered" frame of reference, as opposed to the more specific coordinates of the sensory (retina) and effector (muscle) organs. According to this view, we have an internal neuronal map of the spatial locations of all of the items on my desk in front of us. That map remains coherent and unchanged regardless of which way we are looking—or, for that matter, which way the entire body is oriented. The advantage of such a system is that it provides a generic source of spatial information that can be used to guide all movements, which is independent of the state of the sense organs or muscles. The disadvantage is that, because of its independence from sense organs and muscles, a generic reference frame is extremely difficult to compute.

Numerous studies conducted over the past few decades have evaluated the hypothesis that space is transformed from sensation to action via a unified reference frame. Neuropsychologists have examined the effects of damage to brain regions that lie between early visual processing and motor control—specifically the parietal and premotor areas of the cerebral cortex. The typical consequence of such damage is "neglect," in which subjects ignore stimuli that appear in certain regions of visual space (Figure 19) (for review see Mesulam, 1999). (Neglect is distinguished from blindness by the fact a neglect patient can clearly see a neglected stimulus if his or her attention is drawn to it.) If there were a single unified map of space, one would expect that neglect would be manifested in the same part of the spatial map—always to the right side of the observer, for example. On the contrary, results indicate that neglect can be present in any of a number of different spatial reference frames (retinal coordinates, body part coordinates, object-based coordinates, world-based coordinates), suggesting that there are multiple spatial maps, which may serve specialized functions (for review see Colby and Goldberg, 1999).

Neurophysiological data also support the hypothesis that there are multiple types of spatial maps used to transform information from sensory to motor coordinates. This issue has been explored extensively by Richard Andersen and colleagues, who recorded from neurons in the parietal cortex in search of a representation of visual space in head-centered coordinates (which would, in principle, be useful for directing movements of the eyes). Andersen discovered instead that these neurons possess "gain fields," by which the amplitude of the response to a visual stimulus is modulated systematically by the direction of gaze (Andersen et al., 1985). Because these neuronal responses take eye position into account, it is in principle possible to deduce the spatial location of the visual stimulus from the activity of a population of such neurons, regardless of where the eyes are looking (for review see Andersen et al., 1993). This information can then be used to guide movements to the stimulus.

Recent physiological experiments by two groups—Carl Olson and Sonya Gettner, and Michael Graziano and Charles Gross—provide fascinating evidence for more explicit but highly specialized spatial maps that could mediate visual–motor control. Olson and Gettner (1995) recorded from individual neurons in premotor cortex of monkeys and found that the neurons responded if an eye movement was made to a particular *part* of an object, regardless of the spatial location of the object. These neurons thus appear to represent space in an object-based coordinate frame. Graziano and Gross studied single neurons in the premotor cortex that possess both visual and tactile receptive fields. The visual receptive field of each neuron was found to be linked to the spatial location of the tactile receptive field, such that, for example, a neuron that was activated by tactile stimulation of the arm was also activated by a visual stimulus in the vicinity of the arm (Graziano et al., 1994). Remarkably, if the arm moved to a new location, the visual receptive field moved along with it. The visual receptive fields of these neurons thus appear to have been transformed from a retinal frame of reference to a reference frame centered on the position of the arm. Graziano and Gross propose that this arm-centered reference frame may be well suited for orchestrating movements of the arm to stimuli that are near the arm. More generally, they speculate that visual-motor transformations of many types may rely upon specialized body-part centered maps of space, rather than upon a single unified spatial map. Although both of these physiological findings suggest promising new approaches to the study of sensorimotor coordinate transformations, they leave many questions of a mechanistic nature unanswered. Perhaps the most nagging question raised by the Graziano and Gross study concerns the apparently profound spatial mobility of the visual receptive field, which dances across retinal space with every movement of the arm. How are retinal signals dynamically rerouted, as it were, to continuously update the visual receptive field of the premotor neuron, using information about arm position as a guide? This and other related questions will be an important focus of research in years to come.

Part V. Consciousness: A Challenge for the Next Century

Perhaps the greatest unresolved problem in visual perception, in memory, and, indeed, in all of biology, resides in the analysis of consciousness. This is a particularly difficult problem, in part because there is no widespread agreement on exactly what constitutes a successful solution. There is agreement nevertheless that a successful solution will require, at a minimum, insight into two major issues that lie at the heart of the study of consciousness: (1) *awareness* of the sensory world and (2) *volition*, the voluntary control of thoughts and feelings.

In this section we consider awareness by focusing on two of its components: attentional orienting to sensory signals in the presence and in the absence of stimuli (imagery). We shall then go on to consider volition by focusing on the self-regulation of thoughts, feelings, and actions. These two problems are at once relevant to consciousness, yet tractable, and therefore serve to illustrate how consciousness can be dissected biologically.

As with other problems in biology there are both reductionist and holistic approaches to these components of consciousness. A reductionist approach would view these aspects of consciousness from a genetic, synaptic, and cellular level. However, in the case of consciousness it is hard to imagine any solution that would not also require an understanding of the large neural networks that underlie cognition, actions, and emotion. In our view, the appropriate direction in seeking a solution to the problems of consciousness lies in successfully linking understanding at all of these levels, from genes to behavior. In this section we try to illustrate this integrative approach in relation to orienting to sensory stimuli, imagery, and self-regulation. Finally, we examine how far these scientific approaches will take us in understanding the most subjective aspects of consciousness.

Rigorous top–down approaches to consciousness have been limited by the lack of good methods for resolving the activity of populations of cells. The use of neuroimaging methods during the last decade has made it possible to observe the activity of large numbers of neurons in human subjects while they are studied for their awareness of the sensory world and for their voluntary control of thoughts and feelings (Posner and Raichle, 1994, 1998). Cognitive studies using these imaging methods, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), are based upon changes in blood flow and blood oxygenation that occur in localized regions of the brain when neurons increase their activity (Raichle, 1998; Rosen et al., 1998). These methods have now been applied with some success to the study of attentional orienting, visual imagery, and regulation of cognitive and emotional states. In each of these domains, the individual functional components have proven to be surprisingly well localized; however, each of the major functions of consciousness—such as attentional orienting to sensory stimuli and volition—involves not one but several functional components. As a result each function of consciousness appears to involve several networks and these are distributed across a variety of brain areas. Fortunately the enormous complexity of the problems

has been made somewhat more tractable by use of appropriate animal models. In the best case, as with studies of the visual system, it has proven possible to relate neural activity studied at the cellular level in nonhuman primates to the activity of large neural networks studied in the same brain areas but now in human subjects using brain imaging (Tootell et al., 1998). While the results are not definitive, they show that specific aspects of consciousness can even now be analyzed on the cellular level with methods currently available to neuroscience.

Orienting of Attention to Sensory Stimuli

Origins of the Modern Study of Sensory Attention

The modern study of attention can be traced to 1958 and the publication by Donald Broadbent of a monograph entitled *Perception and Communication*. Broadbent proposed that when we focus attention on one object to the exclusion of other surrounding objects, the focus of selective attention requires a filter that holds back messages from unattended channels. According to Broadbent's view, attention is a high-level skill that is so developed in some people as to allow them to perform remarkable feats such as simultaneous translation. This skill allows even untrained subjects to have a role in selecting their environment by attending only to certain stimuli while shutting out others. Although there have been challenges to this view in the four decades that have passed since it was proposed, even Broadbent's strongest critics have embraced his general approach. In the next section we begin to explore the neuronal implementation of the type of selective attention studied by Broadbent.

All Visual Areas, Including Primary Visual Cortex (V1), Can Be Biased by a Shift of Attention

To obtain an idea of how brain areas become involved in selection of a stimulus, consider the task of looking for a file on your computer desktop. If the desk is cluttered with files, you will have to search for the one you want. Such a search may be accompanied by eye movements, but, if the objects are close, the search may involve covert shifts of attention without eye movements. Such visual search tasks involve the coordinated action of the two large-scale brain networks. One network, the ventral visual pathway, which we discussed in the previous section, is concerned with objects and with form recognition, required for obtaining the identity of each file. The second network—located in the posterior parietal cortex of the dorsal visual pathway—is related to the act of shifting attention to the locations *where* the file might be found. Early studies of the dorsal pathway were conducted by Michael Goldberg and Robert Wurtz (1972). They found that cells in the posterior parietal cortex of alert monkeys responded differentially to identical stimuli depending on whether or not the monkey was attending to the stimulus (Figure 18). When the monkey attended, the firing of the cell was much more intense than when the monkey ignored the stimulus. These results provided the first data on the cellular level that neurons in the parietal cortex are correlated with attention to the location of visual objects. With the advent of neuroimaging, it became possible to see the distributed network of brain areas involved in attention

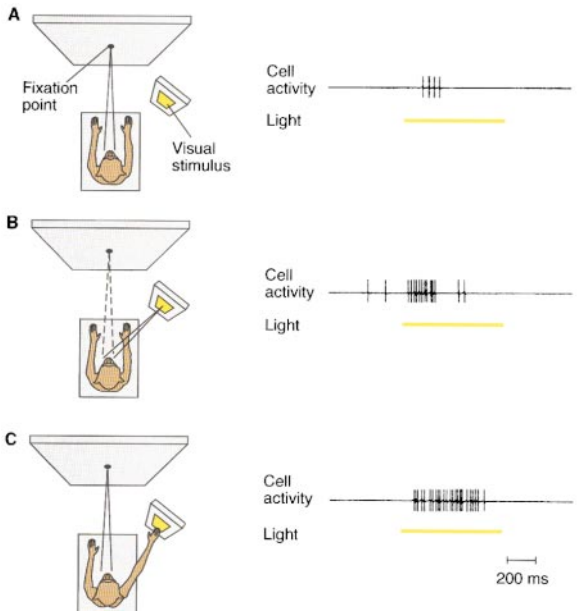

Figure 18. The Influence of Attention on the Response of Cortical Neurons

Neurons in the posterior parietal cortex of a monkey respond more effectively to a stimulus when the animal is attentive to the stimulus.
(A) A spot of light elicits only a few action potentials in a cell when the animal's gaze is fixed away from the stimulus.
(B) The same cell's activity is enhanced when the animal takes visual notice of the stimulus through a saccadic eye movement.
(C) The cell's activity is enhanced further when the monkey touches the spot, even without eye movement.
(From Wurtz and Goldberg, 1989, as illustrated in Kandel et al., 2000.)

in human subjects. This network includes the frontal eye fields, the superior colliculus, and posterior parietal lobe, all of which are also involved in eye movements (Corbetta, 1998).

Studies conducted by Robert Desimone and colleagues have addressed the role of the ventral visual pathway (particularly areas V4 and IT) in attentional control. A typical paradigm involves first establishing the stimulus selectively for a cortical neuron. Suppose, for example, that the neuron under study responded well to a red bar of light and poorly to a green bar when these stimuli were individually placed in the neuronal receptive field. At this point, both stimuli—the red and green bars—would be placed in the receptive field of the cell at the *same time*. If the animal was instructed to attend to the "good" stimulus (red bar) then the neuron responded well. If, however, the animal was instructed to attend to the "poor" stimulus (green bar) then the response was correspondingly poor—*despite the fact that the retinal stimulus was the same in both cases.* Desimone and colleagues interpreted these results as evidence that the receptive field shrinks to conform to the attended stimulus, thereby excluding the unattended stimulus and implementing a filtering mechanisms of the sort proposed by Broadbent (see Desimone and Duncan, 1995 for review).

The exact visual area that will be biased in the manner revealed by these physiological studies appears to depend upon the task required of the subject (Desimone

and Duncan, 1995; Kastner et al., 1999; Posner and Gilbert, 1999). Imaging studies have shown that if people are asked to attend to target motion, activity is increased in a brain area in the dorsal visual pathway sensitive to movement (area MT). Quite different visual areas become active for attention to other stimulus dimensions such as color or orientation (Corbetta et al., 1991). When attention is shifted to a new location, the neural activity of cells in the ventral, object recognition network is increased even before any target is presented at that location (Kastner et al., 1999).

There Is A Fundamental Distinction between Focal and Ambient Attention

Of course there is a sense in which, without even trying to attend, you are conscious of all the objects on your desktop. However, when careful tests are made that involve making changes in a complex visual scene, these tests reveal that when attention is focused on one object, other objects within the scene even large and important ones can be altered without the subject being aware that a change has taken place (Rensink et al., 1997). Thus, while attention can be summoned efficiently to a novel event, there is surprisingly little awareness of changes that occur at loci that are not attended. It, therefore, is useful to distinguish between *focal attention*, which allows reporting of details of the scene, and *ambient attention*, which forms our general awareness of the scene around us. While both are aspects of consciousness, their underlying neurobiological mechanisms may be quite different. It seems likely that ambient attention may depend primarily upon posterior brain areas. By contrast, focal attention, which is often switched between objects based on instructions, may depend on more anterior areas related to voluntary control of action.

There May Be Only a Small Number of Networks Concerned with Attention, and These Can Be Distinguished on the Cellular and Even on the Molecular Level

It seems likely that the neuronal computation that occurs in most cortical areas can be influenced by attention. Indeed, there are a surprisingly large number of sites in the brain where attentional influences can be demonstrated. However, the source of those effects is thought to emanate from a small number of networks that perform different functions.

For example, a novel visual stimulus serves both to alert the organism and to orient attention to the location of the stimulus. This distinction can be demonstrated by using separate cues for alerting and orienting. An alerting cue provides the monkey with information about when a target will occur, but not about where that target will be located. An orienting cue provides the monkey with specific information about where the target will be located and thus allows the subject to move attention to the cued location. Two separate brain networks, both located in the parietal lobe, but using distinctly different chemical transmitter systems are involved in changing the level of alertness and in switching attention toward the stimulus. Thus, the influence of alerting cues is reduced by drugs that block norepinephrine activity, but these drugs do not influence orienting. By contrast, drugs that block cholinergic activity influence orienting

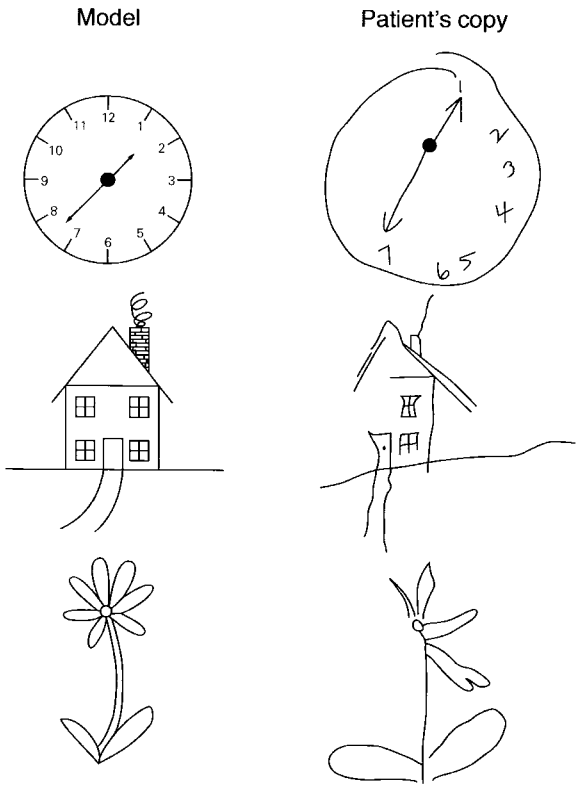

Figure 19. The Contribution of the Posterior Parietal Cortex to Visual Attention?

The three drawings on the right were made from the models on the left by patients with unilateral visual neglect following lesion of the right posterior parietal cortex. (From Bloom and Lazerson, 1988, as illustrated in Kandel et al., 2000.)

to the cue, but do not diminish the alerting effect (Marrocco and Davidson, 1998). These pharmacological studies illustrate how one can separate a simple act of attention to a novel event into two distinct components, and pinpoint both the anatomical systems and the modulatory synaptic mechanisms involved.

Findings in patients confirm the importance of neural systems for alerting and orienting to our normal awareness of the world around us. Strokes that interfere with the blood supply to the posterior parietal cortex on the right side produce an inability to orient attention to the left side, the side opposite the lesion. Patients that suffer from these right-sided lesions will show striking deficits in body image and in their perception of spatial relations. Although their somatic sensations are intact, these patients may ignore (*neglect*) the spatial aspects of all sensory input from the left side of their body as well as of external space, and they will ignore the left half of any visual object with which they are confronted. For example, patients with neglect syndrome will exhibit a severe disturbance in their ability to copy drawn figures. This deficit can be so severe that the patient may draw a flower with only petals on the right side of the plant. When asked to copy a clock, the patient may ignore the digits on the left and try to fill in all the digits on the right, or draw them down the side running off the clock face (Figure 19). These patients also may ignore the left

half of the body and fail to dress, undress, and wash the affected side.

Less dramatic but similar difficulties in orienting accompany loss of parietal neurons due to degenerative disorder such as Alzheimer's dementia (Parasuraman and Greenwood, 1998). In these cases, stimuli going directly to the lesioned area, that would normally produce orienting of attention, may no longer do so and consequently the person may be completely unaware of these stimuli.

Many researchers agree with Francis Crick's view that sensory awareness probably is the most tractable area for a rigorous understanding consciousness at a mechanistic level. As a result, much research has recently been focussed on the study of orienting to sensory stimuli. The discovery that attention can influence activity within primary visual cortex (area 17) has allowed investigators to explore attentional effects within a visual area whose other cellular and physiological features and anatomical characteristics are extremely well characterized (Posner and Gilbert, 1999). The work in area 17 therefore provides an opportunity to specify exactly what cellular structures and functions can be modified by the act of attending.

There also now are methods for exploring the consequences of attention on the natural life of the organism. One direction of current research involves the study of the maturation of orienting mechanisms in the human infants. These studies illustrate that the ability to orient attention to visual stimuli undergoes a substantial maturation during the first year of life. For example, an infant two to four months of age has great difficulty in disengaging from a strong visual attractor. If the attractor is a checkerboard, the difficulty in disengaging may cause the infant to become distressed; if the attractor is the eyes of the mother, the lack of engagement may contribute to the development of parent-infant bonding. As with age, the parietal mechanisms involved in orienting of attention mature, they appear to allow the infant to disengage from strong attractors. Thus, by four months infants can begin to move their eyes in anticipation of the occurrence of a visual stimulus. The ability to show anticipatory eye movements demonstrates an influence of learning on orientation, on where infants attend.

The maturation of the ability to orient to visual stimuli has dramatic consequences for an infant's response to novelty and their ability to know where to look. At four months and older, a parent can regulate negative affect by use of distraction, by orienting the infant to a novel stimulus (Posner and Rothbart, 1998). Much of the development of orienting skills must relate to the maturation of specific pathways between neural areas. The advent of new forms of neuroimaging may allow one to follow the time course of the maturation of the pathways required for the development of orienting and thus give us a new means of exploring the mechanisms of these developmental changes in infants and children (Conturo et al., 1999).

Imagery

Imagery Differs from Perception in Efficiency of Coding

Visual images are an excellent example of a purely mental event and, as such, they are a promising entryway for the neurobiological study of consciousness. Images seem to have a sensory character even though no sensory stimulus is presented. In the early part of the twentieth century visual images could only be studied by the methods of verbal report and by the systematic collection of surveys (Galton, 1907). Because behaviorists thought that the subjective nature of the image would never allow a scientific approach, the study of imagery was largely abandoned. In the period following World War II, imagery again became a focus of study in modern cognitive psychology, and objective experimental methods for probing the characteristics of these mental events were successfully developed (see Kosslyn, 1980 for a review).

It Is Now Possible to Study Brain Mechanisms of Imagery in Humans and Animals

It is now possible to design objective tests of imagery. If a test subject is given the name of a letter (e.g., R) and an angle of orientation (75 degrees) most people can construct a mental image of the R at the correct angle. If people are then shown visual probes of R that may be a real or mirror image R, they can quickly construct a mental image of the R when the probe letter is at the same angle as the image. In fact they are faster to respond to probes that match the angle of the imaged R than to an upright R (Cooper and Shepard, 1973).

But if one tests a subject by asking them to develop a visual image of a somewhat lengthy word, such as "pumpkin," most people believe they can do it until required to perform an objective task of this accomplishment like being asked to spell the word backward. The difficulty encountered when asking a subject to spell backward a word like "pumpkin" led Weber and Harnish (1974) to question whether there really was an image of the word "pumpkin." They then proceeded to compare the performance of subjects when a word stimulus like "pumpkin" was physically present (perceptual condition) and when it had to be created as an image (imagery condition). With short three- or five-letter words, the subjects show the same reaction time for an imagery condition as they show for the perceptual condition. But when the word has more than five letters imagery is much slower than perception (Weber and Harnish, 1974). Thus, imagery can indeed produce a remarkably efficient representation, but it is also rather limited to only about 3–5 separate items. By contrast, most people can hold in memory about 7–8 separate letter names.

It also takes longer to develop a visual images than to provide a name. If you are asked to image each lowercase letter of the alphabet in turn, it will take you 10–20 seconds to go through the alphabet, much longer than if you were to name the letters silently. Creating mental images is thus a complex task that consists of many mental operations. Accordingly, many parts of the brain are involved (Kosslyn, 1994).

The ability to study the brain while people construct and inspect visual images has greatly enhanced the field of mental imagery (Kosslyn, 1994). These studies have revealed that most of the visual areas that are involved in pattern recognition and in orienting of attention also are recruited during visual imagination. The overlap between areas recruited for the perception of real as opposed to imagined visual objects is substantial. It is clear that visual imagination uses the same apparatus

of visual perception and the same systems of visual attention as would be involved if the stimulus were *actually* presented to the sense organ.

This finding also is supported by clinical evidence. Studies of patients with lesions of the right posterior parietal cortex that produce visual neglect in viewing *real objects* also disrupt the experience of imaging the left side of a visual image (Bisiach and Luzzatti, 1978). This defect in imagery was first described in a fascinating study by Bisiach and Luzzatti of a group of patients in Milan, all of whom had injury to the *right* parietal lobe. As the patients were sitting in the hospital's examining room, they were asked to imagine that they were standing in the city's main square, the Piazza del Duomo, facing the cathedral, and to describe from memory the key buildings around the square. These subjects identified from memory all the buildings on the right side of the square (ipsilateral to the lesion) but could not recall the buildings on the left, even though these buildings were thoroughly familiar to them.

The patients were next asked to imagine that they were standing on the opposite side of the square, on the steps of the cathedral, so that right and left were reversed. In this imagined position, the patients were now able to name the buildings they previously had been unable to identify but failed to identify or name the buildings they had previously listed. The patients now described what they previously neglected, and neglected what they had previously described, suggesting that they retained in memory full knowledge of the space. What these patients lost was *access* to memories associated with the side of the body opposite the lesion, no matter which way the patient imagined himself facing.

Study of patients with lesions in the right posterior parietal cortex has yielded three insights. First, these lesions commonly lead to a disturbance in orienting of attention. Second, patients who neglect the left side of their body after damage to the right parietal lobe show a disturbance not only in cortical representation of their own body but also in representation of external space. Specifically, they neglect visual stimuli on that side of the body. Third, patients with neglect syndrome also lack access to memories against which perceptions on the neglected side can be compared. Thus, these patients neglect not only real external objects but also objects in memory. Finally, the lesions not only lead to disorders in perceptive-spatial relationships; they also commonly lead to a disturbance in directed attention (Figure 20A).

It has even been possible to develop an animal model of mental rotation of the sort we described previously (Georgopoulos et al., 1989). Monkeys were taught to move a lever in a direction 90 degrees from a target light. Recording from cells in the motor system shows that immediately after the light comes on, the set of active cells has an equivalent vector that would move the limb directly to the light. However, over a 0.25 second interval, the population of cells changes to compute a vector which is at the proper 90-degree angle. Imaging studies of mental rotation in humans have shown that motor areas of the brain as well as the parietal lobe become involved when rotation involves one's own hand (Parsons and Fox, 1998).

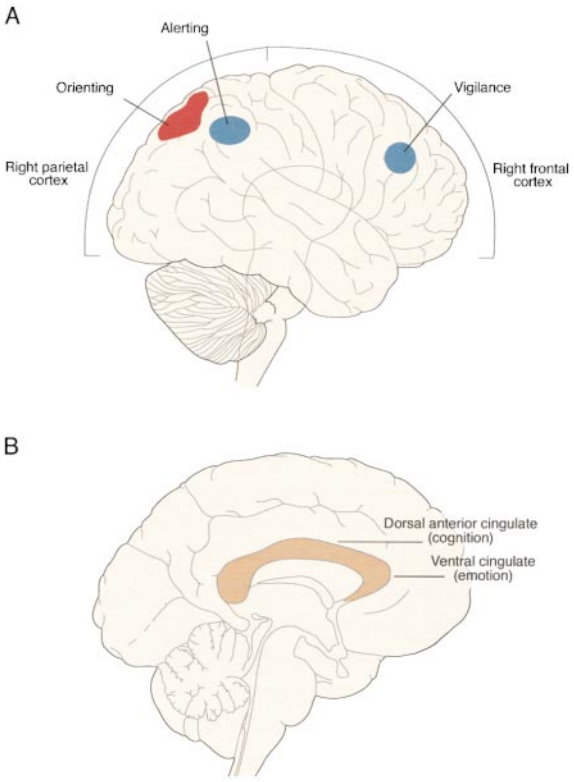

Figure 20. Localization of Alerting and Emotional Function

(A) The localization of alerting and orienting functions in the parietal lobe. A diagram of the lateral surface of the human brain indicating the relation of alerting and orienting mechanisms in the right parietal lobe to the vigilance area in the right frontal cortex. (Courtesy of M. Posner.)

(B) The localization of cortical regions involved in cognitive and emotional states. A diagram of the medial part of the human brain indicating the dorsal anterior cingulate which appears to be involved in the monitoring and/or regulation of cognitive activity and of the more ventral portions of the cingulate that appears to be related to the regulation of emotion. (Adapted from Bush et al., 1998.)

Future Studies of Imagery Can Probe the Influence of Learning and Individual Differences

Traditionally imagery has been defined by subjective report, but that is no longer necessary. It is now possible to know when the visual system has been activated from the top down, even if the person is unaware that any form of imagery has been used. For example, when reading a vivid description, people often create a visual representation of the scene and individual words may automatically evoke images related to their meaning. Some people are aware of these representations, but others deny having any subjective visual experience. By appropriate imaging studies, we can now determine if the representations differ between people according to their reports or whether the representations are the same, but only some reach threshold for a verbal report of awareness.

Normal people can report whether they have created an image, but in some cases, imagery can be pathological as in the voices heard by paranoid schizophrenics which are attributed to outside forces, or in the hallucinations of drug states. Lesions of the frontal midline can

result in attributing control of one's own hand to alien forces (Goldberg, 1985). It seems very likely that high-level attentional networks involving this frontal midline area are a source of knowledge that the information has been internally generated. These networks are normally involved in voluntary control of actions. The pathological belief of alien control of one's thoughts that is found in some schizophrenics may arise from abnormalities in the regulation of these networks. In the next section, we examine the operation of these frontal networks as a means of aiding our understanding of the conscious control of behavior.

Executive Control Includes Volition as a Major Component

Areas of the Frontal Midline Appear to be Important in Voluntary Control of Cognition and Emotion

Normal people have a strong subjective feeling of their intentions. They have a clear sense that they have voluntary control of their own behavior. These subjective feelings of intentions and voluntary control can be freely verbalized. Indeed, asking people about their goals or intentions is probably the single most predictive indicator of their behavior during problem solving (Newell and Simon, 1972). The importance of intention is also illustrated in patients with frontal lesions (Duncan, 1986) or in patients suffering from with mental disorders (Frith and Dolan, 1998), who show disruption in either their voluntary control over behavior or their subjective feelings of control. What are the neural mechanisms of voluntary control?

Norman and Shallice (1986) have argued that an executive attention system is necessary for situations in which routine or automatic processes are inadequate. These nonroutine, nonautomatic executive functions include selection among conflicting inputs, resolution of conflict among responses, and monitoring and correcting errors.

Priming Produced by Automatic Activation without Attention Can Facilitate Reaction Time to the Item When Presented Consciously

The existence of executive control was made more concrete the 1970s and 1980s when cognitive studies first succeeded in separating conscious control of mental events from automatic activation of the same events (Posner, 1978). This separation used priming of a target word by a prime word related in meaning to the target. The method is simple. Subjects are given a task to perform on a target word. For example, they may be asked to classify whether the target word is a meaningful word or not, or to categorize it is as representing a living object or not. On some trials prior to the presentation of the target word, the subject is given a prime word, a word that is flashed briefly on the screen without further comment. The prime may be related to the target (e.g., prime "toy" and target "doll") or unrelated (prime "toy" and target "stop"). Although the prime provides no direct information on how to respond to the target, nonetheless, related primes were found to speed up and unrelated ones to slow down reaction time in comparison to a neutral warning signal that merely tells the person that a target will be coming.

People do not have to attend to the prime or even be aware that it has happened to get the priming effect. Priming still takes place—if the prime word is followed immediately by a visual masking noise (a random visual input) so that subjects are unaware of the identity of the prime word. However, the effects of priming were somewhat different from trials in which the prime word had been carefully attended. Consider a condition when people are given a series of targets that either involve trees or body parts. If ambiguous prime words such as *palm* are followed by a mask so they cannot be reported, they serve to improve the performance on subsequent targets related to both meanings of the word (e.g., tree and hand). However, when the prime *palm* is presented in the context of trees and unmasked, only the meaning related to the category of the previous trials (e.g., tree) is primed (Marcel, 1983).

James Neeley (1978) studied the conscious use of primes by his subjects. In one condition a word from one category (e.g., animal) was presented as a prime word, and subjects were instructed to associate the category "animal" with the category "building." Target words in the category "building" (e.g., window) had faster reaction times than targets in a category unrelated category (e.g., tin). The subject had voluntarily activated the instructed category. If a specific animal target (e.g., dog) was presented after a very short interval so that subjects did not have enough time to switch from the prime category "animal" to the instructed category "building," fast reaction times were made to the word "dog." However, if dog was delayed until after subjects had a chance to execute the switch to the instructed category, the target "dog" would have a slow reaction time since subjects were now attending to the wrong category. Within a second Neeley was able to trace the conscious effort involved in switching categories, by its influence on the reaction time to probes.

There Is More Than One Form of Priming

These findings give a reality to the difference between the voluntary, conscious control of mental events and the same event when driven unconsciously and automatically by input. Priming can be produced in both ways. First, priming can be produced by automatic activation of a pathway without attention, facilitating reaction times for related items. This form of priming can be totally subconscious. Imaging studies have shown that automatic priming of this sort is produced by a reduction of blood flow within the brain area related to processing the target. For semantic priming, this reduction would be within areas of the brain related to the meaning of the word (Demb et al., 1995). It is as if the prime had tuned the neuron pool so that attention of fewer neurons is required to process the target. Possibly, as a consequence of this reduced overall activity, a primed target, although classified rapidly, is often poorly remembered in a later recall or recognition test.

A second form of priming is produced by directing attention to semantic information. This form of attention appears not to depend on the brain area related to the processing of the word but depends upon different frontal networks, and this information is available to the conscious awareness of the person. Within a second, subjects can voluntarily choose an associated category,

and the consequence of that selection is faster processing of related targets and retarded processing of unrelated targets. When a category is attended, items within the category are facilitated in reaction time, but items in other categories are retarded over what they would have been if no priming had taken place. Imaging studies have suggested that attention to a computation increases blood flow within the attended area. Thus, priming may be produced by different brain mechanisms that have quite different consequences for performance. It remains for future studies to tell us how these two priming mechanisms produce what seem like opposite changes at the neural level.

The Dorsal Anterior Cingulate Cortex Is Essential for Executive Control

Suppose you are asked how many objects do you see between the brackets: [two, two, two]. You may at first want to say two, even though the correct answer is three. This is because there is a conflict in your mind between the *meaning* of the word as read and the specified task of saying *how many* words are present. This is one form of the Stroop effect. The most frequent form of Stroop effect occurs when a subject has to name the color of ink in which a word is written when the color of the word conflicts with the word name (e.g., when the word red is written in blue ink). These conflicting tasks involve focal attention to the critical element of the task when that element must be selected in competition with a more dominant element. Imaging studies of the Stroop effect produced by conflict between elements tend to find very strong activity in the dorsal anterior cingulate gyrus (see Figure 20B) often in concert with areas of the basal ganglia and lateral frontal cortex (Bush et al., 1998). For this reason dorsal anterior cingulate gyrus has been thought to be involved in some aspect of focal or executive attention (Carter et al., 1999).

As is the case with humans, rhesus monkeys trained to associate digits with a quantity, show conflict between deciding which of two displays has the greatest number of objects when there is an incompatible relation between the two (e.g., when the larger number of objects is made up of the smaller digit). The monkeys made many more errors on incompatible trials than do humans, despite many hundreds of trials at the task (Washburn, 1994). It is as though the monkeys have somewhat less capacity for avoiding interference, despite very extensive training.

In humans, activity in the anterior cingulate gyrus generally is related to the degree of practice or automation of the task. Perhaps the best example is a task where subjects are required to ascribe a use for each noun in a list (e.g., hammer → pound). There is a conflict between saying the word name aloud and the required task of generating the use of the word. There was strong activation of anterior cingulate when the list was first presented, but with practice on a single list, activity in the cingulate disappears and instead there is activity in the anterior insula, a portion of cortex that lies buried beneath Broca's area (Raichle et al., 1994). Both the anterior insula and Broca's area are closely related to the automatic task of reading the word aloud. Imaging studies have identified two different pathways for producing the use of a word. One pathway is involved when conscious thought is needed to generate a word. This pathway involves the cingulate in conjunction with left lateral areas of the cortex and the right cerebellum. Another, more automatic, pathway is involved when the words are well practiced so that the feeling of conscious search disappears. Now the activity in the cingulate (as well as in the lateral cortex and cerebellum) disappears, and instead one finds activity in Broca's area and in the anterior insula, the structures that are usually involved in the automatic tasks of reading words out loud.

The Ventral and Dorsal Anterior Cingulate Are Concerned with Emotion and Cognition, Respectively

We often think of sensory orienting and memory retrieval as related to focal attention. However, another source of information that frequently engages our attention is emotion. When emotional words are presented in the same conflict tasks described above, a more ventral area of the anterior cingulate becomes active (see Figure 20B). In some neuroimaging experiments, the cognitive and emotional areas of the cingulate seem to be mutually inhibitory (Drevets and Raichle, 1998). Thus, when strong emotions are involved in the task, the dorsal area is less active than at rest and cognitive conflict tasks tend to reduce activity in the more ventral area of the cingulate.

If the dorsal area of the cingulate is involved in selecting dimensions of a stimulus when there is conflict among competing dimensions, a reasonable idea might be that the dorsal area serves a similar selecting function for emotional conflict. Indeed, we have already discussed the idea that orienting of attention in infancy serves as one means by which caregivers seek to distract their infants from the expression of distress. The control of distress is an important concern of early childhood, and caregivers have the task of first regulating emotions in their infant and later teaching the child to regulate its own emotions. Perhaps, areas of the brain that regulate emotion in infancy have acquired the ability to perform the same functions in response to cognitive challenges. If this idea is correct, children who are well advanced in emotional regulation should be at a specific advantage in regulating cognitive conflict.

We know that children differ in their ability to regulate their emotions. This can be elicited from caregivers when they are asked specific questions about the child's ability to control distress, orient attention and be sensitive to pleasures. The dimension of individual variation in regulation has been called *effortful control*. Studies of 6–7 year olds have found that effortful control can be defined in terms of scales measuring attentional focusing, inhibitory control, low intensity pleasure, and perceptual sensitivity (Rothbart et al., 2000). Effortful control is consistently negatively related to a negative affect in keeping with the notion that attentional skill may help attenuate negative affect. Effortful control also is correlated with the performance of two- to four-year-old children in Stroop tasks that require them to handle conflict (Posner and Rothbart, 1998). Effortful control is related both to empathy and to the acquisition of conscience, of a sense of moral behavior. Kochanska has found that individual differences in effortful control have important implications both for the inhibition of antisocial behavior

and for the acquisition of prosocial behavior (Kochanska, 1995). Children who can effectively employ attention to regulate behavior are better able to inhibit prepotent responses (e.g., striking out, stealing) and are better at taking into consideration the effect of their actions on others.

Empathy and a sense of moral behavior or conscience are at the heart of child socialization. The link between the attentional network of the frontal lobe and conscience might make it possible to at least imagine how aspects of morality might be studied on the neuronal level discussed below in relation to disorders.

Disorders that Recruit the Cingulate Cortex Suggest a Connection between Cognition and Emotion

Attention deficit disorder is defined by a set of cognitive and emotional symptoms. This disorder is usually diagnosed in children but often remains present into adulthood. Neuroimaging of adults who suffer from attention deficit disorder has been carried out under circumstances that require them to do a numerical version of the Stroop effect. Here they are asked to respond to the number of items present. When that number is sometimes in conflict with the quantity indicated by the word (e.g., three copies of the word two), adults with attention deficit disorder performed on conflict trials only slightly less efficiently than normals. But unlike the normal controls, adults with attention deficits show no activation of the anterior cingulate. Instead, they show greater activity on incompatible trials in the anterior insula (Bush et al., 1999). As was suggested in the study of word association discussed above, the insula represents a more automatic pathway than the anterior cingulate thus allowing for less effortful control over the task.

Another disorder that produces a disruption of voluntary control as well as other emotional and cognitive problems is schizophrenia. Benes (1998) has reported subtle abnormalities of the anterior cingulate in postmortem analyses of schizophrenic brains. She argues that the problem with the anterior cingulate, in the brains of schizophrenics, may be a shift in dopamine regulation from pyramidal to nonpyramidal cells. She has also argued that these changes in the cingulate are related to circuitry involving the amygdala and hippocampus. The schizophrenia studies provide a lead at the cellular level of the possible disregulation of the anterior cingulate in a second abnormality noted for its attentional deficits.

Both schizophrenia and attention deficit disorder have a genetic basis. Studies of attention deficit disorder families have shown that some of them possess a particular allele of the dopamine 4 receptor (LaHoste et al., 1996; Smalley et al., 1998). These studies provide some potential cellular and genetic links between attentional abnormalities found in various pathologies.

A Future for the Study of Consciousness

We have outlined that the problem of consciousness can be considered to consist of two subproblems: awareness and volition. Studies of orienting and of imagery are concerned with the first, and self-regulation with the second. As future research penetrates the organization of attention networks in the frontal cortex, the two functions could prove to be linked. Studies of complex scenes show that presentation of a stimulus does not lead automatically to awareness of even the most central aspects of the scene (Rensink et al., 1997). Even though subjects report that they are aware of a whole scene, they only become aware of a change when their attention is drawn to a change in the scene. We have suggested that posterior brain areas involved in orienting to sensory stimuli may be closely related to ambient awareness and that focal attention might be more associated with the anterior cingulate and other frontal areas related to voluntary control. Perhaps only as we understand the neural basis of the distinction between *focal attention* to limited aspects of the external world and a more *general ambient awareness* of the general scene will we be able to understand the parts of the brain related to consciousness of sensory events (Iwasaki, 1993).

The exact functions that the anterior cingulate plays in higher level attention are not yet clear. We have learned that even very simple acts of attention such as orienting to sensory stimuli involve a network of brain areas that carry out specific functions. During conflict tasks activation of the anterior cingulate is usually accompanied by activity in lateral frontal cortical areas and in the basal ganglia. It is an important goal to find out what each of these areas contribute.

The functions of attention also relate to issues other than those usually discussed under the term consciousness. Recently, the contribution of biology to understanding the acquisition of high level skills such as those learned in schools has been extensively debated (e.g., Bruer, 1999). In some areas, such as the neural networks involved in reading and arithmetic, progress has been extensive (Dehaene, 1997; Posner et al., 1999). Both skills required attention networks related to those discussed in this section. A better understanding of these mechanisms might help in realizing the goal of a neuroscience-based approach to aspects of education.

Part VI. Coda

What is the future for neural science in the next millennium? We have seen remarkable and rapid progress in understanding neuronal and synaptic signaling. These advances now invite a structural approach to visualize the static and dynamic structures of ion channels, receptors, and the molecular machinery for signal transduction postsynaptically and for vesicle transport, fusion, and exocytosis presynaptically. We also have made some progress in the analysis of the elementary synaptic mechanisms that contribute to memory storage. These studies have revealed that the different memory systems of brain seems to use similar synaptic mechanisms for the storage of both declarative and nondeclarative knowledge. Similarly, we now have achieved an understanding, at least in broad outline, of the development of the nervous system. Specific inducers, morphogens, attractants, and repellants of process outgrowth and synapse organizing molecules have now been defined, providing a molecular reality to concepts that previously were shrouded in mystery.

Progress in these several areas has in turn made possible a molecular-based neurology, a neurology that will, one hopes, finally be able to address the degenerative

diseases of the brain that have for so long eluded our best scientific efforts. In time, advance in these areas may also yield insight into and perhaps solutions for some of the most debilitating diseases confronting medical science—the psychiatric and neurological illnesses of schizophrenia, depression, and Alzheimer's disease. Implicit in this prediction is the expectation that in the future molecular biology will be able to contribute to the system problems of cognitive neural science much as it has recently contributed to signaling, plasticity, and development.

The advances in the cellular understanding of the organization of the somatosensory and visual system by Mountcastle, Hubel, and Wiesel and their followers have helped turn our interest to perception and in the broader sense to cognitive psychology. In turn, contact between cognitive psychology and neural science has given us a new approach to the classical problems of mental function including attention and consciousness. In both early sensory processes and higher cognitive perceptual and motor systems, we find evidence for the localization of components within a broadly distributed network carrying out complex functions. Indeed, one of the early insights into consciousness is that it shares the properties of other cognitive systems in that like vision and action, it can be dissected into components: attention, imagery, and volition. Each component consists of a set of subcomponents that can be localized within a larger, distributed neural system. Having pointed to that similarity, we must nevertheless acknowledge that of all fields in neural science, in fact of all the fields in all of science, the problems of perception, action, memory, attention, and consciousness provide us with the greatest evidence for our lack of understanding as well as the greatest challenge.

Even if one agreed that the scientific agenda outlined here may be adequate to handle the issues of awareness and volition, there is another aspect of consciousness that needs to be confronted and that is the nature of *subjectivity*. The subjective aspect of consciousness is seen by philosophers of mind such as Searle (1993, 1998) and Nagel (1993) as its defining characteristic and the aspect that poses the greatest scientific challenge. Searle and Nagel argue that each of us experiences a world of private and unique experiences and that these seem much more *real* to us than the experiences of others. We experience our own ideas, moods, and sensations—our successes and disappointments, joys and pains—directly, whereas we can only appreciate other people's ideas, moods, and sensations. Are the purple you see and the jasmine you smell identical to the purple that we see and the jasmine that we smell? The fact that conscious experience is uniquely personal and intensely subjective raises the question whether it is ever possible to determine objectively some common characteristics of experience. We cannot, the argument goes, use those same senses to arrive at an objective understanding of experience.

Clearly, we should be prepared for the possibility that there are aspects of consciousness that will not be solved by the approaches discussed in this review. Some might believe that all that is scientific about the study of life is illuminated at all levels from the molecular to the behavioral by what we know about DNA. But others might believe that there are issues about what it means to be a living being that are really not explained by the most detailed account of DNA. Many issues of awareness and voluntary control are likely to be explained at all levels, from genes to behavior. This might constitute a theory of consciousness in much the same way as DNA serves as the basis for any scientific analysis of what constitutes life. Nonetheless, it is at present hard to imagine how the progress discussed above, even if it continues and intensifies, will solve all the issues of the subjective nature of our experience. We leave it to the readers of *Cell* and *Neuron* in the next millennium to determine how much insight about human consciousness will result from the type of work we have discussed here.

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