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A BRIEF HISTORY OF NEUROSCIENCE

The field of knowledge described in this book is neuroscience: the collected multidisciplinary sciences that analyze the nervous system to understand the biological basis for behavior. Modern studies of the nervous system have been ongoing since the middle of the nineteenth century. Neuroanatomists studied the brain’s shape, its cellular structure, and its circuitry; neurochemists studied the brain’s chemical composition, its lipids and proteins; neurophysiologists studied the brain’s bioelectric properties; and psychologists and neuropsychologists investigated the organization and neural substrates of behavior and cognition.

In contrast to these separate investigative pursuits, the term neuroscience was introduced in the mid-1960s to signal the beginning of an era in which each of these disciplines would work together cooperatively, sharing a common language, common concepts, and a common goal: to understand the structures and functions of both the normal and abnormal brain. Neuroscience today spans a wide range of research endeavors from the molecular biology of nerve cells (i.e., the genes encoding the proteins needed for nervous system function) to the biological basis of normal and disordered behavior, emotion, and cognition (i.e., the mental properties by which individuals interact with one another and their environments). For a more complete, but concise, history of the neurosciences, see Kandel and Squire (2000).

Neuroscience is currently one of the most rapidly growing areas of science. Indeed, the brain is sometimes referred to as “the last frontier of biology.” In 1971, 1100 scientists convened at the first annual meeting of the Society for Neuroscience. In 2010, 31,500 scientists participated at the society’s 40th annual meeting, at which more than 15,000 research presentations were made.

THE TERMINOLOGY OF NERVOUS SYSTEMS IS HIERARCHICAL, DISTRIBUTED, DESCRIPTIVE, AND HISTORICALLY BASED

Beginning students of neuroscience may understandably find themselves confused. Nervous systems of many organisms have their cell assemblies and macroscopically visible components named by multiple overlapping and often synonymous terms. With a necessarily gracious view to the past, this confusing terminology could be viewed as the intellectual cost of focused discourse with predecessors in the enterprise. The nervous systems of invertebrate organisms often are designated for their spatially directed collections of neurons responsible for local control of operations, such as the thoracic or abdominal ganglia, which receive sensations and direct motoric responses for specific body segments, all under the general control of a cephalic ganglion whose role includes sensing the external environment.

In vertebrates, the components of the nervous system were named for both their appearance and their location. As noted by Swanson & Bota (2010), and expanded upon in Chapter 2 of this volume, the names of the major parts of the brain were based on creative interpretations of early dissectors of the brain, attributing names to brain segments based on their appearance in the freshly dissected state: hippocampus (shaped like the sea horse) or amygdala (shaped like the almond), cerebrum (the main brain), and cerebellum (a small brain).
NEURONS AND GLIA ARE CELLULAR BUILDING BLOCKS OF THE NERVOUS SYSTEM

This book lays out our current understanding in each of the important domains that together define the full scope of modern neuroscience. The structure and function of the brain and spinal cord are most appropriately understood from the perspective of their highly specialized cells: the neurons, the interconnected, highly differentiated, bioelectrically driven, cellular units of the nervous system, and their more numerous support cells, the glia. Given the importance of these cellular building blocks in all that follows, a brief overview of their properties may be helpful.

Neurons Are Heterogeneously Shaped, Highly Active Secretory Cells

Neurons are classified in many different ways: according to function (sensory, motor, or interneuron), location (cortical, spinal, etc.), the identity of the transmitter they synthesize and release (glutamatergic, cholinergic, etc.), and their shape (pyramidal, granule, mitral, etc.). Microscopic analysis focuses on their general shape and, in particular, the number of extensions from the cell body. Most neurons have one axon, often branched, to transmit signals to interconnected target neurons. Other processes, termed dendrites, extend from the nerve cell body (also termed the perikaryon) to the axon or dendrites. Neurons exhibit the cytological characteristics of highly active secretory cells with large nuclei; large amounts of smooth and rough endoplasmic reticulum; and frequent clusters of specialized smooth endoplasmic reticulum (Golgi apparatus), in which secretory products of the cell are packaged into membrane-bound organelles, termed synaptic vesicles. The proteins of these vesicles have been shown to have specific roles in transmitter storage; vesicle docking onto presynaptic membranes, voltage- and Ca\(^{2+}\)-dependent secretion, and the recycling and restorage of previously released transmitter molecules.

Synaptic Relationships Fall into Several Structural Categories

Synaptic arrangements in the CNS fall into a wide variety of morphological and functional forms that are specific for the neurons involved. The most common arrangement, typical of hierarchical pathways, is either the axodendritic or the axosomatic synapse, in which the axons of the cell of origin make their functional contact with the dendrites or cell body of the target neuron, respectively. A second category of synaptic arrangement is more rare: forms of functional contact between adjacent cell bodies (somasomatic) and overlapping dendrites (dendrodendritic). Within the spinal cord and some other fields of neuropil (relatively acellular areas of synaptic connections), serial axoaxonic synapses are relatively frequent. Here, the axon of an interneuron ends on the terminal of a long-distance neuron as that terminal contacts a dendrite, or on the segment of the axon that is immediately distal to the soma, termed the initial segment, where action potentials arise. Many presynaptic axons contain local collections of typical synaptic vesicles with no opposed specialized synaptolemma. These are termed boutons en passant. The release of a transmitter may not always occur at such sites.

Synaptic Relationships also Belong to Diverse Functional Categories

As with their structural representations, the qualities of synaptic transmission can also be functionally categorized in terms of the nature of the neurotransmitter that provides the signaling; the nature of the receptor molecule on the postsynaptic neuron, gland, or muscle; and the mechanisms by which the postsynaptic cell transduces the neurotransmitter signal into transmembrane changes. So-called “fast” or “classical” neurotransmission is the functional variety seen at the vast majority of synaptic and junctional sites, with a rapid
onset and a rapid ending, generally employing excitatory amino acids (glutamate or aspartate) or inhibitory amino acids (γ-aminobutyrate, GABA, or glycine) as the transmitter. The effects of those signals are largely attributable to changes in postsynaptic membrane permeability to specific cations or anions and the resulting depolarization or hyperpolarization, respectively. Other neurotransmitters, such as the monoamines (dopamine, norepinephrine, serotonin) and many neuropeptides, produce changes in excitability that are much more enduring. Here the receptors activate metabolic processes within the postsynaptic cells—frequently to add or remove phosphate groups from key intracellular proteins; multiple complex forms of enduring postsynaptic metabolic actions are under investigation. The brain’s richness of signaling possibilities comes from the interplay on common postsynaptic neurons of these multiple chemical signals.

The Operative Processes of Nervous Systems are also Hierarchical

As research progressed, it became clear that neuronal functions could best be fitted into nervous system function by considering their operations at four fundamental hierarchical levels: molecular, cellular, systems, and behavioral. These levels rest on the fundamental principle that neurons communicate chemically, by the activity-dependent secretion of neurotransmitters, at specialized points of contact named synapses.

At the molecular level, emphasis is on the interaction of molecules—typically proteins that regulate transcription of genes, their translation into proteins, and their posttranslational processing. Proteins that mediate the intracellular processes of transmitter synthesis, storage, and release, or the intracellular consequences of intercellular synaptic signaling, are essential neuronal molecular functions. Such transductive molecular mechanisms include the neurotransmitters’ receptors, as well as the auxiliary molecules that allow these receptors to influence the short-term biology of responsive neurons (through regulation of ion channels) and their longer-term regulation (through alterations in gene expression). Completion of the human, chimpanzee, rat, and mouse genomes can be viewed as an extensive inventory of these molecular elements, more than half of which are thought to be either highly enriched in the brain or even exclusively expressed there. Genes unique to human brains have been reported and appear to be most frequently expressed during the brain’s development. Recent progress has made clear that the introns (the sections of the genome that contain DNA code that does not lead to production of specific proteins; these noncoding sequences are the largest part of the genome) are important functional control elements that regulate which genes are expressed and which transcribed messenger RNAs are translated into proteins.

At the cellular level of neuroscience, the emphasis is on interactions between neurons through their synaptic transactions and between neurons and glia. Much current cellular-level research focuses on the biochemical systems within specific cells that mediate such phenomena as pacemakers for the generation of circadian rhythms or that can account for activity-dependent adaptation. Research at the cellular level strives to determine which specific neurons and which of their most proximate synaptic connections may mediate a behavior or the behavioral effects of a given experimental perturbation.

At the systems level, emphasis is on the spatially distributed sensors and effectors that integrate the body’s response to environmental challenges. There are sensory systems, which include specialized senses for hearing, seeing, feeling, tasting, and balancing the body. Similarly, there are motor systems for trunk, limb, and fine finger motions and internal regulatory systems for visceral regulation (e.g., control of body temperature, cardiovascular function, appetite, and salt and water balance). These systems operate through relatively sequential linkages, and interruption of any link can destroy the function of the system.

Systems-level research also includes research into cellular systems that innervate the widely distributed neuronal elements of the sensory, motor, or visceral systems, such as the pontine neurons with highly branched axons that innervate diencephalic, cortical, and spinal neurons. Among the best studied of these divergent systems are the monoaminergic neurons, which have been linked to the regulation of many behavioral outputs of the brain, ranging from feeding, drinking, thermoregulation, and sexual behavior. Monoaminergic neurons also have been linked to such higher functions as pleasure, reinforcement, attention, motivation, memory, and learning. Dysfunctions of these systems have been hypothesized as the basis for some psychiatric and neurological diseases, supported by evidence that medications aimed at presumed monoamine regulation provide useful therapy.

At the behavioral level of neuroscience research, emphasis is on the interactions between individuals and their collective environment. Research at the behavioral level centers on the integrative phenomena that link populations of neurons (often operationally or empirically defined) into extended specialized circuits, ensembles, or more pervasively distributed “systems” that integrate the physiological expression of a learned, reflexive, or spontaneously generated behavioral response. Behavioral research also includes the operations of higher mental activity, such as memory, learning, speech, abstract reasoning, and consciousness. Conceptually, “animal models” of human psychiatric diseases are based

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on the assumption that scientists can appropriately infer from observations of behavior and physiology (heart rate, respiration, locomotion, etc.) that the states experienced by animals are equivalent to the emotional states experienced by humans expressing these same sorts of physiological changes.

As the neuroscientific bases for some elemental behaviors have become better understood, new aspects of neuroscience applied to problems of daily life have begun to emerge. Methods for the noninvasive detection of activity in certain small regions of the cerebral cortex can now resolve areas that are active simultaneously and presumed to act together in networks of cortical assemblies. These concurrently active networks have been linked to discrete forms of mental activity. Such advances have given rise to the concept that it is possible to understand where in the brain the decision-making process occurs or to identify the kinds of information necessary to decide whether or not to act. The detailed quantitative data that now exist on the details of neuronal structure, function, and behavior have driven the development of computational neurosciences. This new branch of neuroscience research seeks to predict the performance of neurons, neuronal properties, and neural networks based on their discernible quantitative properties.

Some Principles of Brain Organization and Function

The central nervous system is most commonly divided into major structural units, consisting of the major physical subdivisions of the brain. Thus, mammalian neuroscientists divide the central nervous system into the brain and spinal cord and further divide the brain into regions readily seen by the simplest of dissections. Based on research that has demonstrated that these large spatial elements derive from independent structures in the developing brain, these subdivisions are well accepted. Mammalian brain thusly is divided into hindbrain, midbrain, and forebrain, each of which has multiple highly specialized regions within it. In deference to the major differences in body structure, invertebrate nervous systems most often are organized by body segment (cephalic, thoracic, abdominal) and by anterior–posterior placement.

Neurons within the vertebrate CNS operate either within layered structures (such as the olfactory bulb, cerebral cortex, hippocampal formation, and cerebellum) or in clustered groupings (the defined collections of central neurons, which aggregate into “nuclei” in the central nervous system and into “ganglia” in the peripheral nervous system, and in invertebrate nervous systems). The specific connections between neurons within or across the macro-divisions of the brain are essential to the brain’s functions. It is through their patterns of neuronal circuitry that individual neurons form functional ensembles to regulate the flow of information within and between the regions of the brain.

CELLULAR ORGANIZATION OF THE BRAIN

Present understanding of the cellular organization of the CNS can be viewed simplistically according to three main patterns of neuronal connectivity.

Three Basic Patterns of Neuronal Circuitry Exist

*Long hierarchical neurons* typically constitute the primary sensory and motor pathways. Here the transmission of information is highly sequential, and interconnected neurons are related to one another in a hierarchical fashion. Primary receptors (in the retina, inner ear, olfactory epithelium, tongue, or skin) transmit first to primary relay cells, then to secondary relay cells, and finally to the primary sensory fields of the cerebral cortex. For motor output systems, the reverse sequence exists with impulses descending hierarchically from the motor cortex to the spinal motoneuron. It is at the level of the motor and sensory systems that beginning scholars of the nervous system will begin to appreciate the complexities of neuronal circuitry by which widely separated neurons communicate selectively. This hierarchical scheme of organization provides for a precise flow of information, but such organization suffers the disadvantage that destruction of any link incapacitates the entire system.

*Local circuit neurons* establish their connections mainly within their immediate vicinity. Such local circuit neurons frequently are small and may have relatively few processes. Interneurons expand or constrain the flow of information within their small spatial domain and may do so without generating action potentials, given their short axons.

*Single-source divergent neurons* operate within the hypothalamus, pons, and medulla. From their clustered nuclear locations, these neurons extend multiple branched and divergent connections to many target cells, almost all of which lie outside the brain region in which the neurons of origin are located. Neurons with divergent circuitry could be considered more as interregional interneurons rather than as sequential elements within any known hierarchical system. For example, different neurons of the noradrenergic nucleus, the locus coeruleus (named for its blue pigmented color in primate brains), project from the pons to either the cerebellum, spinal cord, hypothalamus, or
several cortical zones to modulate synaptic operations within those regions.

Glia Are Supportive Cells to Neurons

Neurons are not the only cells in the CNS. According to most estimates, neurons are outnumbered, perhaps by an order of magnitude, by the various nonneuronal supportive cellular elements. Nonneuronal cells include macroglia, microglia, and cells of the brain’s blood vessels, cells of the choroid plexus that secrete the cerebrospinal fluid, and meninges, sheets of connective tissue that cover the surface of the brain and comprise the cerebrospinal fluid-containing envelope that protects the brain within the skull.

Macroglia are the most abundant supportive cells; some are categorized as astrocytes (nonneuronal cells interposed between the vasculature and the neurons, often surrounding individual compartments of synaptic complexes). Astrocytes play a variety of metabolic support roles, including furnishing energy intermediates and providing for the supplementary removal of excessive extracellular neurotransmitter secretions (see Chapter 13). A second prominent category of macroglia is the myelin-producing cells, the oligodendroglia. Myelin, made up of multiple layers of their compacted membranes, insulates segments of long axons bioelectrically and accelerates action potential conduction velocity. Microglia are relatively uncharacterized supportive cells believed to be of mesodermal origin and related to the macrophage/monocyte lineage. Some microglia reside quiescently within the brain. During periods of intracerebral inflammation (e.g., infection, certain degenerative diseases, or traumatic injury), circulating macrophages and other white blood cells are recruited into the brain by endothelial signals to remove necrotic tissue or to defend against the microbial infection.

The Blood–Brain Barrier Protects Against Inappropriate Signals

The blood–brain barrier is an important permeability barrier to selected molecules between the bloodstream and the CNS. Evidence of a barrier is provided by the greatly diminished rate of access of most lipophobic chemicals between plasma and brain; specific energy-dependent transporter systems permit selected access. Diffusional barriers retard the movement of substances from brain to blood as well as from blood to brain. The brain clears metabolites of transmitters into the cerebrospinal fluid by excretion through the acid transport system of the choroid plexus. The blood–brain barrier is much more permeable in the hypothalamus and in several small, specialized organs (termed circumventricular organs; see Chapters 34 and 39) lining the third and fourth ventricles of the brain: the median eminence, area postrema, pineal gland, subfornical organ, and subcommissural organ. The peripheral nervous system (e.g., sensory and autonomic nerves and ganglia) has no such diffusional barrier.

The Central Nervous System Can Initiate Limited Responses to Damage

Because neurons of the CNS are terminally differentiated cells, they cannot undergo proliferative responses to damage, as can cells of skin, muscle, bone, and blood vessels. Nevertheless, small populations of previously unrecognized stem cells have been shown capable of undergoing regulated proliferation and differentiation into neurons and astrocytes. These stem cells, more prominent in rodent than primate brains, provide a natural means for selected neuronal replacement in some regions of the nervous system. As a result, neurons have evolved other adaptive mechanisms to provide for the adaptive responses to environmental demands and can be brought into play for maintenance of function following injury. These adaptive mechanisms range from activity-dependent regulation of gene expression, to modification of synaptic structure, function, and can include actual localized axonal sprouting, changes in dendritic spine numbers and shapes, and new synapse creation. These adaptive mechanisms endow the brain with considerable capacity for structural and functional modification well into adulthood. This plasticity is not only considered to be activity dependent but also to be reversible with disuse. Plasticity is pronounced within the sensory systems (see Chapter 23) and is quite prominent in the motor systems as well. The molecular mechanisms employed in memory and learning may rely upon very similar processes as those involved in structural and functional plasticity.

ORGANIZATION OF THIS TEXT

With these overview principles in place, which are detailed more extensively in Section II, we can resume our preview of this book. Another major domain of our field is nervous system development (Section III). How does a simple epithelium differentiate into specialized collections of cells and ultimately into distinct brain structures? How do neurons grow processes that find appropriate targets some distance away? How do nascent neuronal activity and embryonic experience shape activity?

Sensory systems and motor systems (Sections IV and V) encompass how the nervous system receives information
from the external world and how movements and actions are produced (e.g., eye movements and limb movements). These questions range from the molecular level (how are odorants, photons, and sounds transduced into informative patterns of neural activity?) to the systems and behavioral level (which brain structures control eye movements and what are the computations required by each structure?).

An evolutionarily old function of the nervous system is to regulate respiration, heart rate, sleep and waking cycles, food and water intake, and hormones to maintain internal homeostasis and to permit daily and longer reproductive cycles. In this area of regulatory systems (Section VI), we explore how organisms remain in balance with their environment, ensuring that they obtain the energy resources needed to survive and reproduce. At the level of cells and molecules, the study of regulatory systems concerns the receptors and signaling pathways by which particular hormones or neurotransmitters prepare the organism to sleep, to cope with acute stress, or to seek food or reproduce. At the level of brain systems, we ask such questions as what occurs in brain circuitry to produce thirst or to create a self-destructive problem such as drug abuse?

In recent years, the disciplines of psychology and biology have increasingly found common ground, and this convergence of psychology and biology defines the modern topics of behavioral and cognitive neuroscience (Section VII). These topics concern the so-called higher mental functions: perception, attention, language, memory, thinking, and the ability to navigate in space. Work on these problems traditionally has drawn on the techniques of neuroanatomy, neurophysiology, neuropharmacology, and behavioral analysis. More recently, behavioral and cognitive neuroscience has benefited from several new approaches: the use of computers to perform detailed formal analyses of how brain systems operate and how cognition is organized; noninvasive neuroimaging techniques, such as positron emission tomography and functional magnetic resonance imaging, to obtain dynamic images of the living human brain in action; and molecular biological methods, such as single gene knockouts in mice, which can relate genes to brain systems and to behavior. An especially attractive area of current investigation is the use of fluorescent proteins, such as enhanced green fluorescent protein, to demarcate individual neuronal trajectories, visualizing the “connectome,” the collection of all neuronal circuits. An offshoot of this approach is to create transgenic animals in which neurons expressing specific neurotransmitter receptors can be visually identified in living brain slices for precise investigation of synaptic properties. A third example, termed optogenetics, causes light-sensitive proteins to be expressed in discrete neuronal circuits to be activated or inhibited in intact behaving experimental animals, including nonhuman primates.

This textbook is for anyone interested in neuroscience. In preparing it we have focused primarily on graduate students just entering the field, understanding that some of you will have majored in biology, some in psychology, some in mathematics or engineering, and even some, like me, in German literature. It is hoped that through the text, the explanatory boxes, and, in some cases, the supplementary readings, you will find the book to be both understandable and enlightening. In many cases, advanced undergraduate students will find this book useful as well.

Medical students may find that they need additional clinical correlations that are not provided here. However, it is hoped that most medical scholars at least will be able to use our textbook in conjunction with more clinically oriented material. Finally, to those who have completed their formal education, it is hoped that this text can provide you with some useful information and challenging perspectives, whether you are active neuroscientists wishing to learn about areas of the field other than your own or individuals who wish to enter neuroscience from a different area of inquiry. We invite all of you to join us in the adventure of studying the nervous system.

CLINICAL ISSUES IN THE NEUROSCIENCES

Many fields of clinical medicine are directly concerned with the brain. The branches of medicine tied most closely to neuroscience are neurology (the study of the diseases of the brain), neurosurgery (the study of the surgical treatment of neurological disease), and psychiatry (the study of behavioral, emotional, and mental diseases). Other fields of medicine also make important contributions, including radiology (the use of radiation for such purposes as imaging the brain—initially with x-rays and, more recently, with positron emitters and magnetic waves) and pathology (the study of pathological tissue). To make connections to the many facets of medicine that are relevant to neuroscience, this book includes discussion of a limited number of clinical conditions in the context of basic knowledge in neuroscience.

The Spirit of Exploration Continues

Less than a decade into the twenty-first century, the Hubble space telescope continues to transmit information about the uncharted regions of the universe and
scientists were rapidly able to predict approximately which functions had already been established. In this way, gene products were assigned to families of similar proteins whose sorting, the computers could next assign the genes and identify the beginning and ending of sequences likely to encode proteins. Furthermore, the computer systems could then sort those proteins by similarity of sequences (motifs) within their amino acid building blocks. After sorting, the computers could next assign the genes and gene products to families of similar proteins whose functions had already been established. In this way, scientists were rapidly able to predict approximately how many proteins could be encoded by the genome (all of the genes an individual has). Whole genome data are now available for humans, for some nonhuman primates, for rats, and for mice.

Scientifically, this state of information has been termed a “draft” because it is based on a very dense, but not quite complete, sample of the whole genome. What has been determined still contains a very large number of interruptions and gaps. Some of the smaller genomes, whose beginning and ending are most certain, could be thought of as parts in a reassembled Greek urn, held in place by bits of blank clay until further excavation is done. However, having even this draft has provided some important realities.

Similar routines allowed these genomic scholars to determine how many of those mammalian genes were like genes we have already recognized in the smaller genomes of other organisms mapped out previously (yeast, worm (Caenorhabditis elegans), and fruit fly (Drosophila melanogaster)) and how many other gene forms may not have been encountered previously. Based on current estimates, it would appear that despite the very large number of nucleotides in the human and other mammalian genomes, about 30 times the length of the worms and more like 15 times the fruit fly, mammals may have only twice as many genes—perhaps some 30,000 to 40,000 altogether. Compared to other completed genomes, the human genome has greatly increased its representation of genes related to nervous system function, tissue-specific developmental mechanisms, and immune function and blood coagulation. Importantly for diseases of the nervous system that are characterized by the premature death of neurons, there appears to have been a major expansion in the numbers of genes related to initiating the process of intentional cell death, or apoptosis. Although still controversial, genes regulating primate brain size have been reported, but links to intellectual capacity remain unproven.

Two major future vistas can be imagined. To create organisms as complex as humans from relatively so few genes probably means that the richness of the required proteins is based on their modifications, either during transcription of the gene or after translation of the intermediate messenger RNA into the protein. These essential aspects of certain proteins account for a small number of brain diseases that can be linked to mutations in a single gene, such as Huntington’s disease (see Chapter 31). Second, though compiling this draft inventory represents a stunning technical achievement, there remains the enormously daunting task of determining, for example, where in the brain’s circuits specific genes normally are expressed and how that expression pattern may be altered by the demands of illness or an unfriendly environment. That task, at present, is one for which there are as yet no tools equivalently as powerful as those used to acquire the flood of sequence

THE GENOMIC INVENTORY IS A GIANT STEP FORWARD

Possibly the single largest event in the history of biomedical research was presented in published form in February 2001: the initial inventory of the human genome. By using advanced versions of the powerful methods of molecular biology, several large scientific teams have been able to take apart all of an individual’s human DNA in very refined ways, amplify the amounts of the pieces, determine the order of the nucleic acid bases in each of the fragments, and then put those fragments back together again across the 23 pairs of human chromosomes.

Having determined the sequences of the nucleic acids, it was possible to train computers to read the sequence information and spot the specific signals that identify the beginning and ending of sequences likely to encode proteins. Furthermore, the computer systems could then sort those proteins by similarity of sequences (motifs) within their amino acid building blocks. After sorting, the computers could next assign the genes and gene products to families of similar proteins whose functions had already been established. In this way, scientists were rapidly able to predict approximately how many proteins could be encoded by the genome (all of the genes an individual has). Whole genome data are now available for humans, for some nonhuman primates, for rats, and for mice.

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data with which we are now faced. This stage has been referred to as the end of “naive reductionism.”

In the fall of 2005, a six-nation consortium of molecular biologists announced the next phase of genomic research. The new focus will be toward refining the initial inventories to compare whole genomes of healthy and affected individuals for a variety of complex genetic illnesses (the HapMap project). Complex genetic diseases, such as diabetes mellitus, hypertension, asthma, depression, schizophrenia, and alcoholism, arise through the interactions of multiple short gene mutations that can increase or decrease one’s vulnerability to a specific disease depending on individual life experiences. Ultimately, as the speeds of genome sequencing improve still further and the cost is reduced, it may be possible to predict what diseases will be more likely to affect a given person and to predict lifestyle changes that person could undertake to improve his or her opportunities to remain healthy. Given the recently recognized importance of the intronic sequences of the genome in determining which genes are expressed, so-called “Next-Gen” or “deep sequencing” methods of very high speed and resolution are forcing investigators to re-examine the genetic basis of heritable disorders. Increasing attention is being devoted to epigenetic regulation of gene expression by means of chemical modification of gene sequences and their expression.

In order to benefit from the enormously rich potential mother lode of genetic information, next we must determine where these genes are expressed, what functions they can control, and what sorts of controls other gene products can exert over them. In the nervous system, where cell–cell interaction is the main operating system in relating molecular events to functional behavioral events, discovering the still murky properties of activity-dependent gene expression will require enormous investment.

THE CREATION OF KNOWLEDGE

Over the years, a generally accepted procedure for conducting research has evolved. This process involves examining the existing literature, identifying an important question, and formulating a research plan. Often, new experimental pathways are launched when one scientist reads with skepticism the observations and interpretations of another and decides to test their validity. Sometimes, especially at the beginning of a new series of experiments, the research plan is purely “descriptive”—for example, determining the structure of a protein or the distribution of a neurotransmitter in the brain. Descriptive initial research is essential to the subsequent inductive phase of experimentation, the movement from observations to theory, seasoned with wisdom and curiosity. Descriptive experiments are valuable both because of the questions that they attempt
to answer and because of the questions that their results allow us to ask. Information obtained from descriptive experiments provides a base of knowledge on which a scientist may draw to develop hypotheses about cause and effect in the phenomenon under investigation. For example, once we identify the distribution of a particular transmitter within the brain or the course of a pathway of connections through descriptive work, we may then be able to develop a theory about what function that transmitter or pathway serves.

Once a hypothesis has been developed, the researcher then has the task of designing and performing experiments that are likely to disprove that hypothesis if it is incorrect. This is referred to as the deductive phase of experimentation, the movement from theory to observation. Through this paradigm the neuroscientist seeks to narrow down the vast range of alternative explanations for a given phenomenon. Only after attempting to disprove the hypothesis as thoroughly as possible can scientists be adequately assured that their hypothesis is a plausible explanation for the phenomenon under investigation.

A key point in this argument is that data may only lend support to a hypothesis rather than provide absolute proof of its validity. In part, this is because the constraints of time, money, and technology allow a scientist to test a particular hypothesis only under a limited set of conditions. Variability and random chance may also contribute to the experimental results. Consequently, at the end of an experiment, scientists generally report only that there is a statistical probability that the effect measured was due to intervention rather than to chance or variability.

Given that one can never prove a hypothesis, how do “facts” arise? At the conclusion of their experiments, the researchers’ first task is to report their findings to the scientific community. The dissemination of research findings often begins with an informal presentation at a laboratory or departmental meeting, eventually followed by presentation at a scientific meeting that permits the rapid exchange of information more broadly. One or more research articles published in peer-reviewed journals ultimately follow the verbal communications. Such publications are not simply a means to allow the authors to advance as professionals (although they are important in that respect as well). Publication is an essential component of the advancement of science. As we have already stated, science depends on sharing information, replicating and thereby validating experiments, and then moving forward to solve the next problem. Indeed, a scientific experiment, no matter how spectacular the results, is not completed until the results are published. More likely, publication of “spectacular” results will provoke a skeptical scientist into doing an even more telling experiment, and knowledge will evolve.

RESPONSIBLE CONDUCT

Although individuals or small groups may perform experiments, new knowledge is ultimately the product of the larger community. Inherent in such a system is the need to be able to trust the work of other scientists—to trust their integrity in conducting and reporting research. Thus, it is not surprising that much emphasis is placed on the responsible conduct of research.

Research ethics encompasses a broad spectrum of behaviors. Where one draws the line between sloppy science and unethical conduct is a source of much debate within the scientific community. Some acts are considered to be so egregious that despite personal differences in defining what constitutes ethical behavior, the community generally recognizes certain research practices as behaviors that are unethical. These unambiguously improper activities consist of fabrication, falsification, and plagiarism. Fabrication refers to making up data, falsification is defined as altering data, and plagiarism consists of using another person’s ideas, words, or data without attribution. Each of these acts significantly harms the scientific community.

Fabrication and falsification in a research paper taint the published literature by undermining its integrity. Not only is the information contained in such papers misleading in itself, but other scientists may unwittingly use that information as the foundation for new research. If, when reported, these subsequent studies cite the previous, fraudulent publication, the literature is further corrupted. Thus, through a domino-like effect, one paper may have a broad negative impact on the scientific literature. Moreover, when fraud is discovered, a retraction of the paper provides only a limited solution, as there is no guarantee that individuals who read the original article will see the retraction. Given the impact that just one fraudulent paper may have, it is not surprising that the integrity of published literature is a primary ethical concern for scientists.

Plagiarism is also a major ethical infraction. Scientific publications provide a mechanism for establishing priority for a discovery. As such, they form the currency by which scientists earn academic positions, gain research grants to support their research, attract students, and receive promotions. Plagiarism denies the original author of credit for his or her work. This hurts everyone: the creative scientist is robbed of credit, the scientific community is hurt by the disincentive to share ideas and research results, and the individual who has plagiarized—like the person who has fabricated or falsified data—may well find his or her career ruined.

In addition to the serious improprieties just described, which are in fact extremely rare, a variety of much more frequently committed “misdemeanors” in
the conduct of research can also affect the scientific community. Like fabrication, falsification, and plagiarism, some of these actions are considered to be unethical because they violate a fundamental value, such as honesty. For example, most active scientists believe that honorary authorship—listing as an author someone who did not make an intellectual contribution to the work—is unethical because it misrepresents the origin of the research. In contrast, other unethical behaviors violate standards that the scientific community has adopted. For example, although it is generally understood that material submitted to a peer-reviewed journal as part of a research manuscript has never been published previously and is not under consideration by another journal, instances of retraction for dual publications can be found on occasion.

**Scientific Misconduct Has Been Formally Defined by U.S. Governmental Agencies**

The serious misdeeds of fabrication, falsification, and plagiarism generally are recognized throughout the scientific community. These were broadly recognized by federal regulations in 1999 as a uniform standard of scientific misconduct by all agencies funding research. What constitutes a misdemeanor is less clear, however, because variations in the definitions of accepted practices are common. There are several sources of this variation. Because responsible conduct is based in part on conventions adopted by a field, it follows that there are differences among disciplines with regard to what is considered to be appropriate behavior. For example, students in neuroscience usually coauthor papers with their advisor, who typically works closely with them on their research. In contrast, students in the humanities often publish papers on their own even if their advisor has made a substantial intellectual contribution to the work reported. Within a discipline, the definition of acceptable practices may also vary from country to country. Because of animal use regulations, neuroscientists in the United Kingdom do relatively little experimental work with animals on the important topic of stress, whereas in the United States this topic is seen as an appropriate area of study so long as guidelines are followed to ensure that discomfort to the animals is minimized.

The definition of responsible conduct may change over time. For example, some protocols that were once performed on human and animal subjects may no longer be considered ethical. Indeed, ethics evolve alongside knowledge. We may not currently be able to know all of the risks involved in a procedure, but as new risks are identified (or previously identified risks refuted), we must be willing to reconsider the facts and adjust our policies as necessary. In sum, what is considered to be ethical behavior may not always be obvious, and therefore we must actively examine what is expected of us as scientists.

Having determined what is acceptable practice, we then must be vigilant. Each day neuroscientists are faced with a number of decisions having ethical implications, most of them at the level of misdemeanors: Should a data point be excluded because the apparatus might have malfunctioned? Have all the appropriate references been cited and are all the authors appropriate? Might the graphic representation of data mislead the viewer? Are research funds being used efficiently? Although individually these decisions may not significantly affect the practice of science, cumulatively they can exert a great effect.

In addition to being concerned about the integrity of the published literature, we must be concerned with our public image. Despite concerns over the level of federal funding for research, neuroscientists are among the privileged few who have much of their work funded by taxpayer dollars. Highly publicized scandals damage the public image of our profession and hurt all of us who are dependent on continued public support for our work. They also reduce the public credibility of science and thereby lessen the impact that we can expect our findings to have. Thus, for our own good and that of our colleagues, the scientific community, and the public at large, we must strive to act with integrity.

**SUMMARY**

You are about to embark on a tour of fundamental neuroscience. Enjoy the descriptions of the current state of knowledge, read the summaries of some of the classic experiments on which that information is based, and consult the references that the authors have drawn on to prepare their chapters. Think also about the ethical dimensions of the science you are studying; your success as a professional and the future of our field depend on it.

**References**


**Suggested Readings**


Floyd E. Bloom

I. FUNDAMENTAL NEUROSCIENCE
CHAPTER 14

Shared Mechanisms of Disease

Harald Sontheimer

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1. INTRODUCTION

By now, the reader of this book and probably every student of neurological illness will have heard some hypothesized disease mechanisms multiple times, in seemingly very different diseases. For example, glutamate toxicity appears to be involved in just about any disease where neurons die, be that as a result of stroke, genetic mutation in amyotrophic lateral sclerosis (ALS), or brain cancer. Similarly, the accumulation and aggregation of proteins, either inside or outside of cells, seem to occur quite abundantly in neurological disease. Even when genes are identified that unequivocally cause disease, as is the case with mutated superoxide dismutase 1 (SOD1) causing ALS, or with mutations in the methylcytosine-binding protein 2 (MECP2) gene underlying Rett syndrome, the resulting pathology is often complex and not readily explained by the mutated gene, and sometimes is not understood at all. Indeed, most of the animal models used, even those that accurately model the disease, are poor predictors for subsequent treatment of patients.

This chapter will attempt to synthesize commonalities that bridge multiple neurological disorders with the hope that common mechanisms may teach us important principles. Some of these findings, however, may be exaggerated, in part because scientists have selectively studied certain mechanisms across the spectrum of diseases, thereby overlooking important ones that are not shared. Nevertheless, a critical examination allows us to synthesize at least some true conceptual similarities that are shared by many diseases of the nervous system. A summary overview that identifies some of the hallmarks of disease, separating the disease groups as static, primary and secondary progressive neurodegeneration, developmental illnesses, and neuropsychiatric disease, is provided in Table 1.

2. NEURONAL DEATH

At first glance, neuronal death appears to be the most defining characteristic of most neurological illnesses. Cell death may occur almost instantaneously following a stroke or traumatic insult, or with a delayed onset that defines primary progressive neurodegenerative diseases, including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and ALS. Neuronal death also characterizes what we classified as secondary neurodegenerative diseases, namely brain tumors, multiple sclerosis (MS), and nervous system infections. Neuronal death may be indirect and concealed, and therefore is a less prominent attribute in some disorders. For example, the white matter loss that characterizes MS is associated with axonal dysfunction and ultimately causes axonal death as the loss of myelin removes important metabolic and trophic support. Other diseases, too, present with less conspicuous white matter loss, as in schizophrenia and ALS, and therefore we must surmise that axons may die in these diseases as well.

The question then becomes to what extent neuronal loss is the cause or the consequence of disease. This is more than a rhetorical question, as it guides future therapeutic strategies. Clearly, the neurological symptoms of a disease are directly related to neuronal loss, and, in fact, the symptoms often allow us to directly identify the affected neuronal cell population. For example, the motor weaknesses and gait imbalances in poliomyelitis and ALS selectively affect the motor neurons in the cortex and spinal cord and present with hyperreflexia, while the gait imbalances in neurosyphilis are due to sensory neuron loss in the spinal cord and present with areflexia.

But let us consider a bacterial infection of the meninges, meningitis, which initially presents with muscle stiffness of the neck and may lead to severe headaches or even coma. In the end stage of disease, neuronal
### VI. COMMON CONCEPTS IN NEUROLOGICAL AND NEUROPSYCHIATRIC ILLNESSES

#### TABLE 1  Common Hallmarks of Neurological Diseases

<table>
<thead>
<tr>
<th>Hallmark</th>
<th>Acute/Static</th>
<th>Progressive Neurodegenerative</th>
<th>Secondary Neurodegenerative</th>
<th>Development</th>
<th>Neuropsychiatric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stroke</td>
<td>Trauma</td>
<td>Epilepsy</td>
<td>MS</td>
<td>Gliona</td>
</tr>
<tr>
<td>Neuronal death</td>
<td>Yes</td>
<td>yes</td>
<td>minor</td>
<td>secondary</td>
<td>Yes</td>
</tr>
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<td>White matter involvement</td>
<td>yes</td>
<td>yes</td>
<td>?</td>
<td>primary</td>
<td>Primary</td>
</tr>
<tr>
<td>Glutamate toxicity</td>
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<td>yes</td>
<td>some</td>
<td>primary</td>
<td>yes</td>
</tr>
<tr>
<td>Protein aggregates</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>primary</td>
<td>yes</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>secondary</td>
<td>secondary</td>
<td>secondary</td>
<td>primary</td>
<td>secondary</td>
</tr>
<tr>
<td>Rare familial forms</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>fully penetrant</td>
<td>yes/NIK</td>
</tr>
<tr>
<td>Cell autonomous</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Non-cell autonomous</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Impaired BDNF</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Epigenetic</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>Yes</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Major</td>
<td>Major</td>
<td>Minor</td>
<td>some</td>
<td>Major</td>
</tr>
<tr>
<td>Animal model/therapeutic value</td>
<td>Several/</td>
<td>Several/</td>
<td>Several/</td>
<td>Several/</td>
<td>Several/</td>
</tr>
</tbody>
</table>
cell death is common, yet at no point in the disease are neurons anything but innocent bystanders. We assume the same to be the case in MS, where neurons may never be the direct target of activated lymphocytes, but eventually become victims. The same case could be made for cerebrovascular infarct and central nervous system (CNS) trauma, both of which affect brain tissue in a rather nondiscriminatory fashion, yet, owing to the unique dependence of neurons on oxidative metabolism, they are the most vulnerable cell population and are the first to die as energy supply becomes limited. To take this reasoning even further, we learned that in some neurodegenerative or neurodevelopmental diseases, for example ALS or Rett syndrome, introducing the mutated, disease-causing genes into astrocytes or microglia is sufficient to cause disease even when the neurons continue to make the wild-type protein. Here too, neurons ultimately die, but once again they appear to be collateral damage rather than the principal cell targeted by disease. So while neuronal cell loss is a common feature of neurological disease, and occurs almost without exception at some stage of the illness, one must not make the mistake of focusing too heavily on mechanisms of neuronal cell death to understand the underlying disease mechanisms. Instead, we must accept the fact that neuronal death may occur in many different ways as a final stage of disease, and is mechanistically a consequence of disease rather than its cause.

3. GLUTAMATE TOXICITY

One must also be careful about being overly fixated on any singular molecular cause of neuronal cell death, particularly if death is caused by an extrinsic chemical such as glutamate (Glu). Without question, Glu excitotoxicity is one of the most common threads, appearing in essentially all neurological disorders. Not only does excessive Glu kill neurons, it can also kill oligodendrocytes and thereby cause white matter injury as well. Astrocytes, by contrast, are unaffected by even very high glutamate concentrations.

Glu toxicity was first proposed from extensive studies on the health effect of monosodium glutamate, which is used as a food-flavoring agent. It was found to kill neurons in infant monkey brain regions, particularly those not protected by the blood–brain barrier (BBB). Within the following two decades, Glu emerged as a major culprit in numerous disorders including stroke, HD, and ALS. Indeed, we have since learned that ~90% of all neurons in the brain express Glu receptors and all of those neurons are, in principle, susceptible to Glu toxicity.

3.1 Why is Glutamate Toxic?

As we discussed in Chapter 4 (Aging and Alzheimer disease), glutamate excitotoxicity derives from the peculiar properties of the Glu receptors involved in learning. One may argue that we entered into an “evolutionary bargain” where we accepted this vulnerability to gain a mechanism for cells to learn that two stimuli or pieces of information are related. More specifically, neurons utilize two different types of Glu receptors (Figure 1). Fast signaling occurs via AMPA/kainate receptors that primarily flux Na+ into the cell, thereby depolarizing the postsynaptic cell upon ligand binding. The second type of Glu receptor is the NMDA receptor (NMDA-R), which fluxes both Na+ and Ca2+. In fact, the primary role of NMDA receptors is to flux Ca2+, and thus their permeability for Ca2+ is about 5-fold greater than that for Na+. Importantly, NMDA-Rs are usually inactive, since they are blocked by intracellular Mg2+ ions that are lodged inside the permeation pore. Only when the cell becomes depolarized does this Mg2+ ion temporarily dislodge, allowing Ca2+ and Na+ to enter the cell.
If a postsynaptic terminal receives coincident signals paired less than 50ms apart, the first stimulus will depolarize the cell, thereby relieving the Mg$^{2+}$ block, and the second signal will now trigger a Ca$^{2+}$ influx. This leads to activation of Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and induces long-term changes in receptor number on the cell surface. For the next minutes to hours, this causes a potentiation of all signals that travel through this synapse,
14. SHARED MECHANISMS OF DISEASE

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3.2 Role of Ca\(^{2+}\)

The physiological range for Ca\(^{2+}\) to act as a second messenger in the neuronal cytoplasm is about 100–1000 nM. Above this range, Ca\(^{2+}\) activates destructive enzymes such as proteases, phospholipases, endonucleases, and caspases, which begin to cleave proteins, DNA, and membrane lipids (Figure 1). Also, upon Ca\(^{2+}\) entry, mitochondria open a large transition pore through which they release cytochrome C, which in turn initiates the apoptotic death cascade. Since extracellular Ca\(^{2+}\) concentration is ~1 mM under normal conditions, cells must maintain a steep, 1000–10,000-fold concentration gradient for Ca\(^{2+}\) across the plasma membrane. This gradient is maintained through several energy-dependent Ca\(^{2+}\) transport systems. Any loss of energy, such as that seen during an ischemic stroke, puts the cells at immediate risk for a pathological rise in intracellular Ca\(^{2+}\) due to the loss of this tightly maintained gradient. Of course, this can also occur with prolonged exposure of neurons to elevated concentrations of glutamate. Even a change as small as an excess of only 50–100 μM glutamate for just 2 min is sufficient to cause essentially irreversible neuronal death 24 h later.

Note that any chronic depolarization of neurons causes uncontrolled influx of Ca\(^{2+}\) into their presynaptic terminals via both voltage-gated Ca\(^{2+}\) channels and NMDA-Rs. The additional massive release of synaptic Glu when the presynaptic terminal is depolarized then creates a “perfect storm” scenario in which neurons die as a consequence of the Glu they themselves release.

Other cells can also contribute to Glu toxicity. For example, we learned in Chapter 9 that brain tumors release massive amounts of glutamate and deliberately kill neurons to create room for tumor growth. In addition, whenever inflammation occurs and activated microglia are present, microglia release glutamate as well.

Although astrocytes are not subject to Glu toxicity, Glu readily kills oligodendrocytes and this appears to involve the same principal mechanisms. As we learned in Chapter 8, oligodendrocytes express NMDA-Rs in their myelin, as well as a subtype of AMPA receptor that is also Ca\(^{2+}\) permeable. During normal activity, axons release glutamate as a physiological stimulus to maintain the myelin sheath. Pathological dysregulation of glutamate then becomes toxic to the myelin.
3.3 Astrocytic Glutamate Transport

Because Glu has tremendous toxic potential, neurons are typically protected from uncontrolled rises in extracellular Glu. This is accomplished by Glu transporters expressed on astrocytic processes that surround glutamatergic synapses, illustrated in Figure 2, where the astrocytic processes are colored in blue. Any spillage of Glu from the synaptic cleft binds to one of two excitatory amino acid transporters expressed by astrocytes. Excitatory amino acid transporter 2 (EAAT2) is the most abundant Glu transporter in the mature brain and, in fact, is the most abundant of all brain proteins. EAAT2 has a high affinity for Glu (\( \sim 12 \mu M \)) and imports Glu into astrocytes by harnessing the Na\(^+\) gradient as an energy source. This gradient is electrogenic; three Na\(^+\) ions and one H\(^+\) ion are transported into the cell along with each Glu\(^-\) molecule. During this process, one K\(^+\) ion is also counter-transported out of the cell (Figure 3), creating a situation in which there is a net effect of two extra positive charges entering the cell (four positive and one negative charge entering, minus one positive charge leaving, yields two positive charges entering). These two extra positive charges facilitate Glu uptake into the cell, as the transporter can now effectively harness both the chemical and electrical gradient. It is worth mentioning, however, that the transporter operates very slowly, transporting only 14 ions/sec (compared to other ion channels that flux \( >10^6 \) ions/sec) and therefore has a very low capacity. To compensate for this limited capacity, the density of EAAT2 Glu transporters on astrocytes is extraordinarily high, approximately 1000–10,000 transporters/\( \mu m^2 \), such that binding of Glu to transporters will be sufficient to capture all extra Glu being released from synapses, even if no transport occurs. The system is designed so that under normal conditions no spillage of Glu should occur, unless, of course, astrocytes fail to express a sufficient number of them. Therefore it stands to reason that in any disease where we observe Glu toxicity, astrocytes must be compromised in their ability to neutralize this threat. This is actually the case in many neurological diseases, including stroke, trauma, MS, ALS, PD, HD, and glioma, where a loss of EAAT2 expression or function has been demonstrated in patient

**FIGURE 2** Ensheathment of glutamatergic synapse (arrow) by astrocytes (blue) in the hippocampus shown by electron microscopy. Astrocytic processes are strategically placed to prevent spillage of glutamate from the synaptic cleft. *Modified with permission from Ref. 5 (Figure 1).*

**FIGURE 3** Schematic of the astrocytic EAAT2 transporter. Transport of each negatively charged Glu is coupled to three Na\(^+\) and one H\(^+\) into the cell, with counter-transport of one K\(^+\) for a net charge difference of two positive charges. This charge adds an electrical gradient to the chemical gradient, allowing more effective Glu uptake. It also makes it more difficult for the transporter to run in reverse. *Reproduced with permission from Ref. 3.*
tissue and/or animal models of disease. In fact, in ALS, motor neuron death can be attenuated by experimentally restoring EAAT2 expression using ceftriaxone, an antibiotic that acts as a transcriptional regulator of EAAT2 and enhances its expression in astrocytes up to 3-fold. This drug has shown promise in a clinical trial for ALS (ClinicalTrials.gov, NCT00349622), and ceftriaxone may have the potential to be more broadly used to mitigate glutamate excitotoxicity in other diseases.

3.4 Extrasynaptic NMDA Receptors

The role of NMDA-Rs in learning and memory, as well as glutamate toxicity, is more complex than was described above. NMDA-Rs are expressed not only on postsynaptic terminals but also at extrasynaptic sites located at a distance from the next synapse (Figure 4). These receptors can sense Glu spilling from adjacent synapses if astrocytes do not capture all the Glu. The extrasynaptic NMDA-Rs have a different subunit composition from synaptic ones. All NMDA-Rs are heterotetramers (composed of four different subunits) that share two obligatory GluN1 subunits, typically with a combination of two additional GluN2 subunits. These come in two isoforms, GluN2A and GluN2B. GluN2A is the most common subunit of NMDA-R at synapses, whereas GluN2B is commonly found at extrasynaptic sites. These subunits both promote different signaling cascades, which in turn exert different effects on the cell. Synaptic GluN2A-containing receptors enhance LTP, the cellular equivalent of learning, and activate cellular signals that promote cell survival. By contrast, activation of extrasynaptic GluN2B-containing receptors contribute to long-term depression (LTD), the cellular equivalent of apoptosis.

FIGURE 4 Extrasynaptic NMDA receptors containing the GluN2B subunit activate apoptotic cell death pathways whereas synaptic receptors containing the GluN2A subunit are largely prosurvival.
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4. PROTEIN AGGREGATES AND PRION-LIKE SPREAD OF DISEASE

All of the classical neurodegenerative diseases are histopathologically defined by intracellular or extracellular protein aggregates. Examples of the most common aggregates observed in AD, PD, ALS, HD, and prion disease are illustrated in Figure 5. For each disease, the protein that causes the aggregate is different. However, a shared commonality is that accumulating proteins differ in their tertiary structure so as to be misfolded and insoluble. Most typically, the tertiary structure changes from an alpha helix to a beta-sheet conformation, which makes the proteins sticky and hydrophobic. Seemingly small changes in the primary structure of a protein can have a profound effect on its tertiary structure. For example, under normal conditions, the cleavage of beta-amyloid causes water-soluble nontoxic amyloid. Yet, in the case of AD, a difference in only two or three amino acids affects beta-amyloid cleavage at the 42 position and causes toxic amyloid plaques. In HD, up to 35 polyglutamines produce a normal huntingtin protein that is highly water soluble, while 36–39 can cause disease in some individuals, and 40 or more repeats guarantee protein inclusions and disease in every affected individual.

Another commonality is that protein aggregates tend to develop well before a person becomes symptomatic. In AD, for example, noninvasive imaging allows detection of amyloid deposits up to 15 years prior to disease onset. Not surprisingly, it is therefore widely assumed that protein aggregates cause disease, a notion that is heavily debated and is far from certain. The only example where protein inclusions have been shown to be necessary and sufficient for disease is in HD, where the removal of deposits
after the disease is already fully manifest causes a nearly complete reversal of symptoms. While we assume that only the mutated, hyperphosphorylated, or misfolded proteins accumulate, it would be a mistake to assume that their loss of function is directly responsible for disease. Instead, one must consider that protein aggregates also contain numerous other proteins, including wild-type protein, transcription factors, DNA/RNA regulatory proteins, chaperone proteins involved in protein folding and trafficking, or proteins that confer resistance to cellular stress. It is likely that the sequestration of these supportive proteins by protein aggregates contributes to the functional losses observed in neurons as these proteins are prevented from participating in their normal function.

Protein aggregates may resemble temporary or permanent storage sites for junk proteins in the cell. In all likelihood the aggregates per se are not doing irreversible harm, but result from an inability of the cell to remove junk proteins in a timely fashion. Therefore, a problem in protein homeostasis or proteostasis exists where the production of new protein is in disequilibrium with removal of old or nonfunctioning protein. This leads to a “toxic gain of function” attributed to inclusion bodies but largely explained by a lack of participation of proteins that are caught in these aggregates. Should commonalities prevail, the removal of the protein aggregates therefore

FIGURE 5 Protein aggregates in neurodegenerative disease. (A) Senile amyloid plaques in the neocortex of a patient with Alzheimer disease. (B) Neurofibrillary tangles in the hippocampus of a patient with frontotemporal dementia with parkinsonism (FTDP-17). (C) Lewy body in the substantia nigra of a patient with Parkinson disease. (D) Intranuclear polyglutamine inclusion in the neocortex of a patient with Huntington disease. (E) Ubiquitinylated inclusion in spinal cord motor neuron of patient with ALS. (F) Protease-resistant PrP prion protein in the cerebellum of a patient with Creutzfeldt-Jakob disease. Reproduced with permission from Ref. 10.
should restore neuronal health, and this has been conclusively demonstrated only for HD. Note that protein aggregates do not form in secondary neurodegenerative disorders or acute insults, and are also absent in neuropsychiatric illnesses.

4.1 Proteostasis

Understanding what leads to the formation of aggregates in the presymptomatic stages of disease holds tremendous promise and potential in preventing disease. This concept serves as the rationale behind a large National Institutes of Health-supported AD prevention trial that is enrolling 100 controls and 100 subjects who harbor the presenilin-1 (PSEN1) E280A mutation that causes familial early onset of AD by age 44. The participant’s cognitive status will be assessed prior to disease onset and throughout continuous treatment with the monoclonal antibody crenezumab (Genentech) to clear beta-amyloid before it can form aggregates.

The notion that impaired proteostasis is at least responsible for intracellular protein accumulation is very attractive. A network of proteins called the proteostasis network maintains an orderly balance of protein synthesis and degradation. Such proteins include molecular chaperones that help newly synthesized polypeptides to assume the correct three-dimensional shape, i.e., tertiary and quaternary structure. It also involves proteins that dispose of old, nonfunctional, mutated, or misfolded proteins. Such proteins are degraded by two parallel systems. Small peptides and proteins are tagged by polyubiquitin marks attached by ubiquitin ligases and transported to the proteasome, an intracellular complex that proteolytically cleaves proteins into their amino acid constituents (see also Box 5.1, Chapter 5). Protein aggregates that are too large to fit into the core of the proteasome aggregate into autophagosomes, which then fuse with the cell’s lysosomes, where proteins are enzymatically degraded (see Figure 7.9, Chapter 7). If this equilibrium is disrupted by either increased supply or reduced disposal, junk proteins accumulate and give rise to inclusion bodies.

4.2 Prion-Like Spread of Misfolded Protein

Essentially all diseases that present with protein aggregates show considerable local spread within tissue. For example, in ALS it is typical for adjacent motor neurons to become gradually affected by disease. In AD, disease spreads gradually from the hippocampus to the entorhinal cortex, then encroaches on the frontal lobe and spreads toward the parietal brain. This propagation of disease is consistent with a toxic agent moving from cell to cell. Inclusion bodies are very stable beta-sheet structures, similar to mutated prions, which cause mad cow or Creutzfeldt–Jakob disease, which we discussed in Chapter 10. Mutated prions propagate by imposing their shape on wild-type prion proteins in their vicinity, causing them to assume the same shape. It has been postulated that neurodegenerative disease may also spread within the nervous system in a prion-like fashion, as misfolded mutated proteins coax wild-type proteins in adjacent cells to assume their misfolded state by acting as a template. At least for AD, a prion-like spread of disease has been demonstrated in mice by inoculating mice that overexpress a mutant form of APP with Aβ from brain homogenates into only one brain hemisphere. This was sufficient to cause widespread Aβ deposits in both hemispheres consistent with a prion-like mode of propagation. If true across all diseases with misfolded proteins, this would be a frightening proposition, as prions have been shown to be resistant to just about any chemical intervention. What raises doubt for the generalization of this mechanism to all diseases with protein aggregates is the fact that at least in HD, the inclusions dissolve and disease
reverses when the production of new mutated huntingtin protein is experimentally stopped. If the mutated huntingtin protein behaved as prion, it could not be disposed of by the proteasome. Nevertheless, a prion-like spread of neurodegeneration cannot yet be excluded for AD, PD, and ALS.

5. MITOCHONDRIAL DYSFUNCTION

The brain is the organ with the highest relative energy use. Weighing only 2% of the body’s weight, the adult brain uses 20% of all calories consumed. The reason for this incredible energy use is the sustained high neuronal activity. Action potentials (AP) require maintenance of ionic gradients across the axonal membrane. For Ca$^{2+}$ to function as second messenger, it needs to be kept at a concentration 10,000-fold below the extracellular concentration by pumping it out of the cell or into organelles. In addition, neurotransmitters need to be recycled and packaged into synaptic vesicle. These processes all directly or indirectly consume energy in the form of ATP. In most organs, ATP can be generated glycolytically in the cytoplasm or through aerobic metabolism in the mitochondria. The former process is wasteful, as it only captures 3 mol of ATP per 1 mol glucose. Hence, the brain utilizes the more efficient oxidative metabolism exclusively, yielding 36 mol ATP per 1 mol glucose. Oxidative phosphorylation exclusively occurs in the mitochondria, and these organelles are found abundantly throughout most neurons. The mitochondrial tricarboxylic acid (TCA) cycle, also known as citric acid or Krebs cycle, not only provides effective aerobic energy production but also generates some important amino acid precursors for the synthesis of transmitters and proteins. Oxidative phosphorylation utilizes glucose and oxygen delivered via the cerebral vasculature. Failure to provide either oxygen or glucose or both causes a rapid loss of ATP, depleting the cells’ energy stores within less than 120 s. This occurs in stroke, where loss of blood flow causes a rapid cessation of energy production. As is evident from the sudden onset of symptoms in stroke, neuronal function ceases very quickly. Moreover, if the energy disruption persists, glutamate excitotoxic death, discussed above, will ensue. Even in the continued presence of energy substrates, ATP production by the mitochondria can become impaired, causing a similar loss of neuronal function.

The possibility that mitochondrial dysfunction may contribute to neurological disease first surfaced in studies of HD and PD. In PD, mitochondrial poisons such as the pesticide rotenone, which inhibits complex I of the respiratory chain, cause Parkinsonian movements. Similarly, an early animal model of HD used the mitochondrial poisons 3-nitropropionic acid or malonate to elicit Huntington-like motor dysfunction in mice. The idea emerged that environmental toxins may exert deleterious function by inhibiting mitochondrial function. However, much stronger evidence for concrete disease-causing contribution of mitochondrial dysfunction came from genetic studies.

5.1 Genetic Mitochondrial Defects in Parkinson Disease

Although familial forms of PD are uncommon, several genes have been identified that cause rare familial forms of PD. Two of these (Table 1) are directly related to mitochondrial health, namely Parkin and PTEN-induced kinase 1 (PINK1). Mutations in either gene cause recessive early-onset PD. Parkin encodes an E3 ligase that attaches ubiquitin residues to proteins or organelles, marking them for degradation. PINK1 encodes a protein that is expressed on the surface of mitochondria, where it interacts with Parkin to provide continuous surveillance of mitochondrial health. In normal mitochondria, PINK1–Parkin interactions signal a healthy
organelle. As mitochondria become sick, their membrane potential, which indirectly harnesses the power to generate ATP, deteriorates. This is sensed by PINK1, which then phosphorylates Parkin, causing it to mark the mitochondrial for degradation (Figure 5.16, Chapter 5). When either gene is mutated, however, this surveillance fails and the neuron begins to accumulate faulty mitochondria that are unable to generate ATP effectively. This leads to an increased release of a highly reactive oxygen species (ROS) that can harm DNA and cellular proteins. Importantly, mitochondria harbor their own mitochondrial DNA to produce most essential proteins required for the synthesis of ATP. The presence of oxygen radicals puts this mitochondrial DNA at risk to produce faulty enzymes, jeopardizing the role of the mitochondria in energy production.

5.2 Mitochondrial Defects in Other Neurodegenerative Diseases

Defective mitochondrial enzymes are commonly observed in PD and HD neurons and even in fibroblast or muscle cells from patients. As a result, the mitochondria become very inefficient in converting glucose to ATP. In HD, the formation of intracellular inclusion bodies impairs the transport of mitochondria along the axon toward sites of high energy demand. This is also the case in AD, where intracellular hyperphosphorylation of tau destabilizes the microtubules that form the guiderails of axonal transport. As a result, mitochondria can no longer be effectively transported along the axon.

In HD, mitochondria often look noticeably sick. They are swollen, containing vacuoles and disturbed cisternae. Inclusions of mutated huntingtin protein can be found lodged under the outer mitochondrial membrane, where they impair the function of the mitochondrion. This causes measurable decreases in activity of key enzymes in the TCA cycle and complex II–IV activity. Owing to the ineffective use of glucose, HD patients show increased compensatory caloric intake.

Similar changes in the activity of mitochondrial enzymes are found in mitochondria isolated from AD patients. In such cases, pyruvate dehydrogenase and isocitrate dehydrogenase are both impaired, and the expression of complexes I and IV is reduced. Increased glucose uptake is seen in patients with AD by positron emission tomography (PET) imaging and assumed to be a compensatory response.

As already mentioned above, any impairment in mitochondrial function has the potential to generate reactive oxygen radicals, which can damage mitochondrial DNA. These, in turn, generate faulty enzymes that further contribute to the ill health of the mitochondria. In ALS, a common mutation of both familial and sporadic disease affects the SOD1 gene, which is essential for the mitochondrial detoxification of oxygen radicals produced in the context of oxidative phosphorylation. Mutant SOD1 causes swelling of the mitochondria, impairs respiratory complexes, disrupts Ca\(^{2+}\) homeostasis, and leads to markedly reduced ATP production.

Mitochondrial dysfunction appears uniquely critical in diseases involving neurons that have very high AP discharge rates. This is particularly the case in the midbrain, where movement control pathways fire at rates of tens of APs/s, and some neurons have pacemaker activity, such as in the substantia nigra. These cells are literally on the edge, with a very close balance needed to be able to sustain the energy demand with the energy they produce. Even a small decrement in efficiency of their mitochondria would quickly impair their function.

While PD, HD, AD, and ALS are the diseases in which mitochondrial dysfunction is most strongly implicated in disease, this appears to be a much more universal pattern, and likely also contributes to acute and nonprogressive conditions such as stroke, trauma, and epilepsy. To what extent mitochondrial dysfunction
is a consequence of disease or contributes to its cause remains to be elucidated. Thus far, a strong causal role has only been demonstrated in familial PD that presents with Parkin or PINK1 mutations.

5.3 Impaired Redox Status

Even in normal cells, mitochondrial respiration is a major cause for unpaired ROS. The most prevalent mitochondrially produced ROS is the superoxide radical. Even in a normal cell, the mitochondria do not work flawlessly and thus up to 2% of all electrons that pass through the respiratory chain are prematurely reduced. Since these ROS are highly reactive, the cells try to capture and neutralize them. This is done by antioxidant molecules such as vitamin E and glutathione, and by enzymes such as SOD that degrade the superoxide radical to H$_2$O$_2$ and water. SOD1 mutations are found in up to 7% of patients with ALS, making it a leading genetic cause of disease, and the only genetic cause that we know to affect redox status in neurological disease. That said, there is not a single disease that does not implicate a challenged redox environment as a contributing factor to disease, be that stroke or trauma, frank degeneration or infection. It is important to note, however, that while under these conditions an imbalance in the production and neutralization of ROS may contribute to cell damage, it is likely to be not causal of disease but rather an aggravating circumstance.

5.4 Improving Mitochondrial Function to Ameliorate Disease

To compensate for mitochondrial dysfunction, several strategies have been pursued in animal models and human clinical trials. First, phosphocreatine is a high-energy substrate that is utilized by nerve and muscle cells to generate ATP. Phosphocreatine supplementation extends the life expectancy of mice carrying mutated SOD1. Nutritional supplementation, explored in a pilot clinical trial for PD, failed to show improvements. However, high doses of creatine monohydrate slowed cortical atrophy in HD patients, and this trial was recently expanded to a multicenter phase III study (clinical trial registration #NCT00712426).

Another attempt to improve overall mitochondrial health is through supplementation with CoQ10, which is an endogenous substrate for the respiratory chain. It is often sold as an antioxidant dietary supplement and has shown neuroprotective qualities in various mouse models of neurodegenerative disease. Ongoing clinical studies are evaluating CoQ10 in PD, HD, and ALS. As is often the case, small early pilot studies show promising results, yet when expanded to larger patient cohorts the effect disappears. At present there is no strong evidence that CoQ10 supplementation improves outcome for any of the neurodegenerative diseases.

Finally, dietary supplements with cellular antioxidants including glutathione, vitamins C and E, omega 3 fatty acids, and beta-carotene have undergone experimentation in many diseases, yet none have proven to be effective. Given the ready access to these nutritional supplements, they continue to be widely used by patients nonetheless.

6. IN SPITE OF OBVIOUS DISEASE HERITABILITY, GENETIC CAUSES OFTEN REMAIN ELUSIVE

The majority of nervous system disorders, with possibly the only exceptions being stroke, trauma, and infections, are suspected to have genetic causes. However, in only a few instances have mutations in single genes been shown to cause disease. Examples discussed in this book include HD, Rett syndrome, and fragile X syndrome. It is much more common for disease-associated genes to characterize just a small subset of individuals within
affected families, yet these familial forms of disease are phenotypically often indistinguishable from sporadic disease. Therefore, we assume that familial and sporadic diseases are by and large identical and that eventually other genetic alterations will be discovered that explain the disease. Such is the case for three of the classic neurodegenerative diseases, ALS, AD, and PD. In each instance, studies of gene mutations in rare familial diseases have informed us about disease mechanisms and have allowed us to model the disease in animals by introducing similar mutations. This approach has also yielded model systems in which to test therapeutic drugs. Table 2 lists some prominent examples of genes that give rise to familial disease in a small percentage of affected individuals.

On the other side of the spectrum are diseases such as depression and schizophrenia, where any attempt to identify disease-causing genes, combinations of genes, or even susceptibility genes has thus far provided a complicated and largely unsuccessful result.

Nevertheless, we know from epidemiological data that these diseases “run in families” and that disease risk increases quite significantly if any other family member also has the disease. With schizophrenia, the risk of a sibling of an affected individual developing schizophrenia is 10%. Even for multiple sclerosis, where we understand very little about the genetics of the disease and overall disease etiology, sibling studies suggest significant heritability, with a 33% chance for identical twins to develop disease and still a 5% change in risk if a sibling suffers from MS.

6.1 Gene–Environment Interactions

These diseases teach us that genetic factors alone are, in most cases, insufficient to cause disease. Instead, genes generate disease susceptibility that only manifests under the right (or, shall I say, wrong) conditions at the wrong time. Moreover, most diseases are polygenic, probably involving a number of susceptibility genes and possibly a combination of environmental factors. Note that by environment we are not only implying a person’s physical environment but also the cellular environment as depicted in Figure 6, which includes nutritional status, redox environment, temperature, cellular stressors, and many other unknown factors.
VI. COMMON CONCEPTS IN NEUROLOGICAL AND NEUROPSYCHIATRIC ILLNESSES

6.2 Monogenetic Diseases

For only three diseases discussed in this book do we know a single gene that alone is responsible for a disease. These examples are HD, Rett syndrome, and fragile X syndrome. In HD, a microsatellite CAG expansion on chromosome 4 gives rise to a mutated protein (huntingtin) that causes progressive neurodegeneration. In Rett syndrome, mutations in the methyl-CpG-binding protein 2 (MeCP2) gene that regulates chromatin compaction alters the transcription of numerous downstream signals, including brain-derived neurotrophic factor (BDNF), thereby causing a progressive X-linked neurodegeneration. In fragile X, a microsatellite (CGG) expansion causes methylation and transcriptional repression of the fragile X mental retardation gene 1 (FMR1), which encodes an RNA-binding protein inhibiting RNA translation into proteins. Note that in two of these diseases, Rett syndrome and fragile X, the affected gene and protein per se are entirely normal and only their quantity is altered. In HD, the mutation causes abnormal folding and protein inclusions, similar to those seen in PD and ALS.

Genes for the above diseases were identified through linkage analysis long before whole genome sequencing became available. As we learned for HD, large families spanning multiple generations of affected individuals were analyzed to find common regions on the genome. The chromosomal stretches were then narrowed down to smaller and smaller segments to eventually identify a single gene. This approach only works for diseases caused by a single gene.

Through a heroic effort, and involvement of many laboratories, a large-scale cloning effort completed the sequencing of the entire human genome in 2003. So why, in spite of the much-heralded ability to sequence a human’s entire genome, have we

FIGURE 6 Environmental influences and gene expression interactions. Conceptual model of candidate pathways contributing to neurodegeneration. Candidate pathways influencing the balance of neuronal survival and degeneration are displayed within broader functional groups based on their major site or mode of action (intracellular mechanisms, local tissue environment influences, systemic influences, and mechanisms related to neurodevelopment and aging). The pathways and overarching functional groups in this model are highly related and can have overlapping or interacting components that can collectively modulate neurodegenerative processes. Reproduced with permission from Ref. 17.
been unable to define a genetic cause for most of these disorders? For starters, we do not have whole genome sequencing data for many people yet. This is still a time-consuming and costly endeavor. It will probably be feasible and affordable within another decade or two, yet even then it is not likely to become a standard diagnostic procedure. To put it in perspective, an analysis must be done for two copies of 23 chromosomes harboring 25,000 genes encoded by 3.2 billion base pairs. Of these, 99.9% will be identical for most individuals. Among the remainder, many differences will be irrelevant. Others will affect genes of still unknown function. The cloning of the first human genome took 13 years and cost approximately $3 billion. Even if it were feasible to obtain every person’s full genomic sequence, the primary obstacles remain for bioinformatics to identify meaningful differences and for biologists to figure out which of the changes cause proteins to show altered function in disease. We are certainly still trying to find the needle in the haystack.

6.3 Polygenetic Diseases and Genome-Wide Association Study

To deal with current constraints regarding the ability to sequence and align entire genomes, the most common gene mining approach used to date is called a genome-wide association study (GWAS). Through this approach, the assumption is made that certain small genomic changes, namely single nucleotide polymorphisms (SNPs, pronounced snip), occur randomly in our genome, albeit with a relatively low frequency. Typically, such changes do not alter the amino acid and protein that is encoded, since the nucleotide code is highly redundant. For example GTT, GTC, GTA, and GTG each encode for the same amino acid (valine). In this example, two of the three nucleotides can differ and yet still produce the same amino acid and will therefore not change the resulting incorporated protein. GWAS typically compare two groups of people, those with disease and those without (Figure 7). They search for changes that occur nonrandomly and therefore are more likely in people affected by the same disease compared to controls. If an SNP can be identified to be more common in a patient with disease than in a control group, we define the SNP to be associated with disease, and it will mark a region on the genome that appears to influence the disease risk. Such a search can be done without having a gene candidate in mind, or by restricting the search to a genomic region that encodes for a candidate gene or protein suspected of contributing to the disease.

But even using GWAS, the degree of genetic variability in the population requires us to analyze huge data sets. For diseases that are common and homogeneous in their presentation, it should ultimately be possible to identify the underlying susceptibility genes and factors, unless they involve networks of many genes. Indeed, with enhanced bioinformatics capabilities, GWAS data can now be analyzed to search for changes in genes that are within a cellular pathway or network. Nevertheless, for rare diseases, such as ALS, and heterogeneous diseases, such as depression or autism, gene mining through GWAS may still be a futile effort. In depression, for example, 17,000 cases were insufficient to yield statistical power. Hence, the fact that we have failed to identify new gene candidates for these diseases is largely explained by technical issues rather than by the absence of a genetic disease cause.

It is important to recognize that an absence of a clear monogenetic cause of disease makes it difficult to establish a good, highly reproducible animal model of disease in which new therapeutics can be studied.

7. EPIGENETICS

If the polygenetic nature of neurological disease were not complex enough, we now have to contend with epigenetic changes as well. This emerging field provides important insight that explains the above-mentioned interactions of
Importantly, epigenetic mechanisms can confer lasting, even trans-generational environmental risk or protection for developing disease even if a genetic risk is present. In its simplest form, epigenetics refers to persistent but reversible, heritable changes in gene expression without alteration of the DNA itself. Therefore, the gene(s) of interest are not directly affected, but the ability of the gene to be transcribed into protein is regulated via a number of modifications at the level of chromatin or DNA. These modifications are called epigenetic “marks,” and the two most common marks are schematically illustrated in Figure 8.

First, a common epigenetic regulation involves changes in the interaction of chromatin with histones. Histones serve to compact chromatin and conserve space, as they allow for 146 bp of DNA to be tightly wrapped around each histone octamer like a spool of thread (Figure 8(A)). The histone tails protruding from this spool interact with the chromatin. Modification to these tails by acetylation, methylation, or phosphorylation (Figure 8(B)) determines whether the chromatin becomes accessible for transcription by RNA-polymerases or not. For example, acetylation, or attachment of an acetyl group to a lysine residue on the histone, causes the chromatin to relax. This makes the DNA accessible for transcription, and thus acetylation results in transcriptional activation. Acetylation is carried out by a group of enzymes called histone acetyltransferases (HATs). Their activity is antagonized by histone deacetylases (HDACs), which cause transcriptional repression.

Another common epigenetic mechanism to regulate transcription is DNA methylation (Figure 8(C)). This occurs at specific cytosine residues in the vicinity of guanines, which are encoded by CpG dinucleotides, often called CpG islands. DNA methylation typically causes the methylated gene to be silenced, and therefore protein expression is reduced. DNA methylation is catalyzed by a group of enzymes called DNA
methyltransferases (DNMTs). Humans have five different DNMTs, which recognize the cytosines to be methylated via one of several proteins that contain a methyl-CpG-binding domain (MBD). One of these MBDs is the MECP2 gene mutated in Rett syndrome, discussed further below.

Epigenetic changes are surfacing as important genetic regulators in normal biology but also in disease, as simplistically illustrated in Figure 9. Normally the gene of interest is in an unmethylated state and can be transcribed into protein. In disease, the affected gene is hypomethylated, thereby repressing its transcription. This scenario is described below for brain tumors. However, different scenarios are possible, such as when proteins involved in applying the epigenetic marks themselves become mutated and cause disease, best exemplified by Rett syndrome, discussed in Chapter 11. In most instances, however, the actual epigenetic signaling pathway operating in neurological disease has not been elucidated, nor has it been shown that epigenetic changes are a consequence rather than cause of disease. Remember, however, that this is a very young field of study.
that was, until recently, focused almost exclusively on cancer. The few examples below should illustrate that epigenetic mechanisms may very well explain some of the gene–environment interactions commonly observed in polygenetic, complex neurological illnesses. They certainly already explain the fact that identical twins typically do not show identical diseases. In spite of both twins carrying the same DNA, the actual transcription of DNA into protein can differ absolutely due to regulation by epigenetic marks.

7.1 Epigenetics and Rett Syndrome

Rett syndrome is a severe, X-linked developmental intellectual disability caused by sporadic mutations in the MECP2 gene. Following a short period of seemingly normal development, a number of neurological symptoms appear that include stereotypic hand-wringing motions, motor abnormalities including toe walking, breathing abnormalities, autonomic dysfunction, autistic features, and loss of language.

**FIGURE 9** Epigenetics and neurological disease: In healthy neurons or glia (left), expression of the mRNA of a gene occurs in the presence of an unmethylated promoter CpG island and a set of histone modifications associated that cause an open “chromatin” conformation (e.g., hyperacetylation and methylation of lysine 4 of histone H3). Blue cylinders indicate octamers of histones, consisting of histones H2A, H2B, H3, and H4. These form the nucleosomes, and the double strand of DNA is wrapped around them. In neurological disease (right), a combination of selection and targeted disruption of the DNA methylation and histone-modifier proteins disrupts the epigenetic circumstances in the cell. The aberrant epigenetic inactivation of the disease-associated genes (“closed” chromatin conformation) is associated with dense hypermethylation of the CpG island promoter and the appearance of repressive histone modifications. Epigenetic drugs such as DNA-demethylating drugs and HDAC inhibitors can partially rescue the distorted epigenetic processes and restore gene expression of the neuronal or glial gene by removing chemical modifications (e.g., DNA methylation) and inducing the presence of modifications (e.g., histone acetylation). Ac = acetylation. DNMT = DNA methyltransferase. HAT = histone acetyltransferase. HDAC = histone deacetylase. HDM = histone demethylase. MBD = methyl-CpG-binding domain protein. Met-K4 = methylation of lysine 4. Met-K9 = methylation of lysine 9. Met-K27 = methylation of lysine 27. SWI/SNF = switching/sucrose nonfermenting chromatin-remodeling complex. Reproduced with permission from Ref. 20.
Mutations in MECP2 cause a transcriptional dysregulation of many proteins involved in normal dendritic and spine development, including BDNF. While none of the affected proteins themselves are mutated and are therefore functional, their relative abundance is altered in such an unfavorable way that it leads to disorderly brain development, ultimately causing disease and developmental regression.

7.2 Epigenetics and Brain Tumors

As we discussed in Chapter 9, brain tumors are almost universally fatal and typically do not respond to chemotherapy. This is in part due to the fact that chemotherapeutic drugs tend to damage DNA, yet cancer cells have acquired DNA repair enzymes that effectively fix these damages just as quickly as they are inflicted. One of the most promising current chemotherapeutics for brain tumors is the DNA alkylating agent, temozolomide. While it was found to be completely ineffective in the majority of glioma patients, it provides very favorable outcomes in a subgroup. These 20% of patients carry a hypermethylated, and therefore inactivated, DNA repair enzyme, the O-6-methyl-guanine-DNA methyltransferase (MGMT) tasked with repairing the DNA damage inflicted by temozolomide. It is now possible to screen for the methylation status of the gene encoding for MGMT, and its silencing predicts a favorable temozolomide response whereby the tumor can less effectively escape the DNA damage inflicted by the drug. Note that all patients have the same MGMT gene, yet only a small minority have MGMT epigenetically silenced through methylation.

7.3 Epigenetics and Epilepsy

Epilepsy is a very heterogeneous disease, but is commonly associated with unprovoked recurring seizures. It is generally assumed that an imbalance of GABAergic inhibition and glutamatergic excitation is responsible for seizures, and the general therapeutic strategy is to rectify this balance. One of the most effective drugs to treat seizures, sodium valproate (or valproic acid), has been assumed to show its anti-convulsant properties by enhancing GABAergic inhibition. Surprisingly, however, the therapeutic effect of valproate, while immediate, increases with prolonged use. This is difficult to explain on the basis of GABAergic signaling alone. The solution to the mystery may be an unexpected activity of valproate, which is also a potent HDAC inhibitor. As HDAC activity causes transcriptional repression, inhibition of this enzyme relieves this transcriptional activation, possibly affecting many genes. Consistent with a predominantly epigenetic effect of valproate is the finding that hippocampal neurogenesis, commonly blamed for the cognitive changes associated with epilepsy, is suppressed by valproate. This also opens the possibility that epilepsy is associated with altered epigenetic marks, particularly those affecting the histone association of chromatin, possibly due to aberrant HAT or HDAC function. Valproate is also effective as a mood stabilizer in certain neuropsychiatric conditions such as posttraumatic stress disorder, and may similarly affect epigenetic marks that contribute to aberrant gene expression in these conditions.

7.4 Epigenetic Role in Anxiety and Depression

Our understanding of neuropsychiatric illnesses, particularly depression, is considerably less developed than that of neurodegeneration, epilepsy, and brain tumors. In Chapter 12 we highlight the complex nature of depressive disorders that, in spite of a near absence of known genetic risk factors, are still quite heritable. Depression seems to run in families, with an ~15% chance of a family member developing depression if any first-degree relative has also been affected. Is it possible that affected
individuals share epigenetic marks inherited from their parents that predispose the family to depression? Given several related studies on the heritability of maternal stress and childhood neglect, this seems quite plausible.\textsuperscript{23}

The notion that stress can cause trans-generational changes in behavior was first demonstrated in rats. Offspring of “bad” rat mothers that rarely licked or groomed their pups showed significantly elevated anxiety later in life than pups raised by “good” caring mothers. The latter not only showed lower anxiety but also had decreased expression of the glucocorticoid “stress” receptors that respond to the adrenally released stress hormone corticosterone (equivalent to human cortisol). The reduced expression of corticosterone receptors was the result of gene silencing by DNA hypermethylation.\textsuperscript{24} While increased anxiety in mistreated or neglected offspring may be somewhat intuitive and expected, it was surprising to note that the epigenetic marks responsible for the differences in anxiety were passed on for several generations. Therefore, maternal neglect can have lasting effects not only on the mother’s direct offspring, but also on future generations of her grandchildren as well.

While in the above example the epigenetic marks were the result of a life experience, namely stress or neglect, one must consider the possibility that similar epigenetic marks may arise by chance. As a consequence, some of us may be more resilient to stress than others and some may be more likely to develop depression. These differences in epigenetic marks may well explain why monozygotic twins often differ dramatically in their resilience to stress or their susceptibility to depression.

We are only beginning to understand the profound effects that epigenetic changes may have on human mood and behavior and how life experiences may cause heritable changes in our emotional state without ever causing direct changes to a person’s DNA. The potential consequences are both fascinating and frightening. They certainly open a new avenue for therapeutic intervention that may reversibly turn gene expression on and off simply by altering epigenetic marks.

8. NON-CELL-AUTONOMOUS MECHANISMS

Since Theodor Schwann’s nineteenth-century discovery that cells are independent biological entities that function autonomously, we assumed that diseases, too, present in a cell-autonomous fashion. That is, we expected that neurological disorders resulted from the death or functional change of a defined population of neurons. Hence, these neurons alone are responsible for the disease. As an example, once mutations in the SOD1 gene were found to cause 20% of familial ALS, we assumed that SOD1 causes selective motor neuron loss by impairing the biology of this population of cells and this in turn causes the characteristic motor weakness. This is a reasonable assumption that we now know not to be true.

The gold standard for establishing a single gene as disease causing has been the introduction of the mutated gene into the germline of transgenic mice. This yielded valuable disease models for ALS, where various point mutations in the SOD1 gene produced motor neuron death and shortened life span comparable to human ALS. Similarly, germline expression of mutated tau causes hyperphosphorylation of the tau protein and replicates important aspects of AD. Likewise, the expression of the mutated MECP2 gene responsible for Rett syndrome largely mimics disease in mice. However, we have largely neglected the fact that in each of these instances, the mutated genes are expressed in essentially all cell types of body and brain. Could the mutated genes affect other cells that may contribute to disease? Could these non-neuronal cells possibly be equally or even more important in
disease etiology? This indeed appears to be quite commonly the case. As a result we must radically change our viewpoint from a cell-autonomous to non-cell-autonomous cause of disease. Instead of mutations affecting just a single neuronal population, we must consider their effects on all cell types in the brain and elsewhere in the body as well. This is particularly true for the glial support cells, astrocytes, oligodendrocytes, and Schwann cells, as well as microglia acting as innate immune cells of the brain. In addition, even other seemingly unrelated cells, such as the endothelial cells of the cerebral blood vessels or bone marrow-derived macrophages, may contribute to certain neurological diseases in a non-cell-autonomous fashion.

8.1 Microglia and Disease

In ALS, approximately 20% of all familial cases are caused by mutated SOD1, presumably through toxic protein aggregates, mitochondrial impairment, and possible transcriptional regulation. Overexpression of mutated SOD1 fails to produce disease in mice, unless the mutated SOD1 is also found in microglial cells. Interestingly, selective ablation of mutated SOD1 only in microglial cells (or astrocytes) while leaving the wild-type protein in neurons significantly attenuates the severity of disease and slows its time course.

An even more striking example is Rett syndrome. As discussed in the previous paragraph, the transcriptional regulator MeCP2 changes the expression of a number of downstream genes, including BDNF, causing a progressive degeneration of cognitive function, language, and ambulation, along with peculiar changes in breathing in mice. These phenotypes are accurately reproduced by germline mutation of the MECP2 gene in mice. However, only correcting the mutated gene in microglial cells while leaving the mutated gene in neurons is sufficient to rescue mice from the disease.25

Given our broadening understanding of microglial cells in synaptic development, maintenance, and function, their role in neurological disease should not be that surprising after all.26 It is now clear that microglial cells release factors that are involved in synapse formation and maintenance of synaptic connectivity. During development, pruning of synapses adjusts the number of synapses to match the activity between two cells. It turns out that microglial cells are the effector cells that execute this activity-dependent pruning.27 They do so in a classic complement-dependent fashion, whereby microglial cells produce C3 proteases that lyse synapses tagged with the complement protein C1q. Consequently, impaired synaptic pruning by microglial cells may potentially alter synaptic connectivity in neurodevelopmental disorders like Rett syndrome and perhaps other disorders as well. Indeed, a lack of C1q expression on neurons is sufficient to generate epileptic seizures due to impaired developmental synaptic pruning by microglia.28

8.2 Astrocytes and Disease

For ALS, HD, and Rett syndrome, astrocytes appear to play important roles in disease. In ALS, disease is less severe when the mutated SOD1 protein is corrected in astrocytes. In addition, there appears to be a defect in astrocytic glutamate transport, as the principal transporter EAAT2 shows reduced expression in autopsy tissue from ALS patients and is similarly absent in mouse models of ALS. Yet, simply restoring normal EAAT2 expression in astrocytes can attenuate disease severity and prolong the life of mutant mice, suggesting that a breakdown of astrocyte Glu homeostasis plays an important role in ALS.

In Rett syndrome, the astrocytic MECP2 mutation appears to contribute significantly to disease. When MECP2 function was selectively restored in astrocytes, the dendritic morphology of neurons normalized, presynaptic levels of Glu increased, and even the respiratory abnormalities
improved. Most importantly, the animals lived for over 7.5 months compared to only 10 weeks. How one can explain the astrocytic protection from these diseases, however, remains unclear.

One important role of astrocytes is the regulation of extracellular K+, and this typically occurs through diffusional uptake of K+ via the Kir4.1 K+ channel encoded by the KCNJ10 gene. Changes in [K+] occur with every AP during the repolarization phase, when K+ leaves the cell. Accumulation of extracellular K+ is most pronounced in the vicinity of neurons that fire at high frequency. In HD, astrocytic Kir4.1 expression is significantly reduced in the striatum, which contains medium spiny neurons that fire constantly at high frequency. As a result, extracellular K+ is higher than normal. Restoring normal Kir4.1 expression in astrocytes through viral delivery of the gene is sufficient to attenuate motor dysfunction and prolong life in a mouse model of HD.

While these examples illustrate a co-dependence of neurons on support cells, with somewhat defined sharing of responsibility, we do not yet understand the complex interaction between different populations of brain cells. Instead, it is clear that in addition to finding genes that cause disease, we must also ask in which cell types these genes function. We must expect that the same gene mutation may affect neurons, glia, and vascular cells differently, and that the sum of these effects are needed to explain the disease phenotype. This notion is commonly referred to as “non-cell-autonomous” mechanisms of disease.

9. INFLAMMATION

An inflammatory response is a common characteristic shared among all nervous system disorders. Inflammation is normally a transient process that helps wound clearance and repair, yet in some neurodegenerative diseases inflammation can become a persistent pathological response. Brain inflammation primarily engages microglia and astrocytes, which together form the brain’s innate immune system, but occasionally blood-derived immune cells, macrophages, and lymphocytes contribute as well.

Along with macrophages, microglia share the capability to engulf cells and debris, and are thus often called the resident macrophages of the brain. However, their lineages diverge early in embryonic life when yolk-derived primitive precursor cells give rise to microglia that take residence in the developing nervous system long before the formation of the brain’s vasculature. In their unactivated state, microglial cells are ramified and process-bearing in appearance and constantly patrol the brain for evidence of damage or injury. Once microglia sense injury, they become activated and change their appearance to an ameboid shape (Figure 10). In this activated state, microglial cells are tasked with clearing debris and initiate a healing response by secreting proinflammatory molecules, including TNFα, interferon γ, interleukin 1β, nitric oxide (NO), and ROS. As injury resolves, microglial cells release anti-inflammatory factors that facilitate healing such as insulin-like growth factor 1 and interleukins 4 and 10. Microglia express complement proteins and can present antigens to blood-borne immune cells for the production of antibodies. They are even capable of eliminating entire neurons that are weakened, yet still functioning, in a process called “phagoptosis.”

Astrocytes play an important role in containing sites of injury through the formation of a scar. Akin to the tenacious tissue that seals the skin after an insult, astrocytes seek to compartmentalize injured and normal tissue. At the scar, they appear morphologically transformed, with thickened intertwined processes, and are called reactive astrocytes. Like microglial cells, they participate in the inflammatory response and may indeed regulate the microglial response through the release of cytokines and chemokines.
9. MICROGLIAL ACTIVATION IN PRIMARY NEURODEGENERATIVE DISORDERS

While acute inflammation is common in trauma, stroke, and infections, neurodegenerative diseases are typically associated with persistent, low levels of inflammation that presents similarly for all classical neurodegenerative diseases, AD, PD, HD, and ALS. Common among them is a progressive accumulation of protein aggregates, often preceding disease onset. These aggregates are perceived as foreign substances by microglial cells and astrocytes, which recognize these molecules through the family of Toll-like receptors (TLRs). These are cell-surface receptors that detect structurally conserved molecules typically produced by microbes. Different subtypes of TLRs have well-defined substrate specificity. For example, bacterial lipopolysaccharides are recognized by TLR4, viral DNA is recognized by TLR3, while TLR1 and TLR2 recognize bacterial peptides. Protein deposits including amyloid plaques are primarily detected via TLR4 (Figure 10).

Microglia and astrocytes sense neuronal injury through purinergic P2X7 receptors that are activated by ATP released from injured or dying neurons. In response to either TLR or purinergic activation,
microglia, and to a lesser extent astrocytes, release proinflammatory molecules (TNFα, IL-1β, MCP-1, NO, ROS). These recruit additional microglial cells, astrocytes, and even invading macrophages and T cells to the disease or injury site. Astrocytes can increase the number of microglial cells by stimulating their local proliferation through the release of colony-stimulating factor 1 (CSF1), a growth factor for microglial cells.

In autopsy tissue from AD patients, activated microglia and reactive astrocytes surround amyloid plaques and stain positive for many proinflammatory mediators, including MCP-1, TNFα, IL-1β, and IL-6. Microglial cells detect the presence of Aβ plaques via TLR4 receptors, causing plaque removal by phagocytosis. This suggests that microglial cells make every effort to clear the potentially toxic protein aggregates, and under these conditions, microglia may well be called neuroprotective microglia (Figure 11). However, the clearance of Aβ ultimately fails and plaque burden increases, resulting in a sustained activation of microglia and astrocytes by the plaque. These chronically activated microglial cells now produce ROS molecules, particularly toxic superoxide radicals (O2-) and hydrogen peroxide (H2O2). Superoxide radicals are generated by NADPH oxidase, a membrane-bound enzyme that transfers electrons to molecular oxygen. Superoxide radicals kill bacteria and fungi and are released together with hydrogen peroxide in a “respiratory burst” to kill pathogens. In so doing, these chronically activated, ROS-producing microglial cells also harm adjacent neurons, and can therefore be regarded as neurotoxic microglia.

Abundant evidence suggests that neurotoxic microglia cells contribute to neurodegenerative disease (see schematic in Figure 11). For example, the expression and activity of NADPH oxidase are upregulated in AD and in PD, where its activity contributes to the loss of dopaminergic neurons. In sporadic and familial ALS, the mutation of SOD1, found in 7% of patients, increases the activity of NADPH oxidase, and the increased production of ROS is a likely contributor to the progressive motor neuron death.

As neurons die, they release ATP, which activates P2X7 purinergic receptors on microglial cells, keeping them in their ROS-producing neurotoxic state. Pharmacological inhibition or genetic ablation of the P2X7 receptor suppresses the neurotoxic response of microglial cells, potentially providing a novel avenue to specifically suppress the activation of neurotoxic microglial cells.

Similar microglial activation can be seen in essentially all CNS insults ranging from infection to trauma. In each of these conditions, neuroprotective and neurotoxic properties have surfaced, and it is likely that both operate at the same time. The eventual outcome, i.e., whether neuroprotective or neurotoxic properties prevail, is determined by the various receptor systems on microglial cells that become activated. One important commonality to remember is that microglial cells are one of the most important sources of toxic ROS molecules in the nervous system. Their ROS production is normally beneficial, as it is required for fighting pathogens. It is their chronic activation, in the absence of foreign pathogens, that turns them from friend to foe in neurological disorders.

9.2 Astrocytes and Inflammation

Although astrocytes are not immune cells, they too become activated after injury and during disease, visible by a change in their overall appearance and by an increase in the expression of GFAP. These morphological changes are typically called reactive gliosis and are often associated with the formation of a scar, a physical barrier that seals off a site of injury (Chapter 2, Box 3). The mechanical containment of an injury site by a physical barrier may be considered one of the important contributions of astrocytes to injury and chronic disease. Such scars can be transient or permanent, depending on the insult, and can also be beneficial or detrimental. In spinal cord trauma, for example, the scar has been suggested to be an impediment to axonal regrowth,
yet suppression of scarring increases the size of the lesion. In mesial temporal lobe epilepsy, glial scarring appears to contribute to epileptic seizures, which do not stop until the scar is surgically removed. Importantly, astrocytes also contribute to inflammation through the release of the very same cytokines and chemokines, both proinflammatory and anti-inflammatory, that we already discussed for microglial cells above. Astrocytes use these signals to amplify the initial inflammatory response and recruit additional microglial cells and astrocytes to a site of injury or disease. Reactive astrocytes express major histocompatibility complex class II and can present

FIGURE 11 Inflammation in AD. Amyloid-β peptide, produced by cleavage of amyloid precursor protein (APP), forms aggregates that activate microglia, in part by signaling through Toll-like receptors (TLRs) and RAGE. These receptors activate the transcription factors NF-κB and AP-1, which in turn induce the production of reactive oxygen species (ROS) and drive the expression of inflammatory mediators such as cytokines. These inflammatory factors act directly on cholinergic neurons and also stimulate astrocytes, which amplify proinflammatory signals to induce neurotoxic effects. Apoptosis and necrosis of neurons result in release of ATP, which further activates microglia through the purinergic P2X7 receptor. Microglia can also play protective roles by mediating clearance of Aβ through ApoE-dependent and ApoE-independent mechanisms. Cholinergic neurons in the basal forebrain, the neurons that are primarily affected in AD, are presumed to be important targets of inflammation-induced toxicity, but other types of neurons, such as glutaminergic and GABAergic neurons, may also be affected. Reproduced with permission from Ref. 34.
antigens such as plaque fragments to invading immune cells. Overall, however, their role is most similar to that of an orchestra conductor: fine tuning the immune response though the release of modulator signals, assisting in the removal of glutamate and potassium, clearing edema, and protecting the integrity of the vasculature.

9.3 Blood-Derived Cells and Inflammation

T cells are major contributors to inflammation. Subpopulations of T cells are specifically recruited to sites of injury and invade the CNS. T cells recognize foreign antigens and become activated. In turn, they recruit cytotoxic T cells through the release of inflammatory cytokines. They can also attract B cells to the brain. B cells then generate antibodies that can induce complement-dependent lysis of proteins or cells. In the normal brain, blood-borne immune cells are absent, and their presence in the cerebrospinal fluid is an indication of brain inflammation and disease. Acute injuries that breach the BBB allow the rapid entry of blood-borne immune cells, many of which contribute to the resolution of the injury. However, in many neurological disorders, infections, and neurodegenerative diseases, the integrity of the BBB is lost and lymphocytes and macrophages enter the brain. In multiple sclerosis, the entry of auto-activated T cells is believed to cause disease. In stroke and CNS trauma, the entry of blood cells is an inevitable and uncontrolled by-product, yet in most other disease conditions, the entry of blood-borne immune cells is viewed as disease ameliorating, with bacterial meningitis being an excellent example.

9.4 Directing the Immune Response to Treat Disease

A universal component shared among all neurological disorders is the very presence of an immune response. This is characterized by the activation of the innate immune cells of the brain, both microglia and astrocytes. Both sense foreign particles via Toll-like receptors and dying cells via purinergic receptors. They jointly try to kill pathogens and engulf and remove debris. Astrocytes seal off the acute lesion and release cytokines and chemokines that guide the process, with different molecules operating during different phases of the immunological response. The uncontrolled respiratory burst causing the release of toxic ROS molecules to fight pathogens causes neurons to die, most likely as bystanders rather than targets.

Given how commonly inflammation accompanies nervous system diseases, it is easy to envision how inflammation contributes in a negative way to disease. However, this would be overly simplistic. As with systemic inflammatory responses, one must assume that the tissue repair ultimately serves the purpose of healing rather than destruction. Abnormal inflammation, as is the case in the autoimmune attack of the myelin by activated T and B cells in multiple sclerosis, is a different matter. Here, inflammation is a major pathological component central to disease etiology. Otherwise, however, the universally observed inflammatory response provides an opportunity to ameliorate disease by instructing the innate immune system to be reparative rather than destructive. Thus far, our knowledge is insufficient to do so, but this will certainly change with future research. At present, anti-inflammatory approaches have been attempted across the spectrum of neurological diseases, but by and large they have not been successful. Among the more promising examples is the antibiotic minocycline, which attenuates microglia activation. It is being explored in active clinical trials for just about every neurological disease ranging from bipolar depression to ALS (see www.clinicaltrial.gov).

10. VASCULAR ABNORMALITIES

We have already repeatedly emphasized the brain’s reliance on constant delivery of oxygen and glucose to provide sufficient energy substrates to sustain brain function. Even brief
or focal disruptions can cause irreversible cell death, with stroke and trauma being examples in which vascular occlusions or ruptures directly cause disease. However, more subtle changes in the cerebral vasculature also have the potential to significantly contribute to disease. We must consider several different functional disruptions that may occur singly or in combination, including ischemia, vascular breach and edema, and breach of the BBB with entry of toxic molecules and cells.39

Unlike the vasculature of peripheral organs, the cerebral vasculature is completely impermeable to cells and water-soluble molecules, thereby separating the blood from the cerebrospinal fluid of the brain through the so-called BBB, which we discussed more extensively in Box 2.3, Chapter 2. A major role of the BBB is to keep immune cells from entering the brain and restrict entry of potential toxic molecules such as glutamate or albumin, which are toxic to neurons.

The integrity of the BBB depends on the continuous presence of pericytes and/or astrocytes on the vascular endothelial cells that form the vessel walls. Loss of either of these cells has been shown to decrease the expression of tight junction proteins (TJPs) that glue adjacent endothelial cells together. The loss of TJPs in turn opens microscopic spaces in the vessel wall through which molecules and even blood cells can enter. Protein deposits such as amyloid on blood vessels, or the release of inflammatory molecules and ROS from neurons and microglia in the context of disease, each weaken the BBB. Once the BBB breaks down, toxic molecules such as albumin, thrombin, and plasmin enter the brain. Plasmin degrade the laminin basement membrane, further accelerating vascular degeneration. Ultimately, red blood cells enter through microbleeds, allowing hemoglobin and iron, which are both toxic to neurons, into the brain. These various vascular dysfunctions are schematically illustrated in Figure 12. With a breach of the BBB, serum enters the brain, causing vasogenic edema or swelling of the brain parenchyma. As the integrity of the vessel walls fails, focal ischemia results, thereby depleting energy substrates. This energy depletion affects both neurons and endothelial cells, as each requires ATP to maintain ion and amino acid transporters.

The above scenario plays out to some degree in nearly all neurological illnesses, albeit in some diseases more visibly than in others. A breach of the BBB following stroke or trauma is obvious. However, bacterial or viral infections similarly cause inflammation that induces transient opening of the endothelial BBB. As a result, fluid enters the brain, causing tissue swelling or edema. This can also be observed around brain tumors, where newly formed blood vessels lack tight junctions and thus fluid can enter the peritumoral tissue. Moreover, gliomas associate with the existing brain vasculature, thereby causing a focal breach of the BBB through which immune cells and blood-borne molecules such as glutamate can enter.40

Vascular abnormalities are also prominent in AD, vascular dementia, ALS, and MS,39 where the expression of TJPs that form the BBB decreases. This may be in part due to the activity of extracellular matrix-degrading enzymes such as metalloproteinases (MMPs), which are employed in tissue remodeling. The TJPs are substrates for proteolytic cleavage by MMPs.

An important consideration is the fact that pericyte numbers decrease with age in the normal brain, and consequently their trophic support for the vasculature and the integrity of the BBB wanes. Age alone, therefore, contributes to progressive vascular changes, visible by a reduction in blood flow that can be detected in normal aging individuals by PET.41 Interestingly, people carrying the ε4 allele of the APOE4 apolipoprotein, the major risk factor for AD, show enhanced regional decline in cerebral blood flow compared to people without this allele.42 Amyloid deposits are often found in close association with blood vessels, and may directly contribute
Indeed, noninvasive imaging of cerebral blood flow suggests that amyloid burden correlates with the degree of reduction in cerebral blood flow in presymptomatic patients carrying disease-causing PS1 or APP mutations. To what extent the above age- or pathology-related changes in the vasculature are contributing to disease or are the consequence of disease remains controversial. Clearly, we must consider vascular health as an absolute requirement for healthy aging, and strategies to accomplish this require a better understanding of the brain–vascular interactions. We must particularly improve our understanding of the role of astrocytes and pericytes in maintaining a healthy endothelial vessel wall with intact TJPs. This may provide an untapped opportunity for the future development of drugs that could be beneficial across all neurological illnesses.

**11. BRAIN-DERIVED NEUROTROPHIC FACTOR**

Following the accidental discovery that nerve growth factor (NGF) stimulates the outgrowth of sympathetic sensory nerve fibers in the 1950s, over 50 additional growth factors have been described in the nervous system. They can be divided into five families, namely the neurotrophins, cytokines, transforming growth factor-β, fibroblast growth factor, and insulin-like...
growth factor families. Most growth factors show a degree of cell type specificity, and most operate throughout an individual’s life span. With regard to diseases of the nervous system, BDNF appears to play a particularly prominent role in many of the nervous system disorders, and therefore deserves some expanded discussion.43

**Normal Brain-Derived Neurotrophic Factor Signaling**

Identified in 1982, BDNF is a member of the neurotrophin family, which also includes NGF, neurotrophin-3 (NT3), and neurotrophin-4 (NT4). BDNF is synthesized and released as a 247 amino acid precursor molecule. Following an initial cleavage by proteolytic enzymes such as furin, the pro-BDNF is packaged into secretory vesicles and released into the extracellular space (Figure 13). After its release, the pro-BDNF is further proteolytically cleaved by plasmin or MMPs, giving rise to the mature BDNF. Mature BDNF binds with high affinity and specificity to the tropomyosin-related kinase type B (TrkB) receptor (Figure 14). The TrKB receptor is a tyrosine kinase that mediates its activity by phosphorylation of tyrosine residues on downstream signaling molecules, eventually activating the external receptor kinases ERK1/2 or AKT. Both mature and proBDNF also bind with low affinity to the p75 receptor, a member of the tumor necrosis factor family. The function of p75, which is not a kinase, is less well

**FIGURE 13** BDNF secretion in the CNS. (A) ProBDNF may be processed to mature BDNF by several cellular mechanisms. ProBDNF can be cleaved within the endoplasmic reticulum by furin (1) and in regulated secretory vesicles by proconvertase enzymes (2). If proBDNF reaches the extracellular milieu, it can be processed by plasmin, and the mature BDNF produced can then activate cell-surface TrkB receptors (3). Alternatively, extracellular proBDNF can bind p75NTR and become endocytosed and then cleaved to produce mature BDNF that either activates TrkB within endosomes (5) or is recycled to the cell surface (6). (B) The site of BDNF translation within the neuron may determine the form of BDNF released. BDNF mRNA with a short 3′ UTR accumulates in the neuronal soma, whereas BDNF mRNA with a long 3′ UTR is trafficked to dendrites. The soma supports BDNF cleavage within the Golgi, but the majority of dendrites lack Golgi elements necessary for processing of proBDNF, and therefore proBDNF may be the predominant form released. Reproduced with permission from Ref. 44.
understood. Some research suggests that it may be a decoy receptor that sequesters BDNF if there is too much present. Importantly unlike TRKB, p75 is promiscuous and binds all of the neurotrophins including NGF, NT3, and NT4.

BDNF may be described as a reward factor. BDNF is released in an activity-dependent fashion; the more active a neuron, the more BDNF is synthesized and released. On adjacent cells it enhances dendritic branching and induces growth of spines. Continuous signaling assures the survival of postsynaptic neurons. The presence of BDNF has also been shown to alter synaptic plasticity; most importantly, BDNF enhances LTP, the cellular substrate for learning.

BDNF plays a major role in Rett syndrome, Huntington disease, Parkinson disease, Alzheimer disease, and ALS, and in every case the

**FIGURE 14** Receptors for brain-derived neurotrophic factor (BDNF). BDNF binds with high specificity to the tropomyosin-related kinase receptor type B (TrKB) and to the low-affinity neurotrophin receptor p75. “Mature” BDNF binds with greatest affinity to TRKB, whereas proBDNF binds with a higher affinity to p75. TRKB predominantly supports neuronal survival and expression of several functional genes, including extracellular signal-regulated kinases (ERKs) AKT and cyclic AMP-responsive element-binding protein (CREB). By contrast, separate p75 activation results in proapoptotic signaling. BDNF binding to the transmembrane TRKB leads to dimerization and autophosphorylation of tyrosine sites adjoining the cytoplasmic carboxy-terminal domain; this in turn activates several adaptor proteins that ultimately activate AKT, thus enhancing cell survival. Activation of PLCγ1 results in the generation of inositol-1,4,5-triphosphate (InsP3) and diacylglycerol (DAG), which results in the mobilization of calcium stores and activation of calcium-dependent protein kinases that influence synaptic plasticity.
loss of BDNF causes a reduction in neural activity, LTP, and even cell death. Moreover, BDNF signaling is important in acute injury and trauma, and genetic changes suggest that aberrant BDNF signaling may contribute to depression.

Brain-Derived Neurotrophic Factor and Rett Syndrome

BDNF appears to be the major disease target in Rett syndrome, which is caused by de novo mutations in the MeCP2 protein. The MeCP2 protein regulates gene expression by binding to methylated CpG dinucleotide sequences on chromosomes. After binding to the transcriptional repressor site, MeCP2 recruits HDACs that induce a compaction of chromatin. Chromatin compaction makes the gene inaccessible for transcription. This process particularly affects the BDNF gene, which becomes constitutively repressed by MeCP2 binding to one of its four promoters. In the normal brain, BDNF is transcribed in an activity-dependent manner, whereby neuronal depolarization allows Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels. This Ca\(^{2+}\) influx causes CaMKII-mediated phosphorylation of MeCP2 at serine residue 421, dislocating it from the BDNF promoter, thereby activating transcription. Functional loss of MeCP2 in Rett syndrome therefore impairs the activity-dependent release of BDNF, impairing its effect on learning and dendritic development. Indeed, one of the pathological features of Rett syndrome is a reduced number of abnormal, dysmorphic spines, a phenotype that can be completely rescued by restoring normal BNDF release.\(^{45}\)

Brain-Derived Neurotrophic Factor in Huntington Disease

The main disease pathology of HD is the progressive loss of medium spiny neurons in the striatum. These glutamatergic neurons are part of the indirect movement control pathway that fine-tunes voluntary movements, and their loss causes the abnormal chorea movements of affected patients. Striatal neurons rely on the continuous supply of BDNF they receive from layer 5 cortical motor neurons. In these motor neurons, wild-type huntingtin protein promotes the transcription of BDNF by binding to exon II of the BDNF promoter via REST, a transcriptional repressor. This causes the activation of the BDNF promoter and stimulates BDNF transcription. The synthesized BDNF is then axonally transported through the corticostriatal pathway and released onto the striatal neurons as a trophic factor. In HD patients, the mutated huntingtin protein is unable to promote BDNF transcription, resulting in a loss of BDNF to be released at the corticostriatal synapses. Moreover, protein inclusion bodies disrupt the axonal transport of BDNF to the target synapse. In turn, the striatal neurons involved in controlling voluntary movements are deprived of BDNF and are gradually lost, causing the characteristic movement symptoms.

Brain-Derived Neurotrophic Factor in Parkinson Disease

In PD, the neurons most affected by a loss of BDNF are the same striatal medium spiny neurons mentioned above for HD, but the dopaminergic neurons in the substantia nigra are lost as well. BDNF protein expression is reduced in the midbrain of patients with PD. In monkeys, PD-like symptoms can be induced by infusion of the chemical 1-methyl-4-phenylpyridium, which causes a lesion of the striatum. Subsequent infusion of BDNF rescues both the anatomical and behavioral effects, suggesting that treatments that restore normal BDNF signaling may ameliorate disease in PD patients.

Brain-Derived Neurotrophic Factor in Alzheimer’s Disease

In AD, a progressive loss of cholinergic neurons throughout the cortex underlies the relentless dementia. The synaptic loss in the hippocampus impairs both short- and long-term memory, particularly spatial memory, which is localized in the hippocampus. BDNF is normally produced
by neurons in the entorhinal cortex immediately adjacent to the hippocampus, and is then transported to the hippocampus and released in an activity-dependent manner. A reduction in BDNF can be seen in the entorhinal cortex and the hippocampus in Alzheimer disease. The infusion of BDNF into the hippocampus rescues hippocampal-dependent learning and memory in mouse and monkey models of AD. Ultimately, clinical studies are needed to examine whether an infusion of BDNF or a BDNF mimetic is feasible and efficacious in patients suffering from AD.

**Brain-Derived Neurotrophic Factor and Amyotrophic Lateral Sclerosis**

ALS is characterized by the progressive and selective loss of upper and lower motor neurons in the cortex and spinal cord, causing progressive muscle weakness and paralysis. Approximately 6% of familial cases of ALS present with mutations in the RNA/DNA binding protein fused in sarcoma (FUS). Mutated FUS leads to a splicing defect of the mRNA for BDNF, causing impaired TRKB-mediated BDNF signaling, which likely contributes to the neuronal cell death. Unfortunately, thus far, attempts to supply exogenous BDNF to ALS patients in clinical studies have failed, most likely because BDNF does not reach its target neurons in sufficient quantity.

**Brain-Derived Neurotrophic Factor and Depression**

Depression is among the least well-understood neuropsychiatric disorders. However, one of the few clues we have regarding genetic causes of depression is a SNP in the BDNF gene, which makes individuals more prone to develop depression. More specifically, if the normal valine at position 66 of the BDNF gene is substituted with a methionine, the resulting Met allele (Val-66Met) is less efficiently packaged into secretory vesicles and therefore causes a reduction in activity-dependent BDNF release. Individuals with at least one Met allele have a 50% reduction in the wild-type allele and are at increased risk to develop depression or related affective disorders, suggesting a strong causative role of BDNF signaling in depression. BDNF and TrkB are abundantly expressed throughout the limbic pathways, where activity-dependent BDNF release is believed to contribute to a regulation of emotion. Interestingly, the most effective drug to treat bipolar depression, lithium, has been shown to exert some of its therapeutic benefit via a stimulation of BDNF release from presynaptic terminals. Stress decreases BDNF levels, yet chronic treatment with antidepressant drugs can restore BDNF-mediated signaling, suggesting an additional important role for BDNF as modulator for chronic stress and depression. Note that there is a rich literature on the mood-stabilizing effect of exercise, which suggests that this effect is at least partially mediated via increased release in BDNF.

**Structural Effects**

In addition to these neurodegenerative and neuropsychiatric diseases, acute insults such as stroke and CNS trauma, too, may involve disruption of BDNF signaling or may at least benefit from BDNF supplementation. In the case of spinal cord injury, for example, animal models clearly demonstrate that BDNF enhances axonal sprouting and regeneration. In CNS trauma and stroke, a recovery of function by sprouting processes and enhancing synaptogenesis will certainly benefit recovery. In mouse models of ischemia, infusion of BDNF has been shown to reduce infarct size and speed up recovery of function.

**Feasibility of Brain-Derived Neurotrophic Factor Therapy**

While the cellular mechanisms whereby a deficit in BDNF is somewhat disease specific, a bridging theme is that inadequate BDNF signaling consistently causes impaired neuronal signaling and, commonly, progressive cell death. Therefore,
it seems that a strategy that could employ the supplementation of BDNF from an exogenous source would be almost universally beneficial. This idea, as mentioned above, is supported by studies in mice and nonhuman primates, but has yet to be done in human subjects. Several major challenges exist. First, it is difficult to produce sufficient BDNF in the test tube for treatment. One approach taken to overcome this issue is the production of mimetics, which are artificial peptides that are chemically different from BDNF but bind to the TrKB receptor. Ideally, mimetics have some enhanced properties; for example, they are more diffusible or have a higher affinity for the TrKB receptor. One of these mimetics, LM22A-4, directly activates the same receptor as BDNF (TrkB) and is being pursued to treat Rett syndrome.45

The second challenge is the difficulty of delivering BDNF reliably and in high enough quantity to the targeted neurons, in part because the BDNF molecule diffuses poorly in tissue and does not cross the BBB. To overcome these challenges, gene delivery systems are being developed that package the BDNF gene into adeno-associated viruses, for example, and a number of clinical trials using gene delivery of BDNF are in advanced stages of planning.

As mentioned above, lithium enhances BDNF release in depression, and has been shown to cause a 30% increase in BDNF in patients with mild AD. With this rationale, and the overall safety of lithium, it is now being studied in clinical trials for AD (NCT00088387) and ALS (NCT00790582). Another strategy, pursued in Rett syndrome, is to activate the downstream targets activated by BDNF (Akt, PI3K, and MAPK), using a recombinant insulin-like growth factor 1 (IGF1) currently in clinical trials (NCT01777542).

Remember, however, that one reliable way to augment BDNF levels in the nervous system is exercise. Clearly, once disease is established, an individual may no longer be able to exercise, or exercise may no longer be able to supply sufficient BDNF. Moreover, if the BDNF gene is silenced, for example via a MECP2 mutation, it is unlikely that exercise can solve the problem. However, much of the beneficial effect of exercise in neurological disease may in fact be attributable to the positive effects of BDNF on neuronal health. In lectures to lay audiences, I like to refer to BDNF as an endogenously produced “multivitamin” for the brain. While overly simplistic, this notion may not be far from the truth.

12. CHALLENGES AND OPPORTUNITIES

This quick round-up of common disease mechanisms makes several important observations that are beginning to inform our approach to research and may ultimately change the development of new therapeutic strategies. Most importantly, we have become less neurocentric, and instead are beginning to consider the contribution of non-neuronal cells, and particularly vascular cells, glia, and microglia as well. Neuron-targeted therapies will ultimately fail as they only treat one of the affected cell types. This complicates matters, as the same genetic mutations may have different biological consequences in neuronal and non-neuronal cells.

Mitochondrial health is taking center stage, as these organelles not only fuel all brain activity but also function as modulators of intracellular second messengers, such as Ca2+, NO, and ROS. Additionally, mitochondria also function as arbiters of cell death. Development of drugs that target mitochondria may provide novel ways to protect neurons and non-neuronal cells alike.

Our understanding of inflammation has evolved. It has lost its exclusive negative connotation as we are beginning to see the many positive contributions of inflammatory cells and processes to disease. Once we learn to specifically redirect the brain’s immune cells to their neuroprotective state we may be able to harness the endogenous healing powers of our body to attenuate disease.

Finally, the genetics of disease, believed to be the “holy grail” necessary for our understanding
of disease, continues to frustrate us. The majority of nervous system disorders have complex polygenetic causes, convoluted by superimposed epigenetic regulation. However, there is no doubt that given time, we will gain a handle on the molecular genetics and epigenetics that predispose us to disease. Who said it will be easy? This will take time and patience. However, such insight, particularly as regards our knowledge of epigenetic regulation of disease processes, holds tremendous potential for the development of novel therapeutics to ameliorate or even cure disease.

References


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**References**


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**Suggested Papers or Journal Club Assignments**

**Clinical Papers**


**Basic Papers**


INTRODUCTION

I will start by saying what this article is not about. This is not a commentary about how to perform and interpret an examination of the nervous system. Thus, I will not comment on the importance of obtaining a proper history, or the nuances of eliciting clinical responses as part of the examination. In other words, I will downplay the clinical aspects of my title. Those seeking such information might consider referring to one of many excellent textbooks on the subject.1-3 Rather, I will focus on the application of neuroscience to current questions about diseases of the nervous system. I will emphasize those areas in which my background as a neuroscientist has helped me. Further, I will try to highlight areas where those in neuroscience can, and I hope will, help me as a clinician. This is not an extensive review of many topics; rather, I have focused on a few of the areas with which I am familiar. I am a neurologist, so much of my emphasis is on disorders that fall under the province of neurology. However, I have also included some comments about psychiatry.

As a clinician, I am interested in disease: What can go wrong with the nervous system? The word “disease” or “disorder”, the somewhat softer term we have chosen to use in this textbook, implies a change from a person’s normal state. That change can vary markedly from person to person. For example, I once was asked to evaluate a 70-year-old patient who had been one of the youngest graduates from the Massachusetts Institute of Technology (MIT), at age 14. His family and business associates reported that he was starting to have cognitive problems,
I. AN INTRODUCTION

Of deep brain stimulation (DBS) for a variety of motor and abnormal motor control but also the development have been the basis for understanding not only normal works, originally delineated in the non-human primate, subcortical structures are involved. These motor networks, originally delineated in the non-human primate, subsequently delineated as “Broca’s area”, leads to an expressive aphasia. The concept of specific regions of brain having very specific functions, as happens in the motor system, held until the application of imaging, both computed tomography (CT) and magnetic resonance imaging (MRI), for the correlation of lesions with clinical symptoms. These studies showed that subjects with similar language deficits, including a classical “Broca’s aphasia”, may have markedly different lesions. These observations have enhanced the concept of localization of function so that we now believe that rather than there being specific localization of functions in the brain, there exist networks of function that interrelate not only areas of cerebral cortex, but also deeper brain structures such as thalamus, basal ganglia, and cerebellum. Alterations of these networks are part of the pathophysiology underlying disorders of language, motor function, and consciousness, as well as psychiatric disorders.

The networks of the brain have been more fully established in the motor system, where not only cortical but subcortical structures are involved. These motor networks, originally delineated in the non-human primate, have been the basis for understanding not only normal and abnormal motor control but also the development of deep brain stimulation (DBS) for a variety of motor disorders, particularly Parkinson disease (PD), which will be discussed at length.4

LOCALIZATION OF LESIONS

In classical clinical neurology, the emphasis was on the location of a place in the brain associated with a specific clinical picture. This stems from the pioneering observations of Broca and Wernicke regarding language. Broca, for example, made two observations: people speak with the left side of the brain; and an anterior lesion of cerebral cortex, subsequently delineated as “Broca’s area”, leads to an expressive aphasia. The concept of specific regions of brain having very specific functions, as happens in the motor system, held until the application of imaging, both computed tomography (CT) and magnetic resonance imaging (MRI), for the correlation of lesions with clinical symptoms. These studies showed that subjects with similar language deficits, including a classical “Broca’s aphasia”, may have markedly different lesions. These observations have enhanced the concept of localization of function so that we now believe that rather than there being specific localization of functions in the brain, there exist networks of function that interrelate not only areas of cerebral cortex, but also deeper brain structures such as thalamus, basal ganglia, and cerebellum. Alterations of these networks are part of the pathophysiology underlying disorders of language, motor function, and consciousness, as well as psychiatric disorders.

If you were to ask what has been the primary advance that has changed our approach to the brain, both its normal and abnormal functioning, I would point to the continuing development of imaging of the brain. The major impact of imaging is that we can now study the living brain. The techniques are non-invasive; the subject just lies there and lets the machine do its thing. Whether one is determining the structure of the brain, abnormal growths, such as a tumor, how the brain is functioning, or whether there are abnormal accumulations, as occurs in AD, modern techniques are fast, accurate, safe, and relatively easy to interpret. Further, most results are interchangeable. Studies done in London or Singapore can be analyzed in Baltimore and vice versa.

Structural imaging by CT scanning began in the 1970s, followed by MRI in the 1980s. These techniques made it possible to obtain immediate information about structural abnormalities of the brain and revolutionized our diagnostic approach to stroke, head trauma, and brain tumors. Thus, we could immediately diagnose a patient with a head injury, seizure, or persistent headache. Many emergency rooms now have CT or MRI scanning available. Further advances in MRI have yielded techniques such as diffusion weighted imaging,5 which indicates recent ischemic events in the brain, and diffusion tensor MRI,6 which delineates white matter pathways in the brain.

Functional imaging demonstrates which part of the brain is being used as a subject performs various tasks. Functional imaging studies originally evaluated the use of glucose by positron emission tomography (PET), but now evaluate blood flow by functional magnetic resonance imaging (fMRI). PET still has a role in diagnosis, using labeled ligands to bind to substances in the brain and to receptors, as in the demonstration of Aβ mentioned below.7

SELECTIVE VULNERABILITY OF NEURONAL POPULATIONS

Are the changes in brain function related to changes in the basic abilities of brain systems, or has some external factor disrupted brain processes? In the first category are disease processes in which intrinsic functions of brain cells and systems are altered, such as occurs in neurodegenerative diseases, multiple sclerosis (MS), seizures, or disorders of consciousness and sleep. In the latter category, brain functions are altered by insults that originate
outside the brain such as trauma, infections, effects of radiation, and responses to systemic diseases such as a cardiac arrest or exposure to toxins.

Regardless of type, we seldom encounter a generalized dysfunction of the brain, or “brain failure”, as we do with other organs such as the liver or kidneys. The reaction of the nervous system to both external and internal insults can be surprisingly focused, involving only specific neuronal populations. For example, with a cardiac arrest in which the entire neuronal population has been deprived of oxygen and sustenance, only certain neuronal populations exhibit degeneration, specifically the Purkinje cells in the cerebellum, layers 2, 3, and 5 of the cerebral cortex, or the C-1 portion of the hippocampus, and lack of sustenance from hypoglycemia can have similar specificity (see Chapter 22). Certain viral infections can also lead to specific neuropathology, as in the case of poliomyelitis (see Chapter 27), which affects predominantly anterior horn cells. In genetically determined diseases, in which every cell in the body presumably carries the same gene mutation, only specific sets of neurons may be affected; for example, in a retinal degeneration, only photoreceptor cells; in a hereditary cerebellar ataxia, only Purkinje cells; in Huntington chorea, only neurons in the caudate and putamen. And the specificity need not be with respect to brain region but can also be with regard to neuronal type. For example, although for many years the focus of the neuropathology underlying PD was on the dopamine neurons of the substantia nigra, it now appears that many long, unmyelinated axons are affected both caudal and rostral to the nigra (see Chapter 19).

The neurodegenerative diseases all start by involving specific neuronal populations. Why this selective vulnerability occurs is not known, but it is not limited to humans. There is a reverse side to this question: What protects the non-involved neurons?

**RECOVERY AFTER INJURY**

After acute injuries to the brain there is, in most people, substantial recovery. This recovery is greater in younger people: children recover from an injury that would be devastating to an adult. Recovery is aided by therapy that is specific to the deficit, when started early, but some degree of recovery typically occurs spontaneously.

One of the mysteries in clinical neurology is: How does the brain recover from injury? And why does it sometimes not recover? Two people may have seemingly similar strokes involving the motor area of the right cerebral hemisphere, resulting in the inability to use the left arm and hand. Over the next 3 months subject A makes a slow but consistent recovery, and by 6 months he is using his left hand almost normally. The other, subject B, has made very little recovery, and is still quite disabled at 6 months. What occurred in subject A that did not occur in subject B? There are several possibilities. Perhaps the damage to the motor area was not as great in subject A and the part of the brain that controls the arm and hand was only slightly damaged, and recovered. Another possibility is that some other area of the brain takes over at least some of the functions of the motor area. Perhaps the motor area in the other side of the brain takes over. Or perhaps some area on the same side of the brain that we do not normally associate with motor function steps up.

Dr Steven Zeiler and his colleagues at Johns Hopkins have taken a step towards solving this mystery. In their experiments, they first trained mice to perform the complicated task of reaching for and grasping a food pellet through a narrow slit using one paw. That training took nine to 10 sessions. The next step was to create an artificial stroke, by damaging the motor area controlling the trained paw. As expected, the mice lost their ability to get the food pellets. They next gave the mice “physical therapy”, retraining them to get the pellets starting 2 days after the “stroke”. That worked, and after a similar period of training the mice were back to where they had started, just like human subject A mentioned above.

In the human, one can use imaging to try to see which part of the brain is active, and gain some clue as to how the brain might have reorganized. Such studies have been conducted in people with strokes, but not with consistent results. In the mouse one can look at the brain and see what may be going on. Zeiler first found that the damaged neurons in the motor area had not recovered, so that was not the mechanism. However, another, nearby area of the brain, the medial premotor cortex, an area in front of the usual motor area, had become active. In healthy mice, damaging this medial premotor cortex did not cause any paralysis, so normally this is not a motor-control area, but apparently becomes one as a response to injury. Zeiler and colleagues then damaged the medial premotor cortex in mice with their motor area damaged, and saw the original motor deficit return.

But that is only part of the story. What is going on in the medial premotor cortex? This area contains inhibitory neurons that keep other premotor neurons inactive. After the injury to the motor cortex, these inhibitory neurons become quiet, allowing the other neurons to be more active and to take over motor functions. But why did human subject B not recover? Perhaps his lesion was larger, knocking out both the primary motor area and the equivalent of the premotor area. Or maybe the inhibitory neurons in the premotor area did not get turned off. Or maybe the subject did not receive adequate physical therapy. Addressing the timing, dose, and use of medicines are the next steps.

I. DEVELOPMENTAL DISORDERS
This mouse model has obvious human implications, but many questions remain. In the human, what is the equivalent of the medial premotor area? Can this area be focused on in studies of recovery? Are there similar inhibitory factors that have to be shut off or diminished to allow this human area to take over motor functions? Could this area in the human be suppressed by applying transcranial magnetic stimulation (TMS) to provide inhibition of the inhibitory cells to aid recovery?

Attempts to go from the results of therapy in a mouse model of stroke to an application in humans have a dismal record, as outlined in the discussion of stroke, below. However, most previous studies of animal models have been different. In the most commonly used model, a large stroke is induced in the mouse, and some agents are given to the mouse to try to make the stroke smaller. Many agents achieve that in mice, but not a single one has had any positive effect in humans. The studies I report here are different: they are targeting the cellular mechanisms of recovery. Thus, these observations by Zeiler and his colleagues move the enquiry in a different direction. For example, they ask: How does the brain injury remove the inhibitory influences that normally keep the medial premotor neurons less active? Does this area of the human brain that is the equivalent of the medial premotor area of the mouse become active during recovery? If it could be identified, would suppressing inhibition in the area by applying TMS to provide inhibition to the inhibitory cells in this area aid recovery?

**STEM CELLS IN RECOVERY**

In most of the brain, with the exception of specific areas such as the cerebellum or hippocampus, regeneration of new neurons does not occur and is not a mechanism of recovery. Thus, the possibility of introducing a source of new neurons from stem cells has raised great expectations. Dipping into the stem cell field is not easy, in part because the terminology is confusing and keeps changing. In general, stem cells have different properties depending on their source or origin:

- **Embryonic stem cells** are derived from the embryo, usually mouse or human. These cells are pluripotential; that is, they are able to differentiate into any organ in the body. There is considerable information about the differential factors that will drive embryonic stem cells to form cells of a specific organ such as heart, skin, or brain. The study of embryonic stem cells is where the field first started, and this almost immediately raised ethical issues relating to the use of human embryonic tissue. There has been a period of federal restriction on this research in which the US National Institutes of Health would, at various times, (1) not support this research, (2) allow research but only with certain cell lines, and, currently, (3) allow some research but with regulations. This is a politically charged subject in the USA, with Democrats in the US Congress generally in favor of this research, and Republicans generally opposed.

- **Adult stem cells** are derived from a specific organ and lead to the formation of cells in that organ, which in the brain are neurons, oligodendrocytes, and astrocytes. In the brain, specific cells that can differentiate into more mature neurons and oligodendrocytes reside in two sites: in the wall of the lateral ventricle in the subventricular zone, with migration to the olfactory bulb and beyond; and in the subgranular zone, from which cells migrate to the dentate area of the hippocampus.

- **Induced pluripotent stem cells (iPSCs)** are derived from an adult tissue such as skin, and then “dedifferentiated” to a pluripotential stem cell. These cells have several potential advantages. First, being derived from adult tissue, their use avoids raising the ethical issues associated with embryo-derived cells. Second, they can be derived from the subject who is to receive the stem cells, thus avoiding the immunological mismatches associated with a foreign donor. This approach, like much in the stem cell field, is rapidly evolving. For example, the techniques involved in dedifferentiation that were originally feared to be potentially associated with tumor formation have been modified so this concern has been minimized. However, it is not clear that iPSCs have the same potential as embryonic stem cells. Another potential problem is that a cell from an individual with a disease may have the biological characteristics that were involved with the appearance of the disease in the first place, with the possible result that the iPSCs will reproduce the disease you are trying to treat.

- **Mesenchymal stem cells**, also called multipotent stromal cells, are derived from diverse tissue including bone marrow, adipose tissue, and amniotic fluid. They are pluripotential in that they may be limited to certain cell outcomes. As will be discussed in several of the disease-specific chapters that follow, stem cells, usually mesenchymal stem cells or neural stem cells, have been proposed to promote recovery in a variety of neurological conditions including stroke, MS, spinal cord, and brain injury, PD, and amyotrophic lateral sclerosis (ALS). In addition, they have been proposed in specific roles such as memory improvement in AD, and return of vision in hereditary blindness. Possible benefit may occur when these cells
differentiate into specific cell types such as neurons or oligodendrocytes. However, more commonly, these cells migrate to the site of the disease and release trophic factors that aid recovery.

The stem cells may be introduced by intravenous injection or directly into the brain tissue, as in the spinal cord in ALS. Not only in brain or spinal cord, but also in other tissues such as the heart, stem cells migrate to the site of injury. The mechanisms behind this migration are not known. For example, in animal models of stroke, stem cells given intravenously migrate to the site of injury, whereas in non-stroke animals, they stay in the vascular system. At the injury site, stem cells can differentiate into neurons or oligodendrocytes, release trophic factors which promote recovery, or suppress inflammation.10

Oligodendrocytes have been specifically sought in MS or metabolic diseases affecting the function of oligodendrocytes, such as Pelizaeus–Merzbacher disease. One of the primary lesions in MS, discussed in detail in Chapter 30, is demyelination. In many patients with MS clinical recovery occurs to some degree when remyelination occurs. In animal models of the disease, this remyelination, as well as a decrease in inflammation, is aided by the introduction of human mesenchymal stem cells. These stem cells do not differentiate into oligodendrocytes; rather, they produce hepatocyte growth factor, which is important in promoting both a decrease in inflammation and remyelination. Mesenchymal stem cells and hepatic growth factor are both being considered as therapy in MS in clinical trials.10

There have been several human trials involving injection of stem cells into the spinal cord in patients with ALS. Investigators at Emory University are currently carrying out a phase I safety trial involving 12 patients. No untoward problems have been seen. Thus, these investigators are moving on to a phase II efficacy trial. Investigators are currently carrying out a phase I safety trial involving 12 patients. Not only in brain or spinal cord, but also in other tissues such as the heart, stem cells migrate to the site of injury. The mechanisms behind this migration are not known. For example, in animal models of stroke, stem cells given intravenously migrate to the site of injury, whereas in non-stroke animals, they stay in the vascular system. At the injury site, stem cells can differentiate into neurons or oligodendrocytes, release trophic factors which promote recovery, or suppress inflammation.10

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The actual use of stem cells as therapeutic agents in brain diseases is in its infancy. To the author’s knowledge, no trials have yet shown clear-cut efficacy for stem cells in any brain disease.

Stem cells are an excellent example of the interaction between clinical scientists and more basic neuroscientists. Clinically oriented scientists know, and have access to, the human diseases. They may even have experience in developing animal models of these diseases. However, the basic cell biology of what is going on, as represented by such issues as dedifferentiation, factors involved in differentiation to a specific cell type, migration to an injury site, and mechanism of aiding recovery, are all problems awaiting attention by researchers in basic neuroscience.

**BRAIN TRANSPLANTS**

This is a different issue from stem cell implants. Here, brain tissue is transplanted to the brain as a therapy for a disease. The most striking findings have been in PD, where human fetal substantia nigra has been transplanted into the striatum of patients with PD. In controlled trials, which involved sham surgery in the control groups, a therapeutic response was found in some of the transplanted group. Indeed, the response was too good: some patients had symptoms compatible with overdose of L-dopa, with adventitious movements.12 Unfortunately, two double brain placebo-controlled trials failed to see any efficacy as a result of fetal tissue transplants. However, questions have been raised about the experimental design and thus it remains unclear as to whether the transplantation of fetal brain tissue will be an effective intervention in this – or any – condition.13 In addition, many of the ethical issues raised regarding the use of embryonic stem cells have also been raised regarding the use of fetal tissue.

In any event, these studies raise an important basic science question: Can exogenous cells containing transmitter be transplanted, survive, and provide the needed transmitter to other neurons? Some of these studies suggest that they can but that the exogenous cells are not integrated and are outside the usual control systems, and that this loss of control can be a limiting factor. Therapy with stem cells, differentiated as dopamine-producing neurons, if that becomes possible, may well have the same problem.14

**NEUROLOGY AS A THERAPEUTIC FIELD**

When I first decided to become a neurologist, many years ago, colleagues asked me why I wanted to do that, when all I could do was diagnose, with no therapy to provide. For many disease entities therapeutic possibilities have changed dramatically. New therapeutic approaches have changed our management of MS, stroke, epilepsy, PD, migraine, and human immunodeficiency virus (HIV) disease. In addition, understanding of the underlying disease mechanisms has led to clinical trials in many disorders, including AD, ALS, depression, epilepsy, Huntington disease, PD, schizophrenia, stroke, traumatic brain injury, and genetically determined disorders, to mention just a few. It is true that many of these attempts have shown relatively little efficacy and we still have a long way to go, but progress is being made.

The neurodegenerative diseases have several things in common. First, most are sporadic diseases. There are families with a specific, often dominantly inherited disease as occurs with AD, PD, and ALS, but they are quite rare. Second, they are usually late-onset diseases, with
increasing occurrence after ages 60–70. One hypothesis for neurodegenerative diseases is an extension of the prion hypothesis, first applied to a group of diseases called transmissible spongiform encephalopathies, which includes Creutzfeldt–Jakob disease, kuru, and bovine spongiform encephalopathy (mad cow disease). This hypothesis was put forth by Stanley Prusiner, for which he won the Nobel Prize in 1997. As discussed in Chapter 23, in the prion hypothesis, a normal cellular protein, a prion (from “proteinaceous” and “infection”) self-replicates into an abnormal, misfolded form that is toxic to cells. An important part of the hypothesis is the ability of the abnormal, misfolded protein to induce misfolding in normal prion protein in other cells. This self-replication spreads to other cells, so that a cascade of toxic cells damages the brain. In neurodegenerative diseases there are accumulations of specific proteins such as β-amyloid (Aβ) and tau in AD, and tau in frontotemporal dementia and chronic traumatic encephalopathy after head injury. In PD there is the accumulation of α-synuclein in the Lewy bodies of specific neurons. Recent studies have suggested that all of these proteins can lead to transmissible disease after long incubation periods (years) following intracerebral injection. For example, human brain homogenate from AD patients injected into marmosets resulted in the appearance of Aβ plaques after 3–4 years. This phenomenon was shown to be specific to the Aβ plaques, even synthetic plaques.

**ANIMAL MODELS OF HUMAN DISEASE**

Of importance to basic neuroscientists is the development of *in vitro* and *in vivo* models of human disease. These are important not only in defining basic mechanisms but also in evaluating potential therapies. So far, going from the mouse, even the genetically altered mouse, to the human has been only marginally productive. However, successes have included the development of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD in the non-human primate, which provided a model for evaluating the affects of lesions on PD. Ultimately, these studies resulted in the development of DBS for the human parkinsonian patient.

One of the outstanding examples of failure in going from an animal to human effectiveness has been in the area of stroke. It has been estimated that there have been over 500 trials of “neuroprotective compounds” in models of stroke, mostly in mice and rats. However, only antiplatelet compounds (aspirin and Plavix® [clopidogrel bisulfate]) and recombinant tissue plasminogen activator (tPA) are accepted therapies in humans. There are several reasons for these failures, but as outlined by van der Worp and colleagues, the healthy, younger, male animals usually used do not reflect the aging human with comorbidities, particularly hypertension (in 75%) and diabetes (in >60%). Further, most animal models have built-in differences from what is possible in the human, such as a short time from the inducement of the stroke to onset of therapy (10 minutes in animals), change in stroke volume as an outcome rather than a functional recovery, and short follow-up (1–3 days in animals versus 3 months in humans). Unfortunately, many pharmaceutical companies have abandoned stroke as a therapeutic goal because of previous failures of clinical trials. However, as will be discussed below, there may be ways to improve both the animal model and the clinical application.

**DEVELOPMENT OF NEW DRUGS**

The development of a new pharmacological approach to a disease is a long and extremely expensive process. Estimates suggest that it takes 12 years and costs around 1.2 billion USD from the first research on a drug to its final clinical acceptance. That cost figure can be sharply revised upwards if the pharmaceutical company includes the cost of their drug failures as part of the calculation. Even for a large company like Merck or GlaxoSmithKline, only a few drugs can be supported for expensive clinical trials. Brain drugs must compete with drugs for other diseases such as cancer or heart disease. Not surprisingly, decisions are influenced by recent successes or failures. Thus, if a drug for AD or stroke has failed in a recent trial, the company may be hesitant to conduct further trials of drugs for neurological disorders. That is exactly what is happening, as large pharmaceutical companies (“big pharma”) have decreased their neuroscience operations and clinical trials of drugs for brain diseases.

A challenge to those in clinical neuroscience is to devise ways to simplify this process so that clinical trials can involve smaller numbers of subjects, have defined predictable outcome measures, be of shorter duration, and therefore cost less. For example, the population being studied can be simplified by being more homogeneous, with a better defined clinical course and defined outcomes. We can also learn from investigators in other fields. For example, in oncology various forms of cancer are being defined by their molecular defects. In breast cancer the presence or absence of a hormonal receptor (estrogen or herceptin) defines the population.

In general, in the development of a drug there is a jump from animal results to large trials in the human. As mentioned above, the results in animals may hint at clinical efficacy, but the animal model is often so far from the human disease, or the therapeutic approach in animals so impractical in the human, that using that data as the basis of an extremely expensive human trial is
hazardous. There is a crucial step, or question, missing: Does the stuff do anything in the human?

**CLINICAL TRIALS**

Eventually, a potential treatment for patients has to be evaluated in a clinical trial. In this trial the projected therapy is compared with a control group, which may consist of those with no therapy, standard therapy, or a successful therapy. Ideally, subjects are randomized into the treatment group or control group. This randomization and study evaluation is carried out in a double-blind fashion, with neither the subjects nor the investigators knowing in which group a subject resides. The randomized control group is included to be a measure of the natural progression of the disease, to equalize the effects of gender, age, and local factors, and to minimize the bias of the investigators.

One of the factors that complicates these studies is the "placebo effect": that is, the control group responding in a positive way, as if they are receiving the actual therapy. These placebo responders appear to be using two mechanisms. The psychological one is related to the power of suggestion. If I tell you that what I am giving you will help your problem, you are prepared to respond positively. If I am quite positive, you will also be positive. Your response may have a biological component, such as when a change in neurotransmitter level or the release of endorphins results in a higher pain threshold. Not all subjects respond in the same way. One of my colleagues, Tony Ho, now at AstraZeneca and previously at Merck, ran into this placebo problem while he was directing a trial of a new compound for the treatment of pain. He noticed that some, but not all, subjects in the placebo group were responding almost as well as those receiving the new agent. In his next trial he started by giving the new agent. In his next trial he started by giving the new agent. After a period he removed those who responded to placebo and randomized only the placebo responders in the next stage of clinical trial to treatment or placebo. In other words, he eliminated the placebo responders.

A continuing problem is to devise clinical trials that are applicable to changing approaches to a disease. This arises in the comparison of two approaches to coronary artery disease: surgery with coronary artery bypass grafting (CABG) versus stents. (A stent is a mesh tube that keeps a previously occluded coronary vessel open.) The problem with such studies is that both groups, particularly those receiving stents, have made technical advances, so that a trial that requires 5 years to finish is out of date by the time it is completed. With stents, cardiologists have gone from bare metal stents to drug-eluting stents to prevent rethrombosis. So now there is a need for another study to determine whether the drug-eluting stents are actually better than the old, bare metal stents. Since some of these studies use survival as an outcome, they take time to be completed. The final answer is not in yet, but the results of several studies indicate that the death rate over 6 years is higher in the stent group than in the CABG group, particularly in patients with diabetes, and that the type of stent may not make a difference.

There is no easy answer to the problem of progress in the diagnosis or treatment of a disease while a trial is in progress. This is particularly true in some trials that involve imaging techniques as part of the evaluation of subjects. What do you do about a new imaging technique, such as going from a 1.5T MRI scanner to a 3.0T MRI scanner? A possible solution is to evaluate a sample of subjects using both the older and the new imaging techniques for comparison purposes.

**TRIALS IN ALZHEIMER DISEASE**

Our concept of AD has changed over the years. When I was first training in neurology in the late 1950s, AD was thought of as a rare form of dementia, called a "presenile dementia" because it affected people in their forties and fifties. In the 1970s it became apparent that older people with dementia at that time called "senile dementia" or "hardening of the arteries" had the same pathology as those younger people first described by Alois Alzheimer. In recent years our concepts have changed even more. First, the disease process starts many years before we make the diagnosis based on the appearance of clinical symptoms such as impaired memory. Thus, criteria have recently been established for three phases of the disease: (1) a preclinical phase in which a subject is cognitively normal by history and usual cognitive testing, but has biomarker and pathological evidence of the disease; (2) an intermediate phase in which there is cognitive impairment, but not enough to make a diagnosis of dementia: this phase, referred to as minimal clinical impairment (MCI), is defined by cognitive change and biomarker evidence of underlying disease; and (3) finally, the dementia phase, in which people become increasingly impaired and have biomarkers of the disease. The brain pathology of the dementia phase shows marked neuronal loss, particularly in the regions of the brain where the disease starts, such as the entorhinal cortex and hippocampus. From these early involved sites, the disease spreads to other areas, such as the frontal cortex. Unless some way of providing new, healthy neurons to an involved region is found, reversal of this advanced disease is unlikely. I wonder if one is doing any favors to the patient or their family by treating a severely demented patient when, at best, one might slow the progression to even more severe dementia. However, until
recently it is exactly these demented patients who have been the focus of clinical trials. It would be preferable to treat the phase I, or early phase II subject with the goal of preventing or slowing the disease process in a relatively normal person. To accomplish this, we need to know whether it is possible to predict in a normal or minimally affected population who will go on to progress to MCI or dementia. There are several studies in progress of subjects, some with positive family histories or genetic risk factors, who are evaluated at baseline and then followed for years. In a study being carried out at Johns Hopkins by Marilyn Albert, some of the subjects have been followed for 16 years. Her study, and others, indicates that the combination of cognitive testing, changes on MRI, and cerebrospinal fluid (CSF) findings of biomarkers (see “Biomarkers of Disease”, below) can identify a population that is at risk for progression. However, these studies are applied to a population, and it is not clear whether they can be used with an individual subject.

BIOMARKERS OF DISEASE

In the preceding paragraphs I have referred to biomarkers of AD. Biomarkers are biological markers of a disease process. Some are related specifically to the disease and the disease mechanism, others reflect the effects of the disease, and some are markers of disease presence, but not related to disease mechanism or effects (e.g., prostate-specific antigen in prostate cancer). Currently, five biomarkers in two major categories are used in diagnosing and following disease progression in AD: (1) those related to brain amyloid $A\beta$ deposition, specifically measures of $A\beta$ in CSF and demonstration of amyloid on brain imaging by PET; and (2) those related to neuronal degeneration, including measures of atrophy on MRI structural imaging, measures of hypometabolism on fluorodeoxyglucose positron emission tomography (FDG-PET) and increased levels of tau and phosphorylated tau in CSF. These markers have been used to develop dynamic models of the three phases of AD.

One advance in the area of AD is the ability to demonstrate the presence of amyloid in the living human brain by PET imaging. After years of preliminary work, two investigators, William Klunk and Chester Mathis at the University of Pittsburgh, USA, working with clinicians in Sweden, reported in 2002 and 2004 the demonstration of amyloid in AD subjects, and not in non-AD subjects. Since then, other teams have used the Pittsburgh compound to evaluate subjects with possible AD. What originally looked like a clear-cut marker of the disease has become complicated. The major problem is that about 20–30% of those considered cognitively normal are positive for amyloid on PET scanning. It is not clear what these positives represent. If one waits long enough will they progress to show clinical indications of MCI or AD? In other words, are they representatives of the first, preclinical group mentioned above? Or is amyloid on PET scanning an imperfect marker? There is also an important ethical question raised by such results: Does one tell the individual of the finding, given that it may be a false positive, and even if it is not, we do not currently have a treatment to offer to patients beyond cognitive and physical exercise? This and other ethical dimensions of research are discussed in Chapter 45.

The second problem is that there appears to be a ceiling effect with this compound. In other words, amyloid is detected early in the disease process, but then there is a maximal detection by scanning, even though the disease continues to progress clinically. Thus, it is unlikely that amyloid imaging will be a good marker of attempts to alter the latter stages of the disease.

A third problem that limits the widespread use of the Pittsburgh compound is its use of carbon-11, an isotope with a 20 minute half-life. Therefore, these studies require a cyclotron on site to make the labeled compound and then its immediate use in the scanner. Only a few medical centers have this combination of equipment available. Several companies have focused on a different labeled compound using fluorine-18, which has a half-life of 2 hours, enough time for a compound to be made at a central site and then be made available to those who cannot make their own compound. The first to appear was made by Avid Pharmaceuticals, and has been approved by the US Food and Drug Administration (FDA). There are other fluorine-18 compounds in the pipeline.

A welcome advance would be the demonstration of tau by PET imaging. This would be helpful not only in AD, but also in other tauopathies such as frontotemporal dementia and in the chronic traumatic encephalopathy that occurs in athletes and military personnel after head injuries. In the latter situation tau accumulates in a different distribution from that seen in AD, and without the accompanying amyloid plaques. With the increasing concern about head injuries not only in professional athletes such as football players and boxers but also in younger amateur participants in contact sports, the ability to detect tau in brain quickly and reliably would be a significant breakthrough (see Chapter 16). There is one report of tau imaging in head injury from the University of California – Los Angeles, involving a small number of subjects, and similar studies may be expected in the future. With successful tau imaging, a whole new set of questions would emerge. For example, are there genetic factors that determine a person’s response to head injury in terms of accumulation of tau? If so, are there young people who could be identified as being at higher risk for subsequent behavioral and cognitive problems? Once there, is the accumulation of tau reversible? Are there medications that could modify the accumulation of tau?
There has been considerable interest by the pharmaceutical industry in AD because the potential market for a successful therapeutic compound is enormous. In 2013, it was estimated that 5.2 million people in the USA had AD; worldwide, the number jumps to more than 35 million. Unfortunately, clinical trials, almost all aimed at Aβ as the target, have been negative, and big pharma is getting restless. Thus, a model of interaction has been developed between the US government, in this case the National Institute on Aging, an advocacy group (the Alzheimer’s Association), various pharmaceutical companies, and academia. This group, known as the Alzheimer’s Disease Neuroimaging Initiative, is developing and evaluating clinical, neuropsychological, biomarker, and imaging parameters in subjects with various stages of memory loss, from normal to MCI to AD. Of importance is that tests are carried out in various locations, but analyzed in central sites with standardized methods. The results are available to all researchers and provide background for future clinical trials of specific therapies. In its design, this group has been flexible, trying to take advantage of the rapidly changing techniques in imaging and biomarkers. This model of collaboration could and should be applied to other diseases as well.

**PSYCHIATRIC DISEASE**

As I mentioned at the outset of this chapter, this is not an all-inclusive review of diseases of the nervous system, but focused on areas where I have first hand experience, particularly neurological diseases. However, if you combine those with depression (Chapters 40 and 43), schizophrenia (Chapter 39), anxiety (Chapter 37), obsessive–compulsive disorder (Chapter 38), addiction (Chapter 35), and suicide (Chapter 43) you are considering a large number of subjects. It is estimated that in any given year, 25% of Americans have a diagnosable mental illness, a total that probably exceeds all those with neurological disease. (I put AD in both camps.) These groups with psychiatric diseases have several things in common that set them apart from most neurological diseases at present:

- These entities are defined by their clinical symptoms, obtained by clinical history from the patient or an informant. This leads to controversy about the criteria for diagnosis, particularly at the ends of the age spectrum, in children and elderly people.
- These diseases do not yet have unequivocal biomarkers (with the exception of disorders lying at the interface between neurology and psychiatry, such as AD). Thus, there are no biological aids for diagnosis or measurement of disease course.
- Most have no definitive pathology. One cannot examine the brain at autopsy and determine that the patient suffered from depression or schizophrenia.
- Imaging studies have been helpful in demonstrating structural and functional abnormalities associated with psychiatric disorders, but have not yet yielded clear diagnostic differences. For example, the hippocampus can exhibit atrophy in schizophrenia, major depressive disorder, and post-traumatic stress disorder.
- Whereas many of these disorders have increased prevalence within a family, the genetics is complex at best. This is similar to neurological diseases such as AD, MS, and PD.

Despite these problems, therapy in psychiatric diseases, based on their neuropharmacology, has been more successful than for most neurological diseases. This has been particularly true in schizophrenia and the mood disorders. The treatments for these disorders were originally developed over 50 years ago by serendipity in clinical testing of novel agents, including chlorpromazine, imipramine, and lithium, with unknown mechanisms of action. Subsequent preclinical investigations identified potential mechanisms of action. For example, Julius Axelrod demonstrated that the antidepressant imipramine inhibited the synaptic uptake/inactivation of biogenic amine neurotransmitters, and Arvid Carlsson proposed that the mechanism of action of antipsychotic drugs was blockade of dopamine receptors. Axelrod and Carlsson received Nobel Prizes in Medicine or Physiology for these insights. The pharmaceutical industry capitalized on this knowledge to develop second generation drugs that were associated with greater specificity and fewer side effects.

In depression, the currently used medications act by influencing the transmitters serotonin, norepinephrine, and dopamine. The goal of therapy is to selectively increase the level of these transmitters at the synapse. For example, selective serotonin reuptake inhibitors increase the level of serotonin by inhibiting its reuptake by the presynaptic cell, making more serotonin available to the postsynaptic cell. Commonly used antidepressants such as sertraline, paroxetine, and fluoxetine work by this mechanism. Newer agents are more broadly based in their action, often affecting more than one transmitter.

**GENETICS OF NEUROLOGICAL AND PSYCHIATRIC DISORDERS**

It is hoped that understanding the genetics underlying a disorder will aid diagnosis, elucidating the underlying mechanism, and approaches to therapy. In some diseases, such as the metabolic diseases Tay–Sachs
disease and metachromatic leukodystrophy, this has been the case. However, unlike many neurological disorders that follow Mendelian genetics, psychiatric disorders, AD, and MS are disorders of complex genetics in which multiple risk genes of modest effect interact with the environment to produce the phenotype. The principles of epigenetics are only just being applied to diseases such as schizophrenia. Newer genetic approaches such as the application of Genome-Wide Association Study (GWAS) to specific populations may prove to be helpful. This approach has been useful in establishing a location on chromosome 9p21 in ALS. The usual application of GWAS techniques to a large population of subjects with ALS and controls was unrevealing. However, when the study was focused on a Finnish population, genes localized on chromosome 9 were indicated. Eventually, a hexanucleotide (CPORF72) was found. It is anticipated that advances in genetics will lead to the identification of novel targets for the development of treatments that address the pathophysiology and will be more effective than current therapies.

TEMPERAMENT AND DISEASE

There is an area that spans the overlap between psychiatry and neurology (as well as other areas of medicine) and that is the effect of temperament, mood, or both on the clinical course of a disease. In other words, does a patient’s emotional reaction to a disease affect their response to that disease? One striking example is the effect of depression on outcome after a heart attack. If a patient is depressed 1 month after a heart attack, his or her mortality is three to four times higher over the next year or two. Similarly, depression is associated with poor outcomes after CABG. The mechanisms underlying these associations are not clear. Nor is it established that antidepressive therapy will alter cardiac outcomes. In other instances, depression is associated with poorer outcomes of disease, but it is not clear whether depression is causative as part of the disease process or the relationship in an association. This situation comes up when evaluating the outcomes in MS, AD, and PD, among others.

CONCLUSION

In looking back over my half-century of research and practice in clinical neuroscience, I see successes, failures, and lessons for the future. The major lesson I have learned is that clinical neuroscience has changed from being a descriptive field to being an active intervention field. For example, in stroke the role of a neurologist has gone from arranging physical therapy to considering immediate therapy with tPA, anticoagulants, and possible intra-arterial or surgical therapy, to preventive therapy keeping hypertension and cholesterol under control (see Chapter 30 for further discussion).

Even in an area where clinical progress has been slow, there is hope. In the most severe form of brain tumor, glioblastoma multiforme, despite treatment with both radiation and chemotherapy, life expectancy is not much better now than it was 10 years ago. With no therapy, survival is 4–6 months. With the currently accepted therapy of radiation followed by chemotherapy, survival has been extended to 14–16 months. Some, mainly younger, people live longer, up to 3–5 years. Currently, much attention is being paid to the cells of origin in this tumor, particularly cancer stem cells. The tumor is quite heterogeneous, with both tumor and non-tumor cells. Within the tumor cell population are cells that have the properties of stem cells, referred to as cancer stem cells. These cells account for the uncontrolled growth, resistance to therapy, and migration of tumor cells, and therapy directed at these cells is being tried. This field desperately needs new ideas, and this is one of them.

I have already mentioned our inability to get at the underlying mechanism in neurodegenerative diseases. I do not know whether Prusiner is correct in his prion hypothesis as applied to these diseases, but at least it is a start. The question now is whether the prion hypothesis can lead to a treatment. This is not the only area where basic sciences are contributing approaches to therapy. Other examples are the use of trophic factors, such as nerve growth factor or brain-derived neurotrophic factor, as therapeutic agents, or attempts at gene replacement or modification, particularly in muscular dystrophies.

I will close by commenting on the development of new approaches to the treatment of brain disorders. The dependence on enormously expensive, prolonged, clinical trials is killing us. We need a change in philosophy to include smaller, focused trials that will provide indications of efficacy, and less “me-tooism” in considering new approaches. We must avoid acting like lemmings, following each other off the cliff. At least some participants should be sitting quietly with their feet on their desks, considering what should be the new approach, and then bravely going into the laboratory, followed by carrying out small, focused clinical trials. It is not clear to me that big pharma can adapt. The onus may have to shift back to academia, working in conjunction with smaller, more flexible pharmaceutical entities.

In conclusion, there are reasons to rejoice – we have come a long way; but also reasons for disappointment – there is so much farther to travel. I hope that the readers of this textbook will find the inspiration to join the struggle.
References


I. DEVELOPMENTAL DISORDERS
Knowledge to Fill in the Gaps.

To start, it helps to know which gaps to fill. Because Elsevier Science and Technology Books has unique visibility into the world’s research output, we share insights that keep you a step ahead. And a step closer to your goal.

BE IN THE KNOW: elsevier.com/sciencedirect/books
The brain is not simply an amorphous mass of grayish tissue. It courses with blood and electrical impulses. It regulates the body’s temperature. It tells us how we feel. It allows us to interact with others and the world. It says when to wake up and when to fall asleep. It helps us put our shoes on in the morning. It also is susceptible to...
a host of external influences, including drugs. To better understand the subsequent chapters in this book and to put the medical, biological, and neurobiological mechanisms of drug addiction into context, we must take a step back to define and explain the common components of the body’s central nervous system, from the macro (brain regions) to the micro (neurons, neurotransmitters). Armed with this information, students will be able to appreciate the in-depth knowledge that has been gained from extensive scientific research during the past 100 years, with the hope that they, too, will be able to discover greater intricacies to explain why many individuals succumb to drug addiction.

THE CENTRAL NERVOUS SYSTEM

The human brain consists of two types of cells: roughly 100 billion neurons and a greater number of glia. Neurons are highly specialized cells that have an important and unique functional property that is not shared with any other cells in the body. Neurons communicate with each other through both electrical and chemical mechanisms. More importantly for the theme of this book, neurons communicate through circuits, and these circuits form the structural bases of feelings, thoughts, and behavior, the ultimate functional output of the brain.

Neurons

Neurons have four major components: (1) cell body, (2) axons, (3) dendrites, and (4) synapses (Figure 2.1). The cell body contains the nucleus and receives inputs, providing the machinery for the generation of neurotransmitters and action potentials. An action potential occurs when a neuron’s membrane is depolarized beyond its threshold. This depolarization is propagated along the axon. The axon is the “sending” part of the neuron, and it conducts these action potentials to the synapse to release neurotransmitters. The synapse is a specialized space or contact zone between neurons that allows interneuronal communication. One or more dendrites comprise the “receiving” part of the neuron, providing a massive receptive area for the neuronal surface (Figure 2.2).

Neurons act on other neurons to exert three major functions: inhibition, excitation, and

![FIGURE 2.1 Anatomy of a neuron.](image)
neuromodulation. Inhibition means that one neuron inhibits another neuron, often through the release of an inhibitory neurotransmitter at the synapse. Excitation means that one neuron activates another neuron through the release of an excitatory neurotransmitter at the synapse. Neuromodulation means that a neuron influences neurotransmission, often at a long distance.

**Neurotransmission**

The communication between neurons can be distilled into six major steps of neurotransmission relevant to the neuropharmacology of addiction (Figure 2.3).

Step 1: Neurotransmitter synthesis, involving the molecular mechanisms of peptide precursors and enzymes for further synthesis or cleavage.
Step 2: Neurotransmitter storage.
Step 3: Neurotransmitter release from the axon terminal into the synaptic cleft (or from a secreting dendrite some cases).
Step 4: Neurotransmitter inactivation caused by removal from the synaptic cleft through a reuptake process, or neurotransmitter breakdown by enzymes in the synapse or presynaptic terminal.
Step 5: Activation of the postsynaptic receptor, triggering a response of the postsynaptic cell.
Step 6: Subsequent signal transduction that responds to neurotransmitter receptor activation.

Drugs of abuse or drugs that counteract the effects of drugs of abuse can interact at any of these steps to dramatically or subtly alter chemical transmission to dysregulate or re-regulate, respectively, homeostatic function.

**FIGURE 2.2** Neurons, synapses, and neurotransmitters. A typical example is shown for the neurotransmitter dopamine.
2. INTRODUCTION TO THE NEUROPSYCHOPHARMACOLOGY OF DRUG ADDICTION

Glia

In addition to neurons, the central nervous system contains supporting cells. Supporting cells, generically called glia, can outnumber neurons by a factor of ten. Historically, glia were defined as the “nerve glue” that holds neurons together in the central nervous system. However, glia are now known to have key dynamic functions in the central nervous system, from myelin synthesis, to synapses, to serving as the innate brain defensive system against pathology. Glia consist of three types of supporting cells: oligodendrocytes, astrocytes, and microglia.

Oligodendrocytes synthesize myelin and provide an expedient way, via the myelin sheath, to significantly increase how fast an axon can conduct an action potential. Myelin is a long plasma membrane sheet that wraps around each axonal segment, leaving bare axons between myelin segments, known as the nodes of Ranvier (Figure 2.3). Myelin effectively forms insulation that allows the action potential to jump from node to node, known as salutatory conduction.

Astrocytes are star-shaped cells that have processes (branches) and both physical and biochemical support functions in the central nervous system. They physically isolate neurons and oligodendrocytes with long processes by making a cover over the nodes of Ranvier and covering the surface of capillaries, forming part of the blood-brain barrier. Astrocytes play a

FIGURE 2.3 Synaptic neurotransmission. The figure shows a generalized process of synaptic transmission. (1) Various components of the neurotransmission machinery, such as enzymes, proteins, mRNA, and so on (depending on the neurotransmitter in question) are transported down the axon from the cell body. (2) The axonal membrane is electrically excited. (3) Organelles and enzymes in the nerve terminal synthesize, store, and release the neurotransmitter and activate the reuptake process. (4) Enzymes in the extracellular space and within the glia catabolize excess neurotransmitters released from nerve terminals. (5) The postsynaptic receptor triggers the response of the postsynaptic cell to the neurotransmitter. (6) Organelles within postsynaptic cells respond to the receptor trigger. (7) Interactions between genetic expression and postsynaptic nerve cells influence cytoplasmic organelles that respond to neurotransmitter action. (8) Certain steps are modifiable by events that occur at the synaptic contact zone. (9) The electrical portion of the nerve cell membrane integrates postsynaptic potentials in response to various neurotransmitters and produce an action potential. (10) The postsynaptic cell sends an action potential down its axon. (11) The neurotransmitter is released. The neurotransmitter that is released from the nerve terminal can be modulated by autoreceptors that respond to the neurotransmitter. [Modified with permission from Iversen LL, Iversen SD, Bloom FE, Roth RH. Introduction to Neuropsychopharmacology. Oxford, New York, 2009, p. 26.]
PHARMACOLOGY FOR ADDICTION

What is a Drug, and What is Pharmacology?

The following terms need to be defined for pharmacological discussions of addiction. Pharmacology is the study of the interaction between chemical reagents or drugs and living organisms. A drug is any chemical agent that affects an organism. Obviously, this definition can be murky in the domain of drugs of abuse, when one crosses into the realm of natural preparations that contain psychoactive or psychotropic drug entities. Psychotropic can be defined as an effect of a drug on the mind or behavior. For example, most drugs of abuse are derived from plant preparations. Many of them are alkaloids, such as nicotine in tobacco and caffeine in coffee and tea. An alkaloid is an organic compound that normally has basic chemical properties and contains mostly basic nitrogen atoms. So when does a compound transition from being a foodstuff to a drug? One metric is when it begins to have an identifiable psychotropic effect.

Other terms that are often used in the drug abuse field and should be defined in the context of this book are toxicology, pharmacotherapeutics, pharmacokinetics, and pharmacodynamics. Toxicology is the study of the harmful effects of drugs. Pharmacotherapeutics is the study of the diagnostic or therapeutic effects of drugs. Pharmacokinetics is the study of the factors that determine the amount of a given drug at a given site of action. Pharmacodynamics is the study of how a drug produces its biological effect.

Drug Nomenclature

Drugs generally have three names: a chemical name, a nonproprietary (generic) name, and a proprietary (trade) name. The chemical name describes the chemical structure. For example, 7-chloro-2-methylamino-5-phenyl-3-H-1,4-benzodiazapine-4-oxide is the chemical name for a benzodiazepine called chlordiazepoxide. Chlordiazepoxide is the nonproprietary or generic name, which is given to a drug when it has been demonstrated to have a therapeutic use. A proprietary or trade name is given by a drug company when the drug is patented. Two trade names for chlordiazepoxide are Librium and Mitran.
Drug Classification

Drugs can be classified three ways: behavioral classification, pharmacodynamic classification, and legal classification.

Behavioral classification includes five main categories: stimulants, opioids, sedative hypnotics, antipsychotics, antidepressants, and psychedelics (Table 2.1). Each of these categories is more or less self-explanatory.

- Stimulants include drugs that stimulate or produce arousal and behavioral activation. Examples of stimulants are cocaine, amphetamines, nicotine, and caffeine.
- Opioids are natural, semisynthetic, or synthetic drugs that bind to opioid receptors and produce analgesia. Analgesia can be defined as the reduction of pain or elevation of pain thresholds.
- Sedative hypnotics are drugs that sedate or decrease arousal, producing an anti-anxiety effect, hypnosis, or sleep. Hypnosis is defined as the induction of sleep. Two examples of this class of drugs are alcohol and benzodiazepines.
- Antipsychotics are drugs that are used to treat psychosis and include the classic antipsychotics such as haloperidol (trade name: Haldol), and modern second generation drugs, such as olanzapine (trade name: Zyprexa).
- Antidepressants are drugs that are used to treat major depressive episodes and include selective serotonin reuptake inhibitors, such as fluoxetine (trade name: Prozac) and escitalopram (trade name: Lexapro), among others.
- Psychedelics are drugs that produce psychedelic experiences. Psychedelic can be defined as mind-altering. Another term that is often used to describe this drug class is hallucinogen, but the true meaning of the term hallucination is to experience something that is not there; therefore, the term psychedelic is preferred. Psychedelics include lysergic acid diethylamide (LSD) and psilocybin (derived from psychedelic mushrooms).

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Examples</th>
<th>Neurotransmitters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulants</td>
<td>Caffeine, Nicotine, Cocaine, Methamphetamine</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Opiates</td>
<td>Morphine, Heroin, Meperidine (Demerol)</td>
<td>Enkephalins, Endorphins</td>
</tr>
<tr>
<td>Sedative/Hypnotics</td>
<td>Alcohol, Diazepam (Valium)</td>
<td>GABA</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>Haloperidol</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Fluoxetine (Prozac)</td>
<td>Norepinephrine, Serotonin</td>
</tr>
<tr>
<td>Psychedelics/Hallucinogens</td>
<td>Lysergic acid diethylamide, Psilocybin, Marijuana</td>
<td>Serotonin, Glutamate, Endocannabinoids</td>
</tr>
</tbody>
</table>
A pharmacodynamic classification can utilize the same broad behavioral categories mentioned above and adopt them to describe the pharmacodynamic effects on brain neurotransmission (Table 2.1). For example, stimulants are indirect dopamine agonists. Opioids are direct opioid receptor agonists. Sedative hypnotics directly or indirectly facilitate γ-aminobutyric acid neurotransmission. Antipsychotics are currently dopamine D₂ receptor antagonists and serotonin 5-HT₂ receptor antagonists. Antidepressants are serotonin reuptake inhibitors, norepinephrine reuptake inhibitors, or a combination of serotonin/norepinephrine reuptake inhibitors. Psychodelics all facilitate serotonergic activity either directly or indirectly by increasing serotonin release.

Legal classification involves two categories: prescription vs. nonprescription and drug abuse. The modern era of the legal classification of drugs with abuse potential in the United States began in 1970 with the passage of the Controlled Substances Act. Five Schedules were created. The Department of Justice (Drug Enforcement Administration and Federal Bureau of Investigation) and Department of Health and Human Services (Food and Drug Administration) determine which drugs are on which schedule. The classification decisions are made on the basis of specific criteria for the potential of abuse, accepted medical use in the United States, and the potential for dependence. Drugs are classified on a continuum of increasing abuse potential, with or without a medical use.

Schedule I: No officially recognized medical use, lack of accepted safety for use under medical supervision, high abuse potential, and cannot be legally prescribed in the United States. Examples: heroin, LSD, Δ⁹-THC, and methylenedioxymethamphetamine (MDMA or Ecstasy).

Schedule II: Officially recognized medical use and high abuse potential that may lead to severe psychological or physical dependence. Examples: methamphetamine, morphine.

Schedule III: Officially recognized medical use and abuse potential that is less than Schedules I and II that can lead to moderate or low physical dependence or high psychological dependence. Examples: ketamine, drug products that contain less than 15 mg hydrocodone per unit (Vicodin).

Schedule IV: Officially recognized medical use and low abuse potential compared with Schedule III. Examples: alprazolam (Xanax), diazepam (Valium).

Schedule V: Officially recognized medical use and low abuse potential relative to Schedule IV. These drugs consist mainly of preparations that contain limited amounts of certain narcotics. Example: Robitussin AC cough syrup, which contains less than 200 mg codeine per 100 ml.

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**PHARMACOKINETICS**

**Absorption**

Absorption can be defined as the movement of a drug into the blood stream, which is dramatically affected by the route of drug administration (Table 2.2). By definition, intravenous administration goes directly into the veins, giving instantaneous absorption. Each route of administration has advantages and disadvantages. The intravenous route is highly titratable, hence the amount of drug administered over time can be controlled very carefully. One disadvantage, however, is that there is no turning back. Once absorbed intravenously, there is no easy way to remove the drug from the bloodstream before it enters the brain. The oral route, by contrast, is highly variable and generally less preferred by those who use addictive drugs, but it is generally the safest route of administration. The time course of the effects of a drug is also highly affected by the route of administration (Figure 2.4). Particularly relevant to drugs of abuse is the general principle that faster absorption is associated with a higher likelihood of
2. INTRODUCTION TO THE NEUROPSYCHOPHARMACOLOGY OF DRUG ADDICTION

Abuse. For example, amphetamines have high addiction potential when injected intravenously, but Adderall (a mixture of amphetamine isomers/salts) appears to have low addiction liability when used orally and appropriately for the treatment of attention-deficit/hyperactivity disorder.

The basis of the differences in absorption via different routes of administration depends on several factors. One obvious factor, however, is the number of physical membranes within the body that the drug needs to cross before it can be absorbed into the bloodstream (Figure 2.5). Drugs that enter the body through the gastrointestinal track, skin, or lungs must first cross an epithelial barrier and then the endothelial cells of capillary walls. Drugs that are administered subcutaneously or intramuscularly bypass the epithelial barrier but must also cross the endothelial cells of capillary walls. Epithelial cells line the cavities and structures of the body. Endothelial cells line the interior surface of blood vessels.

**Drug Elimination**

Drugs are eliminated from the body through metabolism in the liver, excretion from the kidneys, or a combination of both (usually metabolism followed by excretion). The classic drug that is largely metabolized is alcohol, and its elimination past a certain level is entirely metabolic and thus conforms to what is known as zero-order kinetics, in which the absolute amount of drug that is removed from the body over time is constant and

---

**TABLE 2.2** Common Routes of Drug Administration

<table>
<thead>
<tr>
<th>Route</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Valuable for emergency use</td>
<td>No turning back</td>
</tr>
<tr>
<td></td>
<td>Titratable</td>
<td>Increased risk of adverse effects</td>
</tr>
<tr>
<td></td>
<td>Suitable for large volumes</td>
<td>Must inject slowly</td>
</tr>
<tr>
<td></td>
<td>Suitable for irritating substances</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Suitable for some insoluble suspensions</td>
<td>Not suitable for large volumes</td>
</tr>
<tr>
<td></td>
<td>Suitable for implantation of solid pellets</td>
<td>Possible pain</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Suitable for moderate volumes</td>
<td>Contraindicated when concomitant with anticoagulants</td>
</tr>
<tr>
<td></td>
<td>Suitable for some irritating substances</td>
<td>Possible pain</td>
</tr>
<tr>
<td>Oral</td>
<td>Reversible</td>
<td>Requires patient cooperation</td>
</tr>
<tr>
<td></td>
<td>Generally safe</td>
<td>Erratic absorption</td>
</tr>
<tr>
<td></td>
<td>Convenient</td>
<td>Possible incomplete absorption</td>
</tr>
<tr>
<td></td>
<td>Economical</td>
<td>Possible instability</td>
</tr>
</tbody>
</table>

**FIGURE 2.4** Time course of blood levels of drug by different routes of administration.
conforms to the capacity of the liver to break down the drug. Thus, zero-order kinetics result from the liver’s metabolism of drugs. For alcohol in a non-tolerant social drinker, the average metabolism for a 70 kg male is approximately 0.01 gram percent (g%) per hour (for more information, see Chapter 6, Alcohol).

The excretion of a drug or drug metabolite follows first-order kinetics, in which a constant percentage of the drug in the blood stream is excreted over time. Such a concept reflects the drug’s half-life. Half-life is defined as the time it takes to remove 50% of the drug from the blood stream. As an example, morphine administered intramuscularly at a dose of 10 mg in a 70 kg male produces a blood level of approximately 70 ng/ml. The half-life of morphine is approximately 2.5h, so, as shown in Table 2.3, at the 7.5h time point, the amount of morphine in the blood is reduced to 8.75 ng/ml. The half-life of some drugs can be changed by adjusting the pH of urine. For example, alkaloids, which constitute all major drugs of abuse with the exception of marijuana (see above), can have their half-life significantly shortened by acidifying the urine using ascorbic acid (vitamin C). This is used by physicians in an emergency room, who will intravenously administer ascorbic acid to a patient who presents with amphetamine-induced psychosis. The process by which this works is called ion trapping, in which there is a build-up of a high concentration of a drug across a cell membrane because of a difference in pH across the membrane. Therefore, a basic drug (one with a high pH) will accumulate in the acid (or low-pH) compartment (for example, acidic
2. INTRODUCTION TO THE NEUROPSYCHOPHARMACOLOGY OF DRUG ADDICTION

In an acidic medium, the drug becomes more ionized (more polar/charged and less lipophilic) and thus is less likely to cross a lipid barrier or membrane.

### Drug Receptors and Signal Transduction

A receptor is a cellular element of an organism with which a drug interacts to produce its effect. Most receptors are proteins, and most drugs bind to a specific binding site (although there are exceptions; for example, alcohol may interact with an ethanol-receptive element, perhaps in a water-containing pocket of receptors; see Chapter 6, Alcohol).

Drugs that bind to receptors and produce an effect are called agonists. Think of an agonist as a key and the receptor as a lock. The key is turned, producing an effect. An antagonist is a drug that binds to receptors and blocks the effect of an agonist. Think of an antagonist as a broken key that goes into a lock, cannot open the lock, and prevents another key from opening the lock. In the body, and particularly the brain, antagonists can produce effects on their own by blocking an endogenous agonist. For example, a dopamine receptor antagonist can block the effects of a dopamine receptor agonist. On its own, however, the antagonist can produce motor initiation deficits by blocking the effects of endogenous dopamine. A drug that binds strongly to the binding site of the receptor with high affinity but is only partially effective (low efficacy) is termed a partial agonist (it partially activates the receptor).

Once a drug binds to a receptor, it must also trigger an effector domain in the receptor that activates various intracellular targets through intermediate components, collectively referred to as a signal transduction cascade. The brain has three major types of receptor binding/effector systems: enzymes, ligand-gated ion channels, and G-protein-coupled receptors. Enzymes and G-protein-coupled receptor systems have intermediate small molecules, called second messengers, which mediate a cascade of biochemical signals that ultimately change the function of cells or neurons in the brain. Ligand-gated ion channels bind some psychoactive agents, such as nicotine, that in turn can directly modulate neuronal excitation by opening or closing ion channels to let in excitatory sodium ions or inhibitory chloride ions. This leads to a fast response (within milliseconds). G-protein-coupled receptors, in contrast, use G proteins to transduce signals to multiple other intracellular proteins in the neuron that ultimately also affect excitability via calcium and potassium channels (Figure 2.6). For example, $G_{\alpha s}$ proteins activate adenylyl cyclase that in turn activates protein kinase A, which can inhibit potassium channels, facilitate excitatory glutamate neurotransmission, and increase neuronal excitability. $G_{\alpha i}$ proteins inhibit adenylyl cyclase and in turn inhibit protein kinase A and neuronal excitability. $G_{\alpha q}$ proteins activate a different enzyme, phospholipase C, causing the release of calcium from intracellular stores and increasing neuronal excitability. These G-protein responses are thought to occur over longer periods of time, from seconds to minutes.

### TABLE 2.3 Drug Elimination

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Concentration in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 0</td>
<td>70 ng/ml in blood</td>
</tr>
<tr>
<td>t = 2.5 h</td>
<td>35 ng/ml</td>
</tr>
<tr>
<td>t = 5 h</td>
<td>17.5 ng/ml</td>
</tr>
<tr>
<td>t = 7.5 h</td>
<td>8.75 ng/ml</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>2.5 h</td>
</tr>
</tbody>
</table>

10 mg morphine, intramuscular injection
FIGURE 2.6 Molecular mechanisms of neuroadaptation. Shown are examples of ligand-gated ion channels such as the γ-aminobutyric acid-A (GABA A) receptor and glutamate N-methyl-D-aspartate (NMDA) receptor (NMR) and G-protein-coupled receptors, such as opioid, dopamine, or cannabinoid CB1 receptors, among others. These receptors modulate the levels of second messengers like cAMP and Ca\(^{2+}\), which in turn regulate the activity of protein kinase transducers. Cocaine and amphetamines, as indirect sympathomimetics, stimulate the release of dopamine which acts at G-protein-coupled receptors, specifically D1, D2, D3, D4, and D5. These G-protein receptors modulate via the α subunit the levels of second messengers like cyclic adenosine monophosphate (cAMP) and Ca\(^{2+}\), which in turn regulate the activity of protein kinase transducers. Such protein kinases affect the functions of proteins located in the cytoplasm, plasma membrane, and nucleus. Among the membrane proteins affected are ligand-gated and voltage-gated ion channels (VGCC). G i and G o proteins also can regulate K+ and Ca\(^{2+}\) channels directly through their βγ subunits. Protein kinase transduction pathways also affect the activities of transcription factors. Some of these factors, like cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), are regulated post-translationally by phosphorylation; others, like Fos, are regulated transcriptionally; still others, like Jun, are regulated both post-translationally and/or transcriptionally. While membrane and cytoplasmic changes may be only local (e.g., dendritic domains or synaptic boutons), changes in the activity of transcription factors may result in long-term functional changes. These may include changes in the gene expression of proteins involved in signal transduction and/or neurotransmission, resulting in altered neuronal responses. For example, chronic exposure to psychostimulants has
Dose-Response Functions

The effects of drugs vary according to the dose administered. The relationship between a given dose and the effects that this specific dose generates is called the dose-effect function (or dose–response curve). In many areas of pharmacology, dose-effect functions are monotonic (the functions move in only one direction), such as the relationship between an opioid dose and the response generated, which is typically analgesia (Figure 2.7). As the dose increases, pain relief increases – up to a point. At a certain level, called the asymptote, additional doses of the drug are unable to produce any additional effects. Dose–effect functions, however, can also be non-monotonic, such as the classic inverted U-shaped curve associated with locomotor activation produced by psychostimulant drugs (Figure 2.8). In such a dose-response function, the decreased effectiveness of cocaine/amphetamine in producing locomotor activity is attributable to an increase in stereotyped behavior.

Dose–effect functions describe two key characteristics of drug action: efficacy and potency. These two terms are often confused. Efficacy is the percentage of a maximum response. This can be seen on the Y-axis of the dose–response function in Figure 2.7. Potency is the dose required to produce a given effect relative to a standard. This can be seen on the X-axis of the dose-response function. Increases or decreases in effectiveness can be observed when comparing the levels reached by acetaminophen and morphine on the Y-axis in Figure 2.9. All three opioids shown in the figure are both more effective than acetaminophen (they produce a higher level of pain relief on the Y-axis). Further inspection of the figure also shows that the three opioids, although they have similar efficacy, have different potencies, reflected by

**FIGURE 2.6 (cont’d)**

been reported to increase the levels of protein kinase A (PKA) and adenylyl cyclase in the nucleus accumbens and decrease the levels of Gαi. Chronic exposure to psychostimulants also alters the expression of transcription factors themselves. CREB expression, for instance, is depressed in the nucleus accumbens by chronic cocaine treatment. Chronic cocaine induces a transition from Fos induction to the induction of the much longer-lasting Fos-related antigens such as ΔFosB. **Alcohol**, by acting on neurotransmitter systems, affects the phenotypic and functional properties of neurons through the general mechanisms outlined in the diagram. Chronic exposure to alcohol has been reported to increase the levels of PKA and adenylyl cyclase in the nucleus accumbens and decrease the levels of Gαi. Chronic exposure to alcohol also alters the expression of transcription factors themselves. CREB expression, for instance, is depressed in the nucleus accumbens and increased in the locus coeruleus by chronic morphine treatment, whereas chronic opioid exposure activates Fos-related antigens such as ΔFosB. **Nicotine** acts directly on ligand-gated ion channels. These receptors modulate the levels of Ca2+, which in turn regulate the activity of protein kinase transducers. Chronic exposure to nicotine has been reported to increase the levels of PKA in the nucleus accumbens. Chronic exposure to nicotine also alters the expression of transcription factors themselves. CREB expression, for instance, is depressed in the amygdala and prefrontal cortex and increased in the nucleus accumbens and ventral tegmental area. **Δ9-Tetrahydrocannabinol (THC)**, by acting on neurotransmitter systems, affects the phenotypic and functional properties of neurons through the general mechanisms outlined in the diagram. Cannabinoids act on the cannabinoid CB1 G-protein-coupled receptor. The CB1 receptor also is activated by endogenous cannabinoids such as anandamide. This receptor modulates (inhibits) the levels of second messengers like cAMP and Ca2+, which in turn regulate the activity of protein kinase transducers. Chronic exposure to THC also alters the expression of transcription factors themselves. CaMK, Ca2+/calmodulin-dependent protein kinase; ELK-1, E-26-like protein 1; PLCβ, phosphlipase C β; IP3, inositol triphosphate; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositite 3-kinase; R, receptor. [Modified with permission from Koob GF, Sanna PP, Bloom FE. Neuroscience of addiction. Neuron, 1998, (21), 467–476.]
Parallel shifts of the dose to the right or left on the X-axis: codeine is less potent than morphine, and morphine is less potent than hydromorphone.

**Therapeutic Ratio**

The therapeutic ratio is an index of the safety of a drug. The way to calculate the therapeutic

![Dose-Response Curve](image1)

**FIGURE 2.7** A typical example of a drug’s dose–response curve. As the dose of the drug increases, its effect increases – up to a certain point.

![Graph](image2)

**FIGURE 2.8** A classic example of an inverted U-shaped dose–response curve. Increasing doses of cocaine progressively increase locomotor activity – up to a point. When the dose gets high enough, locomotor activity starts to decrease. With cocaine, high doses cause stereotyped behavior. [Modified with permission from Baladi MG, Koek W, Aumann M, Velasco F, France CP. Eating high fat chow enhances the locomotor-stimulating effects of cocaine in adolescent and adult female rats. Psychopharmacology, 2012, (222), 447–457.]

![Graph](image3)

**FIGURE 2.9** Comparisons of efficacy and potency between different analgesics. Notice that hydromorphone is more potent than morphine but has similar efficacy. Acetaminophen is both less potent and less effective than the opioid analgesics. [Modified with permission from Levine RR. Pharmacology: Drug Actions and Reactions, 2nd edition. Little, Brown and Company, Boston, 1978.]
2. INTRODUCTION TO THE NEUROPSYCHOPHARMACOLOGY OF DRUG ADDICTION

BASIC NEUROBIOLOGY OF ADDICTION

Dopamine

Two major dopamine systems project to the basal ganglia from cell bodies in the ventral part of the midbrain: the mesocorticolimbic dopamine system and the nigrostriatal dopamine system. The nigrostriatal dopamine system projects from the substantia nigra to corpus striatum. Degeneration of this system is the primary basis for many of the motor dysfunctions associated with Parkinson’s disease. The activation of this system is also implicated in the focused repetitive behavior, called stereotyped behavior, that is associated with high doses of psychostimulants, such as cocaine and methamphetamine (Figure 2.11).

The mesocorticolimbic dopamine system projects from the ventral tegmental area to the nucleus accumbens, olfactory tubercle, amygdala, and frontal cortex. This system has been implicated in psychostimulant-induced locomotor activity, drug reward, and non-drug motivational attributes, such as incentive salience, conditioned reinforcement, and conditioned approach (for further reading, see Schultz, 2006).

Thus, dopamine neurons that project to the forebrain are associated with the initiation of behavior, reward, and motivational processes. Pharmacological manipulations that increase or decrease dopaminergic function provided early evidence of the role of the midbrain dopamine systems in reward. Pharmacological activation of dopamine synaptic activity, for example with the dopamine indirect agonist cocaine or amphetamine, produces behavioral activation, facilitated responding for many reinforcers, and decreased reward thresholds (that is, less stimulation is needed for a subject to perceive a stimulus as rewarding). Conversely, blockade of dopamine function decreases responding for both positive and negative reinforcers. Electrophysiological studies that measure the electrical activity of cells have shown that both unpredictable and predictable stimuli activate the firing of midbrain dopamine neurons. Some argue that mainly appetitive or rewarding events (like eating chocolate, having sex, or winning a card game) activated dopamine neurons in the mesocorticolimbic dopamine system. Such hedonic selectivity of the activation of dopamine neurons

![FIGURE 2.10 Example of how to calculate a drug’s therapeutic ratio.](image)
provides intriguing insights into the conceptualization of what constitutes positive rewards or incentives. Midbrain dopamine neurons may be part of the process by which rewards motivate or guide behavior, referred to as incentive motivation or incentive salience. Positive incentives paired with previously neutral stimuli through activation of the mesocorticolimbic dopamine system facilitate species-specific approach responses or changes in direction toward important incentives via dopamine release. As discussed in the various drug-specific chapters in this book, such incentive salience provides a powerful mechanism by which associations are made between previously neutral stimuli and drugs of abuse that pharmacologically facilitate the release of mesocorticolimbic dopamine.

Five different dopamine receptors, D1 through D5, have been identified. Dopamine acts through these receptors to produce its functional effects. The dopamine receptors fall into two main categories: D1-like (which are coupled to Gi proteins to inhibit adenylate cyclase; these include D1 and D5 receptors) and D2-like (which are coupled to G1 proteins to activate adenylate cyclase; these include D2, D3, and D4). Most pharmacological studies have been performed using agonists and antagonists of D1, D2, and D3 receptors, mainly because agents that are selective for these receptors are available. D1 and D2 receptors are widely distributed throughout the mesocorticolimbic and nigrostriatal dopamine systems. D3 receptors are localized to more specific subregions of the terminals of the mesocorticolimbic dopamine system in the rat, namely the shell subdivision of the nucleus accumbens and the Islands of Calleja.

**Norepinephrine**

Norepinephrine (also known as noradrenaline) is widely distributed in the central nervous system. It is involved in arousal, attention,
stress, anxiety, and mood disorders. Cell bodies for norepinephrine in the brain originate in the dorsal pons and brainstem (Figure 2.12). The dorsal pons contains the locus coeruleus, which is the source of the dorsal noradrenergic pathway to the cortices and hippocampus. The brainstem projections converge in the ventral noradrenergic bundle to innervate or activate the basal forebrain and hypothalamus. Norepinephrine, particularly in the forebrain, is released in the brain during stressful events and plays an important role in the anxiety/stress-like responses associated with drug dependence. Noradrenergic projections from the locus coeruleus play a key role in maintaining attentional homeostasis (regulating arousal/attention setpoint). For example, both increases and decreases in the activity of norepinephrine in the locus coeruleus are associated with disruptions in working memory.

Norepinephrine binds to three distinct receptors: α₁, α₂, and β. The α receptor subtypes are coupled to the inositol phosphate second messenger system via G_q proteins, or they inhibit adenylate cyclase by coupling to the inhibitory G_i protein. The β receptor subtype activates adenylate cyclase by coupling to the G_s protein.

### Opioid Peptides

Opioid peptides – β-endorphin, enkephalin, and dynorphin – are the endogenous ligands that naturally exist in the body and activate opioid receptors. Both opiate and opioid drugs bind to the same opioid receptor. An opiate is an alkaloid that resembles morphine and is derived from the opium poppy. An opioid is any drug (whether synthetic, semisynthetic, or endogenous) that binds to opioid receptors and has morphine-like effects. There are three types of opioid receptors: μ (mu), δ (delta), and κ (kappa).

Opioids have profound analgesic effects and rewarding properties. As such, they have both high medical use potential and high abuse potential. The analgesic effects of opioids are mediated by all three types of receptors. Their rewarding

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effects and dependence and addiction liability, however, are all largely mediated by the \( \mu \) receptor.

\( \beta \)-endorphin, the endogenous ligand for \( \mu \) opioid receptors, is largely derived from proopiomelanocortin cells in the arcuate nucleus in the brain. It is distributed throughout the brainstem and basal forebrain and released from corticotropes in the anterior lobe of the pituitary.

Methionine and leucine enkephalins are endogenous ligands for \( \delta \) opioid receptors. They are derived from the proenkephalin gene and have a widespread distribution in the basal forebrain, including the basal ganglia and midbrain, such as the periaqueductal gray.

Dynorphins can bind to all three opioid receptor subtypes but show a preference for \( \kappa \) receptors. They are derived from the prodynorphin precursor and contain the leucine (leu)-enkephalin sequence at the \( N \)-terminal portion of the molecule. Dynorphins are widely distributed in the central nervous system and play important roles in neuroendocrine regulation, pain regulation, motor activity, cardiovascular function, respiration, temperature regulation, feeding behavior, and stress responsivity. Dynorphin cell bodies and axon terminals are heavily localized to the central nucleus of the amygdala, bed nucleus of the stria terminalis, and nucleus accumbens shell (Figure 2.13). Activation of the dynorphin-\( \kappa \) receptor system produces actions that are similar to other opioids, such as analgesia, but the actions are often opposite to those of \( \mu \) opioid receptors in the motivational domain. For example, \( \mu \) agonists cause euphoria-like effects, and \( \kappa \) agonists produce dysphoric-like effects in animals and humans. Some evidence suggests that they also mediate negative emotional states. As a link between dopaminergic and opioidergic systems, dopamine D\(_1\) receptor activation in the nucleus accumbens shell can phosphorylate (turn on) cyclic adenosine monophosphate response element binding protein (CREB) and subsequently alter gene expression, notably the transcription of proctdynorphin and prodynorphin. Such activation of dynorphin systems has been suggested to contribute to the dysphoric-like syndrome associated with cocaine dependence and feedback to decrease dopamine release. Such enhanced dynorphin action may also drive corticotropin-releasing factor (a key neurotransmitter in the stress system) responses or be driven by activation of this stress hormone (see Chapter 4).

**Corticotropin-Releasing Factor**

Stress is a major factor in drug relapse, and corticotropin-releasing factor (CRF) is key to the body’s stress response. A large, 41-amino-acid polypeptide with a wide distribution throughout the brain, CRF cell bodies are prominent in the paraventricular nucleus of the hypothalamus, basal forebrain (notably the extended amygdala), and brainstem (Figure 2.14). When CRF is administered directly into rodents, it mimics behavioral activation and the stress response, and CRF receptor antagonists generally have anti-stress effects.

Two major CRF receptors have been identified: CRF\(_1\) and CRF\(_2\). CRF\(_1\) receptor activation is associated with activation of the hypothalamic–pituitary–adrenal (HPA) axis neuroendocrine response to stress and increased stress responsiveness at the behavioral and physiological (autonomic) extrahypothalami (outside the HPA axis). The HPA axis is a term that describes the relationship between direct releasing factor/hormonal actions and feedback between the hypothalamus, pituitary gland, and adrenal gland. CRF\(_2\) receptor activation is associated with decreased feeding behavior and decreased stress responsiveness, although there is some controversy in this area. Both CRF receptor subtypes belong to a subfamily of G-protein-coupled receptors. CRF itself has preferential affinity for CRF\(_1\) rather than CRF\(_2\) receptors. Other CRF-related neuropeptides include urocortins, and some of these preferentially bind CRF\(_2\) receptors. The distribution of CRF\(_1\) receptors in the brain is highly consistent across mammalian species in stress-responsive brain regions, including the neocortex, central
FIGURE 2.13  Dynorphin localization. Schematic representation of the distribution of prodynorphin-derived peptides in the rat’s central nervous system determined by immunohistochemistry. Prodynorphin codes for several active opioid peptides containing the sequence of [Leu]enkephalin, including dynorphin A, dynorphin B, and α-neoendorphin. This precursor
is distributed in neuronal systems found at all levels of the neuraxis. Like their proenkephalin counterparts, the prodynorphin neurons form both short- and long-tract projections often found in parallel with the proenkephalin systems. Neuronal perikarya are shown as solid circles, and fiber-terminals are shown as short curved lines and dots. AA, anterior amygdala; ABL, basolateral nucleus of amygdala; AC, anterior commissure; ACB, nucleus accumbens; ACE, central nucleus of the amygdala; ACO, cortical nucleus of amygdala; AD, anterodorsal nucleus of thalamus; AL, anterior lobe of pituitary; AM, anteromedial nucleus of thalamus; AMB, nucleus ambiguus; AME, medial nucleus of the amygdala; AON, anterior olfactory nucleus; ARC, arcuate nucleus; AV, anteroventral nucleus of thalamus; BST, bed nucleus of the stria terminalis; CC, corpus callosum; CGX, cingulate cortex; CM, central-medial nucleus of thalamus; COCH, cochlear nuclear complex; CPU, caudate-putamen; CST, corticospinal tract; DH, dorsal horn of spinal cord; DG, dentate gyrus; DM, dorsomedial nucleus of hypothalamus; DNV, dorsal motor nucleus of vagus; DTN, dorsal tegmental nucleus; ENT, entorhinal cortex; FN, fastigial nucleus of cerebellum; FRX, frontal cortex; GL, glomerular layer of olfactory bulb; GP, globus pallidus; HM, medial habenular nucleus; HPC, hippocampus; IC, inferior colliculus; IL, intermediate lobe of pituitary; IP, interpeduncular nuclear complex; LC, locus coeruleus; LG, lateral geniculate nucleus; LHA, lateral hypothalamic area; LRN, lateral reticular nucleus; MF, mossy fibers of hippocampus; MOUNTAIN, motor facial nucleus; MFG, medial geniculate nucleus; ML, medial lemniscus; MM, medial mammillary nucleus; MNT, mesencephalic nucleus of trigeminal; MVM, medial vestibular nucleus; NCU, nucleus cuneatus; NCX, neocortex; NDB, nucleus of diagonal band; NL, neural lobe of pituitary; NRG, nucleus reticularis gigantocellularis; NRPG, nucleus reticularis paragigantocellularis; NTS, nucleus tractus solitarius; OCX, occipital cortex; OT, optic tract; OTU, olfactory tubercle; PAG, periaqueductal gray; PAX, periamygdaloid cortex; PBN, parabrachial nucleus; PC, posterior commissure; PIR, piriform cortex; PN, pons; POA, preoptic area; PP, perforant path; PV, periventricular nucleus of thalamus; PVN(M), paraventricular nucleus (pars magnocellularis); PVN(P), paraventricular nucleus (pars parvocellularis); RD, nucleus raphe dorsalis; RE, nucleus reuniens of thalamus; RF, reticular formation; RM, nucleus raphe magnus; RME, nucleus raphe medianus; SC, superior colliculus; SCP, superior cerebellar peduncle; SM, stria medullaris thalami; SNC, substantia nigra (pars compacta); SNR, substantia nigra (pars reticulata); SNT, sensory nucleus of trigeminal (main); SON, supraoptic nucleus; SPT, septal nuclei; STN, spinal nucleus of trigeminal; SUB, subiculum; VM, ventromedial nucleus of hypothalamus; VP, ventral pallidum; ZI, zona incerta. [Modified with permission from Khachaturian H, Lewis ME, Schafer MKH, Watson SJ. Anatomy of the CNS opioid systems. Trends in Neurosciences, 1985, (8), 111–119.]
2. INTRODUCTION TO THE NEUROPSYCHOPHARMACOLOGY OF DRUG ADDICTION

Vasopressin

Vasopressin has an hormonal function as an antidiuretic hormone that helps control water balance in the body. It is derived from the posterior pituitary within the HPA axis but also has extrahypothalamic actions in the central nervous system. Vasopressin is distributed widely in the brain outside of the HPA axis, with the highest concentrations in the suprachiasmatic and supraoptic nuclei and substantial levels in the septum and locus coeruleus. Vasopressin neurons innervate the extended amygdala and are derived from cell bodies in the medial bed nucleus of the stria terminalis. The distribution of vasopressin receptors is prominent in the rat’s extended amygdala, with high concentrations in the division of the extended amygdala, medial septum, hippocampus, hypothalamus, thalamus, cerebellum, and autonomic midbrain and hindbrain nuclei. This receptor distribution, concordant with its natural ligand CRF, is consistent with the role for CRF₁ receptors outside the HPA axis in behavioral and physiological (autonomic) stress responses.

FIGURE 2.14 Corticotropin-releasing factor localization. The major CRF-stained cell groups (dots) and fiber systems in the rat brain. Most of the immunoreactive cells and fibers appear to be associated with systems that regulate the output of the pituitary and autonomic nervous system and with cortical interneurons. Most of the longer central fibers course either ventrally through the medial forebrain bundle and its caudal extension in the reticular formation, or dorsally through a periventricular system in the thalamus and brainstem central gray. The direction of fibers in these systems is unclear because they appear to interconnect regions that contain CRF-stained cell bodies. Three adjacent CRF-stained cell groups – laterodorsal tegmental nucleus, locus coeruleus, parabrachial nucleus – lie in the dorsal pons. It is uncertain which of these cell groups contributes to each of the pathways shown and which of them receives inputs from the same pathways. ac, anterior commissure; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CeA, central nucleus of the amygdala; CG, central gray; DR, dorsal raphe; DVC, dorsal vagal complex; HIP, hippocampus; LDT, laterodorsal tegmental nucleus; LHA; lateral hypothalamic area; ME; median eminence; mfb, medial forebrain bundle; MID THAL, midline thalamic nuclei; MPO, medial preoptic area; MR, median raphe; MVN, medial vestibular nucleus; PB, parabrachial nucleus; POR, periculomotor nucleus; PP, peripeduncular nucleus; PVN, paraventricular nucleus; SEPT, septal region; SI, substantia innominata; st, stria terminalis. [Modified with permission from Swanson LW, Sawchenko PE, Rivier J, Vale W. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology, 1983, (36), 165–186.]
in the lateral and supracapsular bed nucleus of the stria terminalis, central nucleus of the amygdala, and nucleus accumbens shell. Vasopressin produces autonomic arousal-promoting effects in brain structures relevant to memory, including the hippocampus. Vasopressin V₁b receptor antagonists have been shown to produce anxiolytic-like and antidepressant-like effects in animal models, and such anxiolytic-like actions were shown to be localized to the amygdala (Figure 2.15).

Neuropeptide Y

Neuropeptide Y (NPY) is a 36-amino-acid polypeptide that is also widely distributed throughout the central nervous system, with particularly high concentrations in the hypothalamus, periaqueductal gray, and extended amygdala (Figure 2.16). Administration of NPY directly into the brain increases feeding behavior, reduces anxiety-like behavior, and augments the effects of sedative hypnotics. The amygdala is a possible site that mediates the anti-stress effects of NPY. Multiple NPY receptor subtypes have been identified, and the Y₁ and Y₂ receptors have been the most implicated in stress actions. The Y₁ receptor has a wide distribution in the rat brain. It is found most abundantly in the cortex, olfactory tubercle, hippocampus, hypothalamus, and thalamus. The distribution of Y₂ receptors is similar to that of the Y₁ receptors, although Y₂ receptors are less abundant in the cortex and thalamus and more abundant in the hippocampus.
Nociceptin

Nociceptin (also known as orphanin FQ) is a 17-amino-acid polypeptide that is structurally related to the opioid peptide dynorphin A. It is the endogenous ligand for the nociceptin opioid (NOP) receptor (formerly referred to as opioid receptor-like-1). Nociceptin does not bind to \( \mu \), \( \delta \), or \( \kappa \) opioid receptors, and no known exogenous or endogenous opioids bind to the NOP receptor. The neuroanatomical distribution of nociceptin and its receptor are distinct from other opioid peptides. The highest density of nociceptin and the NOP receptor is in the cortex, amygdala, bed nucleus of the stria terminalis, medial prefrontal cortex, ventral tegmental area, lateral hypothalamus, nucleus accumbens, and many brainstem areas, including the locus coeruleus and raphe (Figure 2.17).

NOP receptor agonists and antagonists have numerous functional effects that are related to anxiety-like and stress-like states. Nociceptin blocks stress-induced analgesia triggered by the release of endogenous opioids. Nociceptin generally attenuates adaptive behaviors to stress, such as opioid and non-opioid stress-induced analgesia and stress-induced anorexia. Nociceptin and small-molecule synthetic nociceptin analogs have a broad anxiolytic-like profile in animals and also reverse stress-induced anorexia. Nociceptin may play a role in the addiction process that is independent of any classic opioid action. Nociceptin and synthetic NOP receptor agonists decrease the acute rewarding effects of drugs of abuse in the conditioned place preference paradigm. They also block alcohol consumption in a genetically selected line of rats that is known to be hypersensitive to stressors and decrease reinstatement of drug seeking behavior.
FIGURE 2.17  Nociceptin/orphanin FQ localization. Schematic representation of the distribution of nociceptin peptide in the rat central nervous system determined by immunohistochemistry and in situ hybridization. Neuronal perikarya are shown as solid circles, and fiber-terminals are shown as short curved lines and dots. AA, anterior amygdala; ABL, basolateral nucleus of amygdala; AC, anterior commissure; ACB, nucleus accumbens; ACE, central nucleus of the amygdala; ACO, cortical nucleus of amygdala; AD, anterodorsal nucleus of thalamus; AL, anterior lobe of pituitary; AM, anteromedial nucleus of thalamus; AMB, nucleus ambiguus; AME, medial nucleus of the amygdala; AON, anterior olfactory nucleus; ARC, arcuate nucleus; AV, anteroventral nucleus of thalamus; BST, bed nucleus of the stria terminalis; CC, corpus callosum; CGX, cingulate cortex; CM, central-medial nucleus of thalamus; COCH, cochlear nuclear complex; CPU, caudate-putamen; CST, corticospinal tract; DH, dorsal horn of spinal cord; DG, dentate gyrus; DM, dorsomedial nucleus of hypothalamus; DNV, dorsal motor nucleus of vagus; DTN, dorsal tegmental nucleus; ENT, entorhinal cortex; FN, fastigial nucleus of cerebellum; FRX, frontal cortex; GL, glomerular layer of olfactory bulb; GP, globus pallidus; HM, medial habenular nucleus; HPC, hippocampus; IC, inferior colliculus; IL, intermediate lobe of pituitary; IP, interpeduncular nucleus complex; LC, locus coeruleus; LG, lateral geniculate nucleus; LHA, lateral hypothalamic area; LRN, lateral reticular nucleus; MF, mossy fibers of hippocampus; MFN, motor facial nucleus; MG, medial geniculate nucleus; ML, medial lemniscus; MM, medial mammillary nucleus; MNT, mesencephalic nucleus of trigeminal; MVN, medial vestibular nucleus; NCU, nucleus cuneatus; NCX, neocortex; NDB, nucleus of diagonal band; NL, neural lobe of pituitary; NRGC, nucleus reticularis gigantocellularis; NRPG, nucleus reticularis paragigantocellularis; NTS, nucleus tractus solitarius; OCX, occipital cortex; OT, optic tract; OTU, olfactory tubercle; PAG, periaqueductal gray; PAX, periamygdaloid cortex; PBN, parabrachial nucleus; PC, posterior commissure; PIR, piriform cortex; PN, pons; POA, preoptic area; PP, perforant path; PV, periventricular nucleus of thalamus; PVN(M), paraventricular nucleus (pars magnocellularis); PVN(P), paraventricular nucleus (pars parvocellularis); RD, nucleus raphe dorsalis; RE, nucleus reuniens of thalamus; RF, reticular formation; RM, nucleus raphe magnus; RME, nucleus raphe medianus; SC, superior colliculus; SCP, superior cerebellar peduncle; SM, stria medullaris thalami; SNC, substantia nigra (pars compacta); SNR, substantia nigra (pars reticulata); SNT, sensory nucleus of trigeminal (main); SON, supraoptic nucleus; SPT, septal nuclei; STN, spinal nucleus of trigeminal; SUB, substantia; VM, ventromedial nucleus of hypothalamus; VP, ventral pallidum; ZI, zona incerta. [Taken with permission from Koob GF. A role for brain stress systems in addiction. Neuron, 2008, (59), 11–34.]
BRAIN STRUCTURES AND FUNCTIONS RELEVANT TO THE THREE STAGES OF THE ADDICTION CYCLE

As described in Chapter 1, a three-stage framework can be used to explore the behavioral, neurobiological, and treatment perspectives of addiction: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. One can also utilize this framework to understand the basic neuroanatomy and neurocircuitry of addiction.

**Binge/Intoxication Stage – Basal Ganglia**

The binge/intoxication stage heavily involves the basal ganglia. The basal ganglia are considered a key part of the extrapyramidal motor system and are historically associated with a number of key functions, including voluntary motor control, procedural learning related to routine behaviors or habits, and action selection. The basal ganglia include the following structures: striatum, globus pallidus, substantia nigra, and subthalamic nucleus (Figure 2.18). The striatum can be further divided into the ventral striatum and dorsal striatum. The ventral striatum includes the nucleus accumbens, olfactory tubercle, and ventral pallidum. This is a subarea of the basal ganglia that has gained recognition for its involvement in motivation and reward function. The ventral striatum is now considered a major integrative center for converting motivation to action. In the domain of addiction, it mediates the rewarding effects of drugs of abuse. The basal ganglia receive neurochemical inputs (or afferents) from the prefrontal cortex and midbrain dopamine system. They then send neurochemical signals (or efferents) from the globus pallidus to the thalamus, which then relays motor and sensory signals to the cerebral cortex. The functions of the basal ganglia involve a series of cortical–striatal–pallidal–thalamic–cortical loops that encode habits related to compulsive behavior (Figure 2.19).

Positive reinforcement with drugs of abuse occurs when presentation of a drug increases the probability of a response to obtain the drug and usually refers to producing a positive hedonic state. Animal models of the positive reinforcing or rewarding effects of drugs in the absence of withdrawal or deprivation are extensive and well validated. These models include intravenous drug self-administration, conditioned place preference, and decreased brain stimulation reward thresholds (see Chapter 3, Animal Models).

The acute reinforcing effects of drugs of abuse are mediated by brain structures connected by the medial forebrain bundle reward system, with a focus on the ventral tegmental area, nucleus accumbens, and amygdala. Much evidence supports the hypothesis that the mesocorticolimbic dopamine system, projecting from the ventral tegmental area to the nucleus accumbens, is dramatically activated by psychostimulant drugs during limited-access self-administration. This system is critical for mediating the rewarding effects of cocaine, amphetamines, and nicotine. However, although acute administration of other drugs of abuse activates the dopamine systems, opioids and alcohol have both dopamine-dependent and -independent rewarding effects (Figure 2.20). μ opioid receptors in both the nucleus accumbens and ventral tegmental area mediate the reinforcing effects of opioid drugs. Opioid peptides in the ventral striatum and amygdala mediate the acute reinforcing effects of alcohol, largely observed experimentally through the effects of opioid antagonists and in knockout mice. γ-Aminobutyric acid (GABA) systems are activated pre- and postsynaptically in the extended amygdala by alcohol at intoxicating doses, and GABA receptor antagonists block alcohol self-administration. A specific nicotinic receptor, the α4β2 subtype, either in the ventral tegmental area or nucleus accumbens, mediates the reinforcing effects of nicotine.
via actions on the mesocorticolimbic dopamine system. The cannabinoid CB₁ receptor, involving the activation of dopamine and opioid peptides in the ventral tegmental area and nucleus accumbens, mediates the reinforcing actions of marijuana.

Drugs of abuse have a profound effect on the response to previously neutral stimuli to which the drugs become paired; a phenomenon called conditional reinforcement and now linked with the concept of “incentive salience.” Psychostimulants caused rats to show compulsive-like lever pressing in response to a cue that was previously paired with a water reward (for further reading of this seminal finding, see Robbins, 1976).

In a subsequent series of studies that recorded the electrical activity of ventral tegmental area dopamine neurons in primates during repeated presentation of rewards and presentation of stimuli associated with...
reward, dopamine cells fired at the first exposure to the novel reward, but repeated exposure caused the neurons to stop firing during reward consumption and instead fire when they were exposed to stimuli that were predictive of the reward (for further reading, see Schultz et al., 1997). Through the process of conditioning, previously neutral stimuli are linked to either a natural or drug reinforcer and acquire the ability to increase dopamine levels in the nucleus accumbens in anticipation of the reward, thus engendering strong motivation to seek the drug, termed incentive salience.

As noted previously, all drugs of abuse can initially elicit increased physiological dopamine release in the nucleus accumbens. This drug-induced dopamine signaling can eventually trigger neuroadaptations in other basal ganglia brain circuits that are related to habit formation. Key synaptic changes involve glutamate-modulated \( N \)-methyl-D-aspartate (NMDA) receptors and \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in glutamatergic projections from the prefrontal cortex and amygdala to the ventral tegmental area and nucleus accumbens (for further reading, see Kalivas, 2009; Lüscher and Malenka, 2011; Wolf and Ferrario, 2010). The power of initial dopamine release (and activation of opioid peptide systems) upon initial drug taking begins the neuroadaptations that lead to tolerance and withdrawal and triggers the ability of drug-associated cues to increase dopamine levels in the dorsal striatum, a region that is involved in habit formation (for further reading, see Belin et al., 2009) and the strengthening of those habits as addiction progresses. The recruitment of these circuits is significant for the progression through the addiction cycle because such conditioned responses help explain the intense desire for the drug (craving) and its compulsive use when subjects with addiction are exposed to drug cues. Conditioned responses within the incentive salience process can drive dopamine signaling to maintain the motivation to take the drug even when the direct pharmacological effects of the drug lessen.

**Withdrawal/Negative Affect Stage – Extended Amygdala**

The withdrawal/negative affect stage involves key elements of the extended amygdala. The extended amygdala consists primarily of three structures: the central nucleus of the amygdala, bed nucleus of the stria terminalis, and a transition zone in the nucleus accumbens shell (for those interested in learning more about the anatomy of this structure, see Alheid et al., 1995). The extended amygdala can also be divided into two major divisions: central...
division and medial division. These two divisions have important anatomical structural differences and dissociable afferent and efferent connections.

The central division of the extended amygdala includes the central nucleus of the amygdala, central sublenticular extended amygdala, lateral bed nucleus of the stria terminalis, and a transition area in the medial and caudal portions of the nucleus accumbens (Figure 2.21). These structures in the central division have similar morphology (structure), immunohistochemistry (proteins associated with neurotransmission), and connectivity, and they receive afferent connections from limbic cortices, the hippocampus, the basolateral amygdala, the

![Converging Acute Actions of Drugs of Abuse on the Ventral Tegmental Area and Nucleus Accumbens](image)

**FIGURE 2.20** Simplified schematic of converging acute actions of drugs of abuse on the ventral tegmental area (VTA) and nucleus accumbens (NAc). Drugs of abuse, despite diverse initial actions, produce some common effects on the VTA and NAc. Stimulants directly increase dopaminergic transmission in the NAc. Opiates do the same indirectly: they inhibit γ-aminobutyric acid (GABA) interneurons in the VTA, which disinhibits VTA dopamine neurons. Opiates also directly act on opioid receptors on NAc neurons, and opioid receptors, like dopamine (DA) D₂ receptors, signal via Gᵢ proteins. Hence, the two mechanisms converge within some NAc neurons. The actions of the other drugs remain more conjectural. Nicotine seems to activate VTA dopamine neurons directly by stimulating nicotinic cholinergic receptors on those neurons and indirectly by stimulating its receptors on glutamatergic nerve terminals that innervate dopamine cells. Alcohol, by promoting GABAₐ receptor function, may inhibit GABAergic terminals in the VTA and hence disinhibit VTA dopamine neurons. It may similarly inhibit glutamatergic terminals that innervate NAc neurons. Many additional mechanisms (not shown) are proposed for alcohol. Cannabinoid mechanisms seem complex, and they involve the activation of cannabinoid CB₁ receptors (which, like D₂ and opioid receptors, are Gᵢ-linked) on glutamatergic and GABAergic nerve terminals in the NAc and on NAc neurons themselves. Phencyclidine (PCP) may act by inhibiting postsynaptic NMDA glutamate receptors in the NAc. Finally, there is some evidence that nicotine and alcohol may activate endogenous opioid pathways and that these and other drugs of abuse (such as opiates) may activate endogenous cannabinoid pathways (not shown). PPT/LDT, peduncular pontine tegmentum/lateral dorsal tegmentum. [Modified with permission from Nestler EJ. Is there a common molecular pathway for addiction? Nature Neuroscience, 2005, (8), 1445–1449.]
midbrain, and the lateral hypothalamus. The efferent connections from this complex include the posterior medial (sublenticular) ventral pallidum, ventral tegmental area, various brainstem projections, and a considerable projection to the lateral hypothalamus. The extended amygdala includes major components of the brain stress systems associated with the negative reinforcement of dependence. The central division has also been found to receive cortical information and regulate the hypothalamic–pituitary–adrenal stress axis.

The medial division of the extended amygdala consists of the medial bed nucleus of the stria terminalis, medial nucleus of the amygdala, and medial sublenticular extended amygdala. It appears to be more involved in sympathetic (fight-or-flight) and physiological responses and receives olfactory information. Most motivational experimental manipulations that modify the reinforcing effects of drugs of abuse through both positive and negative reinforcement appear to do so by impacting the central division: central nucleus of the amygdala and lateral bed nucleus of the stria terminalis.

Negative reinforcement occurs when the removal of an aversive event increases the probability of a response. In the case of addiction, negative reinforcement involves the removal of a negative emotional state associated with withdrawal, such as dysphoria, anxiety, irritability, sleep disturbances, and hyperkatifeia. Such negative emotional

![Figure 2.21](image-url)

**FIGURE 2.21** Brain regions recruited during the withdrawal/negative affect stage of the addiction cycle. [Modified with permission from Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology Reviews, 2010, (35), 217–238 (erratum: 35: 1051).]
states are thought to derive from two sources: within-system changes and between-system changes.

- **Within-system neuroadaptations in the reward system.** During the development of dependence, the brain systems in the ventral striatum that are important for the acute reinforcing effects of drugs of abuse, such as dopamine and opioid peptides, become compromised and begin to contribute to a negative reinforcement mechanism, in which the drug is administered to restore the decreased function of the reward systems. Within-system changes within medium spiny neurons in the nucleus accumbens during acute withdrawal include decreased long-term potentiation, increased trafficking of AMPA receptors to the surface of neurons, increased adenylate cyclase activity, and increased CREB phosphorylation. Some of these changes may precede or drive between-system neuroadaptations. Neurochemical evidence of within-system neuroadaptations includes the observation that chronic administration of all drugs of abuse decreases the function of the mesocorticolimbic dopamine system. Decreases in neuronal firing rate in the mesocorticolimbic dopamine system and decreases in serotonergic neurotransmission in the nucleus accumbens occur during drug withdrawal. Decreases in the firing of dopamine neurons in the ventral tegmental area have also been observed during withdrawal from opioids, nicotine, and ethanol. Imaging studies in drug-addicted humans have also consistently shown long-lasting decreases in the number of dopamine D₂ receptors in drug abusers compared with controls. Additionally, drug abusers have reduced dopamine release in response to a pharmacological challenge with drugs. Decreases in the number of dopamine D₂ receptors, coupled with the decrease in dopaminergic activity in cocaine, nicotine, and alcohol abusers, results in decreased sensitivity of reward (incentive salience) circuits to stimulation by natural reinforcers. These findings suggest an overall reduction of the sensitivity of the dopamine component of reward circuitry to natural reinforcers and other drugs in drug-addicted individuals (Figure 2.22).

- **Between-system neuroadaptations in the extended amygdala.** The neuroanatomical substrates for many of the motivational effects of drug dependence may also involve between-system neuroadaptations that occur in the ventral striatum and extended amygdala, which includes neurotransmitters associated with the brain stress systems involved in the negative reinforcement of dependence. Several neurotransmitters localized to the extended amygdala, such as CRF, norepinephrine, and dynorphin, are activated during states of stress and anxiety and during drug withdrawal (Figure 2.22). Antagonists of these neurochemical systems selectively block drug self-administration in dependent animals, suggesting a key role for these neurotransmitters in the ventral striatum and extended amygdala in the negative reinforcement associated with drug dependence.

To summarize the roles of positive and negative reinforcement in addiction, the brain reward (incentive salience) system is implicated in both the positive reinforcement produced by drugs of abuse and the negative reinforcement produced by dependence, mediated by dopamine in the ventral striatum. Neuropharmacological studies in animal models of addiction have provided evidence of the dysregulation of specific neurochemical mechanisms in specific positive reinforcement (reward) systems in the ventral striatum (dopamine, opioid peptides, and GABA). Importantly, however, brain stress systems (CRF, dynorphin, and norepinephrine)
FIGURE 2.22 Diagram of the hypothetical “within-system” and “between-system” changes that lead to the “dark side” of addiction. (Top) Circuitry for drug reward with major contributions from mesolimbic dopamine and opioid peptides that converge on the nucleus accumbens. During the binge/intoxication stage of the addiction cycle, the reward circuitry is excessively engaged. (Middle) Such excessive activation of the reward system triggers “within-system” neurobiological adaptations during the withdrawal/negative affect stage, including activation of cyclic adenosine monophosphate (cAMP) and cAMP response element binding protein (CREB), downregulation of dopamine D₂ receptors, and decreased firing of ventral tegmental area (VTA) dopaminergic neurons. (Bottom) As dependence progresses and the withdrawal/negative affect stage is repeated, two major “between-system” neuroadaptations occur. One is activation of dynorphin feedback that further decreases dopaminergic activity. The other is recruitment of extrahypothalamic norepinephrine (NE)-corticotropin-releasing factor (CRF) systems in the extended amygdala. Facilitation of the brain stress system in the prefrontal cortex is hypothesized to exacerbate the between-system neuroadaptations while contributing to the persistence of the dark side into the preoccupation/anticipation stage of the addiction cycle. [Taken with permission from Koob GF. Negative reinforcement in drug addiction: the darkness within. Current Opinion in Neurobiology, 2013, (23), 559–563.]
are also recruited in the extended amygdala to contribute to the negative motivational state associated with drug abstinence, which in turn drives an additional source of negative reinforcement in drug addiction.

**Preoccupation/Anticipation Stage – Prefrontal Cortex**

The preoccupation/anticipation ("craving") stage involves key elements of the prefrontal cortex. The global function of the prefrontal cortex is to mediate executive function. Executive function can be conceptualized as the ability to organize thoughts and activities, prioritize tasks, manage time, and make decisions. To accomplish such complex tasks in the context of the neurobiology of addiction, the prefrontal cortex can be divided into two opposing systems: the Go system and the Stop system. The Go system engages habit systems, possibly even subconsciously and automatically. The Stop system inhibits such systems. The result of the interactions between these two systems produces the well-known impulsivity associated with the addiction process, both during the initiation of drug intake and relapse.

The Go system involves the anterior cingulate cortex and dorsolateral prefrontal cortex. The anterior cingulate cortex facilitates the maintenance and selection of responses, particularly under high attentional demands (like comprehending the information contained in this book), planning (for the midterm exam), self-initiation (getting yourself to class), and self-monitoring of goal-directed behaviors. The functions of the dorsolateral prefrontal cortex involve working memory, planning, and strategy.

The Stop system largely involves the ventrolateral prefrontal cortex and orbitofrontal cortex. The functions of the ventrolateral prefrontal cortex involve response inhibition, sustained attention, memory retrieval, rule generation, and shifting. The functions of the orbitofrontal cortex, including the ventromedial prefrontal cortex, include the assignment of value (valuation) and integration of reward and punishment (Figure 2.23). The anterior cingulate cortex and dorsolateral prefrontal cortex in humans correspond to the anterior cingulate cortex and prelimbic cortex in rats, and the ventromedial prefrontal cortex and orbitofrontal cortex in humans correspond to the infralimbic cortex and orbitofrontal cortex in rats (Figure 2.24).

The preoccupation/anticipation stage of the addiction cycle is a key element of relapse in humans, defining addiction as a chronic relapsing disorder. Although often linked to the construct of craving, the concept of craving per se has been difficult to measure in human clinical studies and often does not correlate with relapse. Nevertheless, the stage of the addiction cycle at which an individual reinstates drug seeking behavior after abstinence remains a challenging focus of neurobiological studies and medication development.

Animal models of craving can be divided into two domains:

**i)** Drug seeking induced by the drug or stimuli paired with drug taking (reward craving), and

**ii)** Drug seeking induced by an acute stressor or state of stress (relief craving; Table 2.4).

Drug-induced reinstatement appears to be localized to a medial prefrontal cortex/ventral striatum circuit mediated by the neurotransmitter glutamate. Cue-induced reinstatement appears to involve the basolateral amygdala, with a possible feed-forward mechanism that goes through the same prefrontal cortex system involved in drug-induced reinstatement. Neurotransmitter systems involved in drug-induced reinstatement include a glutamate projection from the frontal cortex to nucleus accumbens that is modulated by dopamine in the frontal cortex. Cue-induced reinstatement also involves a glutamate projection from the basolateral amygdala and ventral
2. INTRODUCTION TO THE NEUROPSYCHOPHARMACOLOGY OF DRUG ADDICTION

subiculum to the nucleus accumbens. Stress-induced reinstatement depends on activation of both CRF and norepinephrine in the extended amygdala. Protracted abstinence, largely described in alcohol dependence models, involves both an overactive glutamatergic Go system and sensitized CRF systems. Brain CRF stress systems remain hyperactive during protracted abstinence, and this hyperactivity has motivational significance for excessive alcohol drinking.

Executive control over incentive salience is essential to maintain goal-directed behavior and the flexibility of stimulus-response associations. The prefrontal cortex sends glutamatergic projections directly to mesocortical dopamine neurons in the ventral tegmental area, exerting excitatory control on dopamine in the prefrontal cortex. Thus, the ventral part of the prefrontal cortex (the Stop system) can inhibit incentive salience and suppress conditioned behavior when a salient cue is present. It follows that lesions of the prefrontal cortex can induce impulsivity. Withdrawal from alcohol is associated with increased glutamate release in the nucleus accumbens and other brain areas. However, cue-induced reinstatement of psychostimulant-seeking behavior dramatically increases dorsal prefrontal cortex activity (Go system) and glutamate release in the nucleus accumbens. The increased activity of the prefrontal–glutamatergic system during

FIGURE 2.23 Brain regions recruited during the preoccupation/anticipation stage of the addiction cycle. [Modified with permission from Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology Reviews, 2010, (35), 217–238 (erratum: 35: 1051).]
relapse may elicit a dramatic glutamatergic response that may in turn mediate craving-like responses during the preoccupation/anticipation stage.

Behavioral procedures have been developed to reinstate drug self-administration using previously neutral stimuli that are paired with alcohol self-administration or that predict alcohol self-administration. Cue-induced reinstatement can be blocked by opioid receptor antagonists, dopamine D₁ and D₂ receptor antagonists, and glutamate receptor antagonists. Stress exposure can also reinstate responding for drugs in rats that are extinguished from drug-seeking behavior. Such stress-induced reinstatement can be blocked by CRF antagonists, dynorphin antagonists, and norepinephrine antagonists.

Human imaging studies reveal similar circuit dysregulation during the preoccupation/anticipation stage to that demonstrated in animal models. Decreased frontal cortex activity parallels deficits in executive function in neuropsychologically challenging tasks. Individuals with alcoholism exhibit impairments in the maintenance of spatial information, disruption of decision making, and impairments in behavioral inhibition. Such frontal cortex-derived executive function disorders have been linked to the ineffectiveness of some behavioral treatments in individuals with alcoholism. Thus, individual differences in the prefrontal cortical control of incentive salience may also explain individual differences in the vulnerability to addiction. Excessive attribution of incentive salience to drug-related cues and residual hypersensitivity of the brain stress systems may perpetuate excessive drug intake, compulsive behavior, and relapse.

FIGURE 2.24 Correspondence between rat and human brain regions that are relevant to the addiction process. Rats are commonly studied to unveil the neurobiological mechanisms of addiction because they have a well-characterized central nervous system whose neurochemical and molecular pathways in subcortical areas correspond reasonably well to those in humans. ACC, anterior cingulate cortex; PL, prelimbic cortex; IL, infralimbic cortex; OFC, orbitofrontal cortex; INS, insula; dIPFC, dorsolateral prefrontal cortex; vIPFC, ventrolateral prefrontal cortex; DS, dorsal striatum; Thal, thalamus; GP, globus pallidus; NAC, nucleus accumbens; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; HPC, hippocampus. [Modified with permission from George O, Koob GF. Control of craving by the prefrontal cortex. Proceedings of the National Academy of Sciences USA, 2013, (110), 4165–4166.]
Drug addiction involves a three-stage cycle – binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation – that worsens over time and involves allostatic changes in the brain reward and stress systems. Two primary sources of reinforcement, positive and negative reinforcement, have been hypothesized to play a role in this allostatic process (see Chapter 1).

The construct of negative reinforcement is defined as drug taking that alleviates a negative emotional state. The negative emotional state that drives such negative reinforcement is hypothesized to derive from dysregulation of key neurochemical elements involved in the brain reward and stress systems within the ventral striatum, extended amygdala, and frontal cortex. Specific neurochemical elements in these structures include decreases in reward system function (within-system opponent processes), recruitment of the classic stress axis mediated by CRF in the frontal cortex and extended amygdala, and recruitment of aversive dynorphin-κ opioid systems in the frontal cortex, ventral striatum, and extended amygdala (both between-system opponent processes). Acute withdrawal from all major drugs of abuse increases reward thresholds, decreases mesocorticolimbic dopamine activity, increases anxiety-like responses, increases extracellular levels of CRF in the central nucleus of the amygdala, and increases dynorphin in the ventral striatum. CRF receptor antagonists block anxiety-like responses associated with withdrawal. They also block increases in reward thresholds produced by withdrawal from drugs of abuse and blunt compulsive-like drug taking during extended access.

Excessive activation of dopamine receptors in the nucleus accumbens via the release of mesocorticolimbic dopamine or opioid peptide activation of opioid receptors also activates the dynorphin-κ opioid system, which in turn can decrease dopaminergic activity in the mesocorticolimbic dopamine system. Blockade of the κ opioid system can also block the dysphoric-like effects associated with withdrawal from drugs of abuse and block the development of compulsive-like responding during extended access to

**TABLE 2.4 Drug Craving**

<table>
<thead>
<tr>
<th>Drug craving</th>
<th>“Drug craving is the desire for the previously experienced effects of a psychoactive substance. This desire can become compelling and can increase in the presence of both internal and external cues, particularly with perceived substance availability. It is characterized by an increased likelihood of drug-seeking behavior and, in humans, drug-related thoughts.” (United Nations International Drug Control Programme. Informal Expert Group Meeting on the Craving Mechanism (report no. V92–54439T). United Nations International Drug Control Programme and World Health Organization, Geneva, 1992.)</th>
</tr>
</thead>
</table>
| Reward Craving | • Induced by stimuli that have been paired with drug self-administration such as environmental cues.  
• Termed conditioned positive reinforcement in experimental psychology.  
• Animal model: Cue-induced reinstatement where a cue previously paired with access to a drug reinstates responding for a lever that has been extinguished. |
| Relief Craving | • State of protracted abstinence in drug-dependent individuals weeks after acute withdrawal.  
• Conceptualized as a state change characterized by anxiety and dysphoria.  
• Animal model: Residual hypersensitivity to states of stress and environmental stressors that lead to relapse to drug-seeking behavior. |
drugs of abuse, suggesting another powerful brain stress system that contributes to compulsive drug seeking. Thus, the brain reward systems become compromised, and the brain stress systems become activated by acute excessive drug intake. These changes become sensitized during repeated withdrawal, continue into protracted abstinence, and contribute to the development and persistence of addiction. The loss of reward function and recruitment of brain stress systems provide a powerful neurochemical basis for the negative emotional states that are responsible for the negative reinforcement that drives the compulsivity of addiction. Excessive drug taking also activates CRF in the medial prefrontal cortex, paralleled by deficits in executive function that may facilitate the transition to compulsive-like responding. Dysregulation of the prefrontal cortex can impair executive function and drive impulsivity and help perpetuate disinhibition of the brain stress systems. The combination of the facilitation of incentive salience for drugs, reward dysfunction, stress sensitization, and impaired executive function captures most of the addiction phenotype.

Suggested Reading


Several types of cellular elements are integrated to constitute normally functioning brain tissue. The neuron is the communicating cell, and many neuronal subtypes are connected to one another via complex circuitries, usually involving multiple synaptic connections. Neuronal physiology is supported and maintained by neuroglial cells, which have highly diverse functions. These include myelination, secretion of trophic factors, maintenance of the extracellular milieu, and scavenging of molecular and cellular debris. Neuroglial cells also participate in the formation and maintenance of the blood–brain barrier, a multicomponent structure that is interposed between the circulatory system and the brain substance and that serves as the molecular gateway to brain tissue.

NEURONS

The neuron is a highly specialized cell type and is the essential cellular element in the CNS (central nervous system). All neurological processes are dependent on complex cell–cell interactions among single neurons as well as groups of related neurons. Neurons can be categorized according to their size, shape, neurochemical characteristics, location, and connectivity, which determine their particular functional role in the brain. More importantly, neurons form circuits, and these circuits constitute the structural basis for brain function. Macrocircuits involve a population of neurons projecting from one brain region to another region, and microcircuits reflect the local cell–cell interactions within a brain region. The detailed analysis of these macro- and microcircuits is an essential step in understanding the neuronal basis of a given cortical function in the healthy and the diseased brain. Thus, these cellular characteristics allow us to appreciate the special structural and biochemical qualities of a neuron in relation to its neighbors and to place it in the context of a specific neuronal subset, circuit, or function.

Broadly speaking, therefore, there are five general categories of neurons: inhibitory neurons that make local contacts (e.g., GABAergic interneurons in the cerebral and cerebellar cortex), inhibitory neurons that make distant contacts (e.g., medium spiny neurons of the basal ganglia or Purkinje cells of the cerebellar cortex), excitatory neurons that make local contacts (e.g., spiny stellate cells of the cerebral cortex), excitatory neurons that make distant contacts (e.g., pyramidal neurons in the cerebral cortex), and neuromodulatory neurons that influence neurotransmission, often at large distances. Within these general classes, careful analyses of the structural variation of the anatomic features of neurons have led to various categorizations and to the development of the concept of cell type. The grouping of neurons into descriptive cell types (such as chandelier, double bouquet, or bipolar cells) allows the analysis of populations of neurons and the linking of specified cellular characteristics with certain functional roles.

General Features of Neuronal Morphology

Neurons are highly polarized cells, meaning that they develop distinct subcellular domains that subserve different functions. Morphologically, in a typical neuron, three major regions can be defined: (1) the cell body (soma or perikaryon), which contains the nucleus and the major cytoplasmic organelles; (2) a variable number of dendrites, which emanate from the perikaryon and ramify over a certain volume of gray matter and which differ in size and shape, depending on the neuronal type; and (3) a single axon, which extends, in
most cases, much farther from the cell body than the dendritic arbor (Fig. 1.1). Dendrites may be spiny (as in pyramidal cells) or non-spiny (as in most interneurons), whereas the axon is generally smooth and emits a variable number of branches (collaterals). In vertebrates, many axons are surrounded by an insulating myelin sheath, which facilitates rapid impulse conduction. The axon terminal region, where contacts with other cells are made, displays a wide range of morphological specializations, depending on its target area in the central or peripheral nervous system.

The cell body and dendrites are the two major domains of the cell that receive inputs, and dendrites play a critically important role in providing a massive receptive area on the neuronal surface (see also Chapters 16 and 17). In addition, there is a characteristic shape for each dendritic arbor, which can be used to classify neurons into morphological types. Both the structure of the dendritic arbor and the distribution of axonal terminal ramifications confer a high level of subcellular specificity in the localization of particular synaptic contacts on a given neuron. The three-dimensional distribution of dendritic arborization is also important with respect to the type of information transferred to the neuron. A neuron with a dendritic tree restricted to a particular cortical layer typically receives a very limited pool of afferents, whereas the widely expanded dendritic arborization of a large pyramidal neuron receives highly diversified inputs (Fig. 1.2) (Mountcastle, 1978). The structure of the dendritic tree is maintained by surface interactions between adhesion molecules and, intracellularly, by an array of cytoskeletal components (microtubules, neurofilaments, and associated proteins), which also take part in the movement of organelles within the dendritic cytoplasm.

An important specialization of the dendritic arbor of certain neurons is the presence of large numbers of dendritic spines, which are membranous protrusions. They are abundant in large pyramidal neurons and are much sparser on the dendrites of interneurons (see below).

The perikaryon contains the nucleus and a variety of cytoplasmic organelles. Stacks of rough endoplasmic reticulum are conspicuous in large neurons and, when interposed with arrays of free polyribosomes, are referred to as Nissl substance. Another feature of the perikaryal cytoplasm is the presence of a rich cytoskeleton composed primarily of neurofilaments and microtubules. These cytoskeletal elements are dispersed in bundles that extend from the soma into the axon and dendrites.

Whereas dendrites and the cell body are the domains of the neuron that receive afferents, the axon, at the other pole of the neuron, is responsible for...
transmitting neural information. This information may be primary, in the case of a sensory receptor, or processed information that has already been modified through a series of integrative steps. The morphology of the axon and its course through the nervous system are correlated with the type of information processed by the particular neuron and by its connectivity patterns with other neurons. The axon leaves the cell body from a small swelling called the axon hillock. This structure is particularly apparent in large pyramidal neurons; in other cell types, the axon sometimes emerges from one of the main dendrites. At the axon hillock, microtubules are packed into bundles that enter the axon as parallel fascicles. The axon hillock is the part of the neuron where the action potential is generated (see Chapter 12). The axon is generally unmyelinated in local circuit neurons (such as inhibitory interneurons), but it is myelinated in neurons that furnish connections between different parts of the nervous system. Axons usually have higher numbers of neurofilaments than dendrites, although this distinction can be difficult to make in small elements that contain fewer neurofilaments. In addition, the axon may show extensive, spatially constrained ramifications, as in certain local circuit neurons; it may give out a large number of recurrent collaterals, as in neurons connecting different cortical regions; or it may be relatively straight in the case of projections to subcortical centers, as in cortical motor neurons that send their very long axons to the ventral horn of the spinal cord. At the interface of axon terminals with target cells are the synapses, which represent specialized zones of contact consisting of a presynaptic (axonal) element, a narrow synaptic cleft, and a postsynaptic element on a dendrite or perikaryon.

**Synapses and Spines**

**Synapses**

Each synapse is a complex of several components: (1) a presynaptic element, (2) a cleft, and (3) a postsynaptic element. The presynaptic element is a specialized part of the presynaptic neuron’s axon, the postsynaptic element is a specialized part of the postsynaptic somatodendritic membrane, and the space between these two closely apposed elements is the cleft. The portion of the axon that participates in the axon is the bouton, and it is identified by the presence of synaptic vesicles and a presynaptic thickening at the active zone (Fig. 1.3). The postsynaptic element is marked by a postsynaptic thickening opposite the presynaptic thickening. When both sides are equally thick, the synapse is referred to as symmetric. When the postsynaptic thickening is greater, the synapse is asymmetric. Edward George Gray noticed this difference, and divided synapses into two types: Gray’s type 1 synapses are asymmetric, and have clear, round vesicles; Gray’s type 2 synapses are symmetric, and have variably shaped, or pleomorphic, vesicles. The significance of this distinction is that research has shown that, in general, Gray’s type 1 synapses tend to be excitatory, whereas Gray’s type 2 synapses tend to be inhibitory. This correlation greatly enhanced the usefulness of electron microscopy in neuroscience.

In cross-section on electron micrographs, a synapse looks like two parallel lines separated by a very narrow space (Fig. 1.3). Viewed from the inside of the axon or dendrite, it looks like a patch of variable shape. Some synapses are a simple patch, or macule. Macular synapses can grow fairly large, reaching diameters over 1 μm. The largest synapses have discontinuities or holes within the macule, and are called perforated synapses (Fig. 1.3). In cross-section, a perforated synapse may resemble a simple macular synapse, or several closely spaced smaller macules.

The portion of the presynaptic element that is apposed to the postsynaptic element is the active zone. This is the region where the synaptic vesicles are concentrated, and where, at any time, a small number of vesicles are docked and presumably ready for fusion with the presynaptic membrane to release their contents. The active zone is also enriched with voltage gated calcium channels, which are necessary to permit activity-dependent fusion and neurotransmitter release by rapidly increasing calcium concentration (see also Chapter 15).

The synaptic cleft is truly a space, but its properties are essential. The width of the cleft (~20 nm) is critical because it defines the volume in which each vesicle releases its contents, and therefore, the peak concentration of neurotransmitter upon release. The synaptic cleft is spanned by adhesion molecules, particularly on the flanks of the synapse, which is believed to stabilize the cleft.

The postsynaptic element may be a portion of a soma or a dendrite, or, rarely, part of an axon. In the cerebral cortex, most Gray’s type 1 synapses are located on dendritic spines, which are specialized protrusions of the dendrite, and most Gray’s type 2 synapses are located on somata or dendritic shafts. A similar segregation is seen in cerebellar cortex. In non-spiny neurons, symmetric and asymmetric synapses are often less well separated. Irrespective of location, a postsynaptic thickening marks the postsynaptic element. In Gray’s type 1 synapses, the postsynaptic thickening (or postsynaptic density, PSD) is greatly enhanced. Among the molecules that are associated with the PSD are neurotransmitter receptors (e.g., N-methyl-D-aspartate receptors) and molecules with less obvious function, such as PSD-95.
Spines

Spines are protrusions on the dendritic shafts of some types of neurons and are the sites of synaptic contacts, usually excitatory. Use of the silver impregnation techniques of Golgi or of the methylene blue used by Ehrlich in the late nineteenth century led to the discovery of spiny appendages on dendrites of a variety of neurons. The best known are those on pyramidal neurons and Purkinje cells, although spines occur on neuron types at all levels of the central nervous system. In 1896, Berkley observed that terminal axonal boutons were closely apposed to spines and suggested that spines may be involved in conducting impulses from neuron to neuron. In 1904, Santiago Ramón y Cajal suggested that spines could collect the electrical charge resulting from neuronal activity. He also noted that spines substantially increase the receptive surface of the dendritic arbor, which may represent an important factor in receiving the contacts made by the axonal terminals of other neurons. It has been calculated that the approximately 20,000 spines of a pyramidal neuron account for more than 40% of its total surface area (Peters et al., 1991).

More recent analyses of spine electrical properties have demonstrated that spines are dynamic structures that can regulate many neurochemical events related to synaptic transmission and modulate synaptic efficacy. Spines are also known to undergo pathologic alterations and have a reduced density in a number of experimental manipulations (such as deprivation of a sensory input) and in many developmental, neurologic, and psychiatric conditions (such as dementing illnesses, chronic alcoholism, schizophrenia, trisomy 21). Morphologically, spines are characterized by a narrower portion emanating from the dendritic shaft, the neck, and an ovoid bulb or head, although spine morphology may vary from large mushroom-shaped bulbs to small bulges barely discernable on the surface of the dendrite. Spines have an average length of ~2 μm, but there is considerable variability in their dimensions. At the ultrastructural level (Fig. 1.3), spines are the postsynaptic site of asymmetric, excitatory synapses and contain fine and quite indistinct filaments. These filaments most likely consist of actin and α- and β-tubulins. Microtubules and neurofilaments present in dendritic shafts do not enter spines.

FIGURE 1.3 Ultrastructure of dendritic spines and synapses in the human brain. A and B: Narrow spine necks (asterisks) emanate from the main dendritic shaft (D). The spine heads (S) contain filamentous material. Some large spines contain cisterns of a spine apparatus (sa, B). Asymmetric excitatory synapses are characterized by thickened postsynaptic densities (arrows A, B). A perforated synapse has an electron-lucent region amidst the postsynaptic density (small arrow, B). The presynaptic axonal boutons (B) of excitatory synapses usually contain round synaptic vesicles. Symmetric inhibitory synapses (arrow; C) typically occur on the dendritic shaft (D) and their presynaptic boutons contain smaller round or ovoid vesicles. Dendrites and axons contain numerous mitochondria (m). Scale bar = 1 μm (A, B) and 0.6 μm (C). Electron micrographs courtesy of Drs S.A. Kirov and M. Witcher (Medical College of Georgia), and K.M. Harris (University of Texas – Austin).
Mitochondria and free ribosomes are infrequent, although many spines contain polyribosomes in their neck. Interestingly, most polyribosomes in dendrites are located at the bases of spines, where they are associated with endoplasmic reticulum, indicating that spines possess the machinery necessary for the local synthesis of proteins (see also Chapter 5). Another feature of the spine is the presence of confluent tubular cisterns in the spine head that represent an extension of the dendritic smooth endoplasmic reticulum. Those cisterns are referred to as the spine apparatus. The function of the spine apparatus is not fully understood but may be related to the storage of calcium ions during synaptic transmission.

**Specific Examples of Different Neuronal Types**

**Inhibitory Local Circuit Neurons**

**Inhibitory Interneurons of the Cerebral Cortex**

The study of cortical interneurons dates back to the original work of Camillo Golgi when he applied the new technique he had discovered, the *reazione nera* (black reaction) (Golgi, 1873). This method allowed all the components of a neuron to be visualized in histological sections (soma, dendrites and axon). Golgi proposed that neurons could generally be considered to be of two different morphological and physiological types: motor (type I) and sensory (type II) neurons. These motor neurons had long axons that sprout collaterals and they could project beyond the gray matter, whereas by contrast, sensory neurons had short axons that arborized near the parent cell and did not leave the gray matter. This hypothesis was challenged when Santiago Ramón y Cajal entered the scene following his masterful studies of brain structure based on the Golgi method. Cajal argued that it was not physiologically possible to maintain such a distinction and he designated Golgi’s two types as cells with a long axon (projection neurons) and cells with a short axon (intrinsic neurons), avoiding any consideration of their possible physiological roles (Fig. 1.4). Since then, the term short-axon cell has commonly been used as synonymous with interneurons (DeFelipe, 2002).

In general, cortical interneurons have been subdivided into two large groups: spiny non-pyramidal cells and aspiny or sparsely spiny non-pyramidal cells. Spiny non-pyramidal cells represent the typical neurons (including spiny stellate cells) of the middle cortical layers (especially layer IV). These spiny non-pyramidal cells are morphologically heterogeneous,
with ovoid, fusiform and triangular somata, and most of them are excitatory (probably glutamatergic). Their axons are found within layer IV or in the layers adjacent to that in which their soma is located, either above or below. Aspiny or sparsely spiny non-pyramidal cells have axons that remain close to the parental cell body, although prominent collaterals may run out from some of these in the horizontal (parallel to the cortical surface) or vertical dimension (ascending and/or descending to other cortical layers). These interneurons constitute ~15–30% of the total population of neurons and they appear to be mostly GABAergic, representing the main components of inhibitory cortical circuits (Jones, 1993) (Fig. 1.4). Indeed, they can be found in all cortical layers and they characteristically display a tremendous variety of morphological, biochemical and physiological features (Kubota et al., 2011; Markram et al., 2004). However, with few exceptions, a general consensus to name and classify cortical neurons has yet to emerge. This is in part due to the fact that, with some exceptions, there are no rules to establish the essential characteristics that define whether an individual neuron belongs to a given cell type. Indeed, this characterization is also hindered by the fact that most studies of these neurons are generally based on morphological, physiological, or molecular approaches, rather than any combination of these features.

In a more recent attempt to classify these neurons, the Petilla Interneuron Nomenclature Group (Ascoli et al., 2008) proposed a set of terms to organize and describe the anatomical, molecular and physiological features of GABAergic interneurons of the cerebral cortex based on existing data. The anatomical, molecular and physiological classifications divided GABAergic cortical interneurons into several main types, which in turn were further subdivided depending on distinct properties. Briefly, the anatomical classification identified three major neuronal types: cells that target pyramidal cells; cells that do not show target specificity; and cells that specifically target other interneurons. The molecular classification includes five main groups based on the expression of specific biochemical markers: parvalbumin, somatostatin, neuropeptide Y in the absence of somatostatin, vaso-intestinal peptide, and cholecystokinin in the absence of somatostatin and vaso-intestinal peptide. Finally, the physiological classification identified six main types of interneurons: fast spiking neurons, non-adapting/non-fast spiking neurons, adapting neurons, accelerating cells, irregular spiking neurons, and intrinsic bursting neurons. Each of these attempts to classify these neurons has its limitations and the scientific community still lacks a general catalogue of accepted neuron types and names. Nevertheless, standardizing the nomenclature based on the properties of GABAergic interneurons proposed by the Petilla Interneuron Nomenclature Group represents a step in the right direction towards a more comprehensive classification of GABAergic interneurons in the cerebral cortex.

In general, GABAergic interneurons control the flow of information in the cerebral cortex (Fig. 1.5), although there does seem to be some division of labor between the different types of interneurons (Klausberger and Somogyi, 2008). One such example is the class of basket and chandelier cells that are considered as fast-spiking GABAergic interneurons and that express the calcium binding protein parvalbumin. These cells seem to play important roles in controlling the timing of pyramidal cell firing, shaping the network output and the rhythms generated in different states of consciousness (Howard et al., 2005; Klausberger et al., 2003). By contrast, other interneurons containing nitric oxide synthase and various neuropeptides are implicated in neurovascular coupling (Rossier, 2009). In addition, some types of interneurons have been directly implicated in disease, which is particularly evident when we consider that certain alterations to chandelier and basket cells seem to be critical in establishing some forms of human epilepsy (DeFelipe, 1999; Magloczky and Freund, 2005). Finally, the proportion of GABAergic neurons in the cerebral cortex of rodents is lower than in primates, and there are interneurons in primates that are not found in rodents. These observations together with the existence of differences in the developmental origins of GABAergic interneurons in rodents and primates, including humans, seems to indicate that more GABA interneurons and newer forms of GABA interneurons appeared in the primate cortex during the course of evolution (DeFelipe, 2011; Raghanti et al., 2010; Rakic, 2009). All of these features of GABAergic interneurons make their study one of the most exciting and active research fields in relation to how information is processed in the cerebral cortex.

**Inhibitory Projection Neurons**

**MEDIUM-SIZED SPINY CELLS**

These neurons are unique to the striatum, a part of the basal ganglia that comprises the caudate nucleus and putamen. Medium-sized spiny cells are scattered throughout the caudate nucleus and putamen and are recognized by their relatively large size, compared with other cellular elements of the basal ganglia, and by the fact that their distribution is generally sparse. They differ from all others in the striatum in that they have a highly ramified dendritic arborization radiating in all directions and are densely covered with spines. They furnish a major output from the caudate nucleus
and putamen and receive a highly diverse input from, among other sources, the cerebral cortex, thalamus, and certain dopaminergic neurons of the substantia nigra. These neurons are neurochemically quite heterogeneous, contain GABA, and may contain several neuropeptides and the calcium-binding protein calbindin. In Huntington disease, a neurodegenerative disorder of the striatum characterized by involuntary movements and progressive dementia (Chapter 21), an early and dramatic loss of medium-sized spiny cells occurs.

**PURKINJE CELLS**

Purkinje cells are the most salient cellular elements of the cerebellar cortex. They are arranged in a single row throughout the entire cerebellar cortex between the molecular (outer) layer and the granular (inner) layer. They are among the largest neurons and have a round perikaryon, classically described as shaped “like a chianti bottle,” with a highly branched dendritic tree shaped like a candelabrum and extending into the molecular layer where they are contacted by incoming systems of afferent fibers from granule neurons and the brainstem. The apical dendrites of Purkinje cells have an enormous number of spines (more than 80,000 per cell). A particular feature of the dendritic tree of the Purkinje cell is that it is distributed in one plane, perpendicular to the longitudinal axes of the cerebellar folds, and each dendritic arbor determines a separate domain of cerebellar cortex (Fig. 1.1). The axons of Purkinje neurons course through the cerebellar white matter and contact deep cerebellar nuclei or vestibular nuclei. These neurons contain the inhibitory neurotransmitter GABA and the calcium-binding protein calbindin. Spinocerebellar ataxia, a severe disorder combining ataxic gait and impairment of fine hand movements (see Chapter 21), accompanied by dysarthria and tremor, has been documented in some families and is related directly to Purkinje cell degeneration.

**Excitatory Local Circuit Neurons**

**SPINY STELLATE CELLS**

Spiny stellate cells are small multipolar neurons with local dendritic and axonal arborizations. These neurons resemble pyramidal cells in that they are the only other cortical neurons with large numbers of dendritic spines, but they differ from pyramidal neurons in that they lack an elaborate apical dendrite. The relatively restricted dendritic arbor of these neurons is presumably a manifestation of the fact that they are high-resolution neurons that gather afferents from a very restricted region of the cortex. Dendrites rarely leave the layer in which the cell body resides. The spiny stellate cell also resembles the pyramidal cell in that it provides asymmetric synapses that are presumed to be excitatory, and is thought to use glutamate as its neurotransmitter (Peters and Jones, 1984).

The axons of spiny stellate neurons have primarily intracortical targets and a radial orientation, and appear to play an important role in forming links among layer IV, the major thalamorecipient layer, and layers III, V, and VI, the major projection layers. The spiny stellate neuron appears to function as a high-fidelity relay of thalamic inputs, maintaining strict topographic organization and setting up initial vertical links of information transfer within a given cortical area (Peters and Jones, 1984).

**Excitatory Projection Neurons**

**PYRAMIDAL CELLS**

All cortical output is carried by pyramidal neurons, and the intrinsic activity of the neocortex can be viewed simply as a means of finely tuning their output. A pyramidal cell is a highly polarized neuron, with a major orientation axis perpendicular (or orthogonal) to the pial surface of the cerebral cortex. In cross-section, the cell body is roughly triangular (Fig. 1.1), although a large variety of morphologic types exist with elongate, horizontal, or vertical fusiform, or inverted perikaryal shapes. Pyramidal cells are the
major excitatory type of neurons and use glutamate as their neurotransmitter. A pyramidal neuron typically has a large number of dendrites that emanate from the apex and form the base of the cell body. The span of the dendritic tree depends on the laminar localization of the cell body, but it may, as in giant pyramidal neurons, spread over several millimeters. The cell body and dendritic arborization may be restricted to a few layers or, in some cases, may span the entire cortical thickness (Jones, 1984).

In most cases, the axon of a large pyramidal cell extends from the base of the perikaryon and courses toward the subcortical white matter, giving off several collateral branches that are directed to cortical domains generally located within the vicinity of the cell of origin (as explained later). Typically, a pyramidal cell has a large nucleus, and a cytoplasmic rim that contains, particularly in large pyramidal cells, a collection of granular material chiefly composed of rough endoplasmic reticulum and Nissl bodies, as well as, especially in aging, lipofuscin. Although all pyramidal cells possess these general features, they can also be subdivided into numerous classes based on their morphology, laminar location, and connectivity with cortical and subcortical regions (Fig. 1.6) (Jones, 1975).

SPINAL MOTOR NEURONS

Motor cells of the ventral horns of the spinal cord, also called \( \alpha \) motoneurons, have their cell bodies within the spinal cord and send their axons outside the central nervous system to innervate the muscles. Different types of motor neurons are distinguished by their targets. The \( \alpha \) motor neurons innervate skeletal muscles, but smaller motor neurons (the \( \gamma \) motor neurons, forming about 30% of the motor neurons) innervate the spindle organs of the muscles. The \( \alpha \) motor neurons are some of the largest neurons in the entire central nervous system and are characterized by a multipolar perikaryon and a very rich cytoplasm that renders them very conspicuous on histological preparations. They have a large number of spiny dendrites that arborize locally within the ventral horn. The \( \alpha \) motor neuron axon leaves the central nervous system through the ventral root of the peripheral nerves. Their distribution in the ventral horn is not random and corresponds to a somatotopic representation of the muscle groups of the limbs and axial musculature (Brodal, 1981). Spinal motor neurons use acetylcholine as their neurotransmitter. Large motor neurons are severely affected in lower motor neuron disease, a neurodegenerative disorder characterized by progressive muscular weakness that affects, at first, one or two limbs but involves more and more of the body musculature, which shows signs of wasting as a result of denervation.

Neuromodulatory Neurons

DOPAMINERGIC NEURONS

DOPAMINERGIC NEURONS

OF THE SUBSTANTIA NIGRA

Dopaminergic neurons are large neurons that reside mostly within the pars compacta of the substantia nigra and in the ventral tegmental area (van Domburg and ten Donkelaar, 1991). A distinctive feature of these cells is the presence of a pigment, neuromelanin, in compact granules in the cytoplasm. These neurons are medium-sized to large, fusiform, and frequently elongated. They have several large radiating dendrites. The axon emerges from the cell body or from one of the dendrites and projects to large expanses of cerebral cortex and to the basal ganglia. These neurons contain the catecholamine-synthesizing enzyme tyrosine hydroxylase, as well as the monoamine dopamine as their neurotransmitter (see Chapter 7). Some of them contain both calbindin and calretinin. These neurons are affected severely and selectively in Parkinson’s disease—a movement disorder different from Huntington’s disease and characterized by resting tremor and rigidity—and their specific loss is the neuropathologic hallmark of this disorder (see Chapter 21).
The term neuroglia, or “nerve glue,” was coined in 1859 by Rudolph Virchow, who conceived of the neuroglia as an inactive “connective tissue” holding neurons together in the CNS. The metallic staining techniques developed by Ramón y Cajal and del Rio-Hortega allowed these two great pioneers to distinguish, in addition to the ependyma lining the ventricles and central canal, three types of supporting cells in the CNS: oligodendrocytes, astrocytes, and microglia. In the peripheral nervous system (PNS), the Schwann cell is the major neuroglial component.

Oligodendrocytes and Schwann Cells Synthesize Myelin

Most brain functions depend on rapid communication between circuits of neurons. There is a practical limit to how fast an individual bare axon can conduct an action potential (see Chapter 12). Organisms developed two solutions for enhancing rapid communication between neurons and their effector organs. In invertebrates, the diameters of axons are enlarged. In vertebrates, the myelin sheath (Fig. 1.7) evolved to permit rapid nerve conduction (see Chapter 12).

Axon enlargement accelerates action potential propagation in proportion to the square root of axonal diameter. Thus, larger axons conduct faster than small ones, but substantial increases in conduction velocity require huge axons. The largest axon in the invertebrate kingdom is the squid giant axon, which is about the thickness of a mechanical pencil lead. This axon conducts the action potential at speeds of 10 to 20 m/s. As the axon mediates an escape reflex, firing must be rapid if the animal is to survive. Bare axons and continuous conduction obviously provide sufficient rates of signal propagation for even very large invertebrates, and many human axons also remain bare. However, in the human brain with 10 billion neurons, axons cannot be as thick as pencil lead, otherwise heads would weigh one hundred pounds or more.

Thus, along the invertebrate evolutionary line, the use of bare axons imposes a natural, insurmountable limit—a constraint of axonal diameter—to increasing the processing speed of the nervous system. Vertebrates, however, get around this problem through evolution of the myelin sheath, which allows 10- to 100-fold increases in conduction of the nerve impulse along axons with fairly minute diameters.

In the CNS, myelin sheaths (Fig. 1.8) are elaborated by oligodendrocytes. During brain development, these glial cells send out a few cytoplasmic processes that engage adjacent axons and form myelin around them (Bunge, 1968). Myelin consists of a long sheet of oligodendrocyte plasma membrane, which is spirally wrapped around an axonal segment. At the end of each myelin segment, there is a bare portion of the axon, the node of Ranvier. Myelin segments are thus called internodes. Physiologically, myelin has insulating properties such that the action potential can “leap” from node to node and therefore does not have to be regenerated continually along the axonal segment because the myelin membrane sheath covers it. This leaping of the action potential from node to node allows axons with fairly small diameters to conduct extremely rapidly (Ritchie, 1984), and is called saltatory conduction.

Because the brain and spinal cord are encased in the bony skull and vertebrae, CNS evolution has promoted compactness among the supporting cells of the CNS. Each oligodendrocyte cell body is responsible for the construction and maintenance of several myelin sheaths (Fig. 1.8), thus reducing the number of glial cells required. In both PNS and CNS myelin, cytoplasm is removed between each turn of the myelin,
leaving only the thinnest layer of plasma membrane. Due to protein composition differences, CNS lamellae are approximately 30% thinner than in PNS myelin. In addition, there is little or no extracellular space or extracellular matrix between the myelinated axons passing through CNS white matter. Brain volume is thus reserved for further expansion of neuronal populations.

Peripheral nerves pass between moving muscles and around major joints, and are routinely exposed to physical trauma. A hard tackle, slipping on an icy sidewalk, or even just occupying the same uncomfortable seating posture for too long, can painfully compress peripheral nerves and potentially damage them. Thus, evolutionary pressures shaping the PNS favor robustness and regeneration rather than conservation of space. Myelin in the PNS is generated by Schwann cells (Fig. 1.9), which are different from oligodendrocytes in several ways. Individual myelinating Schwann cells form a single internode. The biochemical composition of PNS and CNS myelin differs, as discussed later. Unlike oligodendrocytes, Schwann cells secrete copious extracellular matrix components and produce a basal lamina “sleeve” that runs the entire length of myelinated axons. Schwann cell and fibroblast-derived collagens prevent normal wear-and-tear compression damage. Schwann cells also respond vigorously to injury, in common with astrocytes but

**FIGURE 1.8** An oligodendrocyte (OL) in the central nervous system is depicted myelinating several axon segments. A cutaway view of the myelin sheath is shown (M). Note that the internode of myelin terminates in paranodal loops that flank the node of Ranvier (N). (Inset) An enlargement of compact myelin with alternating dark and light electron-dense lines that represent intracellular (major dense lines) and extracellular (intraperiod line) plasma membrane appositions, respectively.

**FIGURE 1.9** An “unrolled” Schwann cell in the PNS is illustrated in relation to the single axon segment that it myelinates (tube along the bottom of the figure). The nucleus is shown within the cytoplasm at the top of the figure. The broad stippled region is compact myelin surrounded by cytoplasmic channels that remain open even after compact myelin has formed, allowing an exchange of materials among the myelin sheath, the Schwann cell cytoplasm, and perhaps the axon as well.

I. CELLULAR AND MOLECULAR
unlike oligodendrocytes. Schwann cell growth factor secretion, debris removal by Schwann cells after injury, and the axonal guidance function of the basal lamina are responsible for the exceptional regenerative capacity of the PNS compared with the CNS.

The major integral membrane protein of peripheral nerve myelin is protein zero (P0), a member of a very large family of proteins termed the immunoglobulin gene superfamily. This protein makes up about 80% of the protein complement of PNS myelin. Interactions between the extracellular domains of P0 molecules expressed on one layer of the myelin sheath with those of the apposing layer yield a characteristic regular periodicity that can be seen by thin section electron microscopy (Fig. 1.7). This zone, called the intraperiod line, represents the extracellular apposition of the myelin bilayer as it wraps around itself. On the other side of the bilayer, the cytoplasmic side, the highly charged P0 cytoplasmic domain probably functions to neutralize the negative charges on the polar head groups of the phospholipids that make up the plasma membrane itself, allowing the membranes of the myelin sheath to come into close apposition with one another. In electron microscopy, this cytoplasmic apposition appears darker than the intraperiod line and is termed the major dense line. In peripheral nerves, although other molecules are present in small quantities in compact myelin and may have important functions, compaction (i.e., the close apposition of membrane surfaces without intervening cytoplasm) is accomplished solely by P0–P0 interactions at both extracellular and intracellular (cytoplasmic) surfaces.

Curiously, P0 is present in the CNS of lower vertebrates such as sharks and bony fish, but in terrestrial vertebrates (reptiles, birds, and mammals), P0 is limited to the PNS. CNS myelin compaction in these higher organisms is subserved by proteolipid protein (PLP) and its alternate splice form, DM-20. These two proteins are generated from the same gene, both span the plasma membrane four times, and differ only in that PLP has a small, positively charged segment exposed on the cytoplasmic surface. Why did PLP/DM-20 replace P0 in CNS myelin? Manipulation of PLP and P0 in CNS myelin established an axonotrophic function for PLP in CNS myelin. Removal of PLP from rodent CNS myelin altered the periodicity of compact myelin and produced a late onset axonal degeneration (Griffiths et al., 1988). Replacing PLP with P0 in rodent CNS myelin stabilized compact myelin but enhanced the axonal degeneration (Yin et al., 2006). These observations indicate that myelination provides trophic support that is essential for axon survival. Studies of primary demyelinating and genetic demyelinating diseases, such as multiple sclerosis and Charcot-Marie-Tooth disease, indicate that axonal degeneration is the major cause of permanent disability (Nave and Trapp, 2008).

Myelin membranes also contain a number of other proteins such as the myelin basic protein, which is a major CNS myelin component, and PMP-22, a protein that is involved in a form of peripheral nerve disease. A large number of naturally occurring gene mutations can affect the proteins specific to the myelin sheath and cause neurological disease. In animals, these mutations have been named according to the phenotype that is produced: the shiverer mouse, the shaking pup, the rumpshaker mouse, the jumpy mouse, the myelin-deficient rat, the quaking mouse, and so forth. Many of these mutations are well characterized, and have provided valuable insights into the role of individual proteins in myelin formation and axonal survival.

**Astrocytes Play Important Roles in CNS Homeostasis**

As the name suggests, astrocytes were first described as star-shaped, process-bearing cells distributed throughout the central nervous system. They constitute from 20 to 50% of the volume of most brain areas. Astrocytes appear stellate when stained using reagents that highlight their intermediate filaments, but have complex morphologies when their entire cytoplasm is visualized (Fig. 1.10). The two main forms, protoplasmic and fibrous astrocytes, predominate in gray and white matter, respectively (Fig. 1.11). Embryonically, astrocytes develop from radial glial cells, which transversely compartmentalize the neural tube. Radial glial cells serve as scaffolding for the migration of neurons and play a critical role in defining the cytoarchitecture of the CNS (Fig. 1.12). As the CNS matures, radial glia retract their processes and serve as progenitors of astrocytes. However, some specialized astrocytes of a radial nature are still present in adult brain and are thought to be involved in the regulation of synaptic activity.

**FIGURE 1.10** Astrocytes appear stellate when their intermediate filaments are stained (red, GFAP; right panel), but membrane labeling (green, mEGFP; middle panel) highlights the profusion of fine cellular processes that intercalate among other neuropil elements such as synapses and neurons (N) labeled in the overlaid of the middle and right panels shown on the left. Scale bar = 10 μm. Image courtesy of Dr. M.C. Smith.
found in the adult cerebellum and the retina and are known as Bergmann glial cells and Müller cells, respectively.

Astrocytes “fence in” neurons and oligodendrocytes. Astrocytes achieve this isolation of the brain parenchyma by extending long processes projecting to the pia mater and the ependyma to form the glia limitans, by covering the surface of capillaries, and by making a cuff around the nodes of Ranvier. They also ensheathe synapses and dendrites and project processes to cell somas (Fig. 1.13). Astrocytes are connected to each other, and to oligodendrocytes, by gap junctions, forming a syncytium that allows ions and small molecules to diffuse across the brain parenchyma. Astrocytes have in common unique cytological and immunological properties that make them easy to identify, including their star shape, the glial end feet on capillaries, and a unique population of large bundles of intermediate filaments. These filaments are composed of an astroglial-specific protein commonly referred to as glial fibrillary acidic protein (GFAP). S-100, a calcium-binding protein, and glutamine synthetase are also astrocyte markers. Ultrastructurally, gap junctions (connexins), desmosomes, glycogen granules, and membrane orthogonal arrays are distinct features used by morphologists to identify astrocytic cellular processes in the complex cytoarchitecture of the nervous system.

**FIGURE 1.11** The arrangement of astrocytes in human cerebellar cortex. Bergmann glial cells are in red, protoplasmic astrocytes are in green, and fibrous astrocytes are in blue.

**FIGURE 1.12** Radial glia perform support and guidance functions for migrating neurons. In early development, radial glia span the thickness of the expanding brain parenchyma between the ventricle and outer surface depicted on the left. (Inset) Defined layers of the neural tube from the ventricular to the outer surface: VZ, ventricular zone; IZ, intermediate zone; CP, cortical plate; MZ, marginal zone. The radial process of the glial cell is indicated in blue, and a single attached migrating neuron is depicted at the right.
For a long time, astrocytes were thought to physically form the blood–brain barrier (considered later in this chapter), which prevents the entry of cells and diffusion of molecules into the CNS. In fact, astrocytes indeed constitute the blood–brain barrier in nonmammalian species. However, in mammals, astrocytes are responsible for inducing and maintaining the tight junctions in endothelial cells that effectively form the barrier. Astrocytes also take part in angiogenesis, which may be important in the development and repair of the CNS. Their role in this important process is still poorly understood.

Astrocytes Have a Wide Range of Functions

There is strong evidence for the role of radial glia and astrocytes in the migration and guidance of neurons in early development. Astrocytes are a major source of extracellular matrix proteins and adhesion molecules in the CNS; examples are nerve cell–nerve cell adhesion molecule (N-CAM), laminin, fibronectin, cytotactin, and the J-1 family members janusin and tenascin. These molecules participate not only in the migration of neurons, but also in the formation of neuronal aggregates, so-called nuclei, as well as networks.

Astrocytes produce, in vivo and in vitro, a very large number of growth factors. These factors act singly or in combination to selectively regulate the morphology, proliferation, differentiation, or survival, or all four, of distinct neuronal subpopulations. Most of the growth factors also act in a specific manner on the development and functions of astrocytes and oligodendrocytes. The production of growth factors and cytokines by astrocytes and their responsiveness to these factors is a major mechanism underlying the developmental function and regenerative capacity of the CNS.

During neurotransmission, neurotransmitters and ions are released at high concentration in the synaptic cleft. The rapid removal of these substances is important so that they do not interfere with future synaptic activity. The presence of astrocyte processes around synapses positions them well to regulate neurotransmitter uptake and inactivation (Kettenman and Ransom, 1995). These possibilities are consistent with the presence in astrocytes of transport systems for many neurotransmitters. For instance, glutamate reuptake is performed mostly by astrocytes, which convert glutamate into glutamine and then release it into the extracellular space. Glutamine is taken up by neurons, which use it to generate glutamate and GABA, potent excitatory and inhibitory neurotransmitters, respectively (Fig. 1.14). Astrocytes contain ion channels for K⁺, Na⁺, Cl⁻, HCO₃⁻, and Ca²⁺, as well as displaying a wide range of neurotransmitter receptors. K⁺ ions
released from neurons during neurotransmission are soaked up by astrocytes and moved away from the area through astrocyte gap junctions. This is known as spatial buffering. Astrocytes play a major role in detoxification of the CNS by sequestering metals and a variety of neuroactive substances of endogenous and xenobiotic origin.

In response to stimuli, intracellular Ca$^{2+}$ waves are generated in astrocytes. Propagation of the Ca$^{2+}$ wave can be visually observed as it moves across the cell soma and from astrocyte to astrocyte. The generation of Ca$^{2+}$ waves from cell to cell is thought to be mediated by second messengers, diffusing through gap junctions (see Chapters 4 and 9). In the adult brain, gap junctions are present in all astrocytes. Some gap junctions also have been detected between astrocytes and neurons. Thus, they may participate, along with astroglial neurotransmitter receptors, in the coupling of astrocyte and neuron physiology.

In a variety of CNS disorders—neurotoxicity, viral infections, neurodegenerative disorders, HIV, AIDS, dementia, multiple sclerosis, inflammation, and trauma—astrocytes react by becoming hypertrophic and, in a few cases, hyperplastic. A rapid and huge upregulation of GFAP expression and filament formation is associated with astrogliosis. The formation of reactive astrocytes can spread very far from the site of origin. For instance, a localized trauma can recruit astrocytes from as far as the contralateral side to the injury, suggesting the existence of soluble factors in the mediation process. Tumor necrosis factor (TNF) and ciliary neurotrophic factors (CNTF) have been identified as key factors in astrogliosis.

Microglia are Mediators of Immune Responses in Nervous Tissue

The brain traditionally has been considered an “immunologically privileged site,” mainly because the blood–brain barrier normally restricts the access of immune cells from the blood. However, it is now known that immunological reactions do take place in the central nervous system, particularly during cerebral inflammation. Microglial cells have been termed the tissue macrophages of the CNS, and they function as the resident representatives of the immune system in the brain. A rapidly expanding literature describes microglia as major players in CNS development and in the pathogenesis of CNS disease.

The first description of microglial cells can be traced to Franz Nissl (Nissl, 1899), who used the term “rod cell” to describe a population of glial cells that reacted to brain pathology. He postulated that rod-cell function was similar to that of leukocytes in other organs. Ramón y Cajal described microglia as part of his “third element” of the CNS—cells that he considered to be of mesodermal origin and distinct from neurons and astrocytes (Ramón y Cajal, 1913).

Del Rio-Hortega (Del Rio-Hortega, 1932) distinguished this third element into microglia and oligodendrocytes. He used silver impregnation methods to visualize the ramified appearance of microglia in the adult brain, and he concluded that ramified microglia could transform into cells that were migratory, ameboid, and phagocytic. Indeed, a hallmark of microglial cells is their ability to become reactive and to respond to pathological challenges in a variety of ways. A fundamental question raised by del Rio-Hortega’s studies was the origin of microglial cells. Some questions about this remain even today.

Microglia Have Diverse Functions in Developing and Mature Nervous Tissue

On the basis of current knowledge, it appears that most ramified microglial cells are derived from bone marrow-derived monocytes, which enter the brain parenchyma during early stages of brain development. These cells help phagocytosis degenerating cells that undergo programmed cell death as part of normal development. They retain the ability to divide and have the immunophenotypic properties of monocytes and macrophages. In addition to their role in remodeling the CNS during early development, microglia secrete cytokines and growth factors that are important in fiber tract development, gliogenesis, and angiogenesis. They are also the major CNS cells involved in presenting antigens to T lymphocytes. After the early stages of development, ameboid microglia transform into the ramified microglia that persist throughout adulthood (Altman, 1994).

Little is known about microglial function in the healthy adult vertebrate CNS. Microglia constitute a formidable percentage (5–20%) of the total cells in the mouse brain. Microglia are found in all regions of the brain, and there are more in gray than in white matter. The neocortex and hippocampus have more microglia than regions like the brainstem or cerebellum. Species variations also have been noted, as human white matter has three times more microglia than rodent white matter.

Microglia usually have small rod-shaped somas from which numerous processes extend in a rather symmetrical fashion. Processes from different microglia rarely overlap or touch, and specialized contacts between microglia and other cells have not been described in the normal brain. Although each microglial cell occupies its own territory, microglia...
collectively form a network that covers much of the CNS parenchyma. Because of the numerous processes, microglia present extensive surface membrane to the CNS environment. Regional variation in the number and shape of microglia in the adult brain suggests that local environmental cues can affect microglial distribution and morphology. On the basis of these morphological observations, it is likely that microglia play a role in tissue homeostasis. The nature of this homeostasis remains to be elucidated. It is clear, however, that microglia can respond quickly and dramatically to alterations in the CNS microenvironment.

**Microglia Become Activated in Pathological States**

"Reactive" microglia can be distinguished from resting microglia by two criteria: change in morphology and upregulation of monocyte-macrophage molecules (Fig. 1.15). Although the two phenomena generally occur together, reactive responses of microglia can be diverse and restricted to subpopulations of cells within a microenvironment. Microglia not only respond to pathological conditions involving immune activation, but also become activated in neurodegenerative conditions that are not considered immune mediated. This latter response is indicative of the phagocytic role of microglia. Microglia change their morphology and antigen expression in response to almost any form of CNS injury.

**CEREBRAL VASCULATURE**

The cerebral vasculature supports the development and function of the brain. During embryogenesis, the brain vasculature develops via the process of angiogenesis from a preformed perineural vascular plexus. The formation of closed neurovascular networks requires the ordered and coordinated action of neural and endothelial signaling factors, such as vascular endothelial growth factor (VEGF), which is produced in the neuroepithelium and attracts vessel growth into the brain. Other factors, including insulin-like growth factor-1 (IGF-1), basic fibroblast growth factors (bFGFs), interleukin-8 (IL-8), erythropoietin, and angiopoietin-1, promote the recruitment, proliferation, and survival of endothelial cells while simultaneously promoting the proliferation of neural progenitors, neurogenesis, synaptogenesis, and axonal growth. Many neurogenic factors, including nerve growth factor (NGF), brain derived growth factor (BDNF), neuropilin, glial derived growth factor (GDNF), and artemin, as well as components of the extracellular matrix (ECM), affect both neurons and endothelial cells. The vasculature also contributes to neurogenesis by providing a vascular niche for the resident neural progenitor population that exists in neurogenic regions of the adult brain (Ihrie and Alvarez-Buya, 2011).

During angiogenesis, endothelial cells proliferate and migrate to form capillary networks, initially aligning into multicellular, precapillary cord-like structures that form an integrated polygonal network. As the vascular cords mature, lumens form allowing blood flow,

![Figure 1.15](image_url)
and endothelial cells become sequestered from the interstitial matrix by a continuous lamina of ECM that forms a basement membrane or basal lamina. The vascular ECM is a hyaline rich and amorphous substance containing laminins, which are thought to be the primary glycoprotein determinants of basement membrane assembly, along with collagen type IV (predominantly the heterotrimer $[\alpha_1]^2\alpha_2$), perlecain (heparan sulfate proteoglycan-2), nidogens, collagen type XVIII, and fibronectin (Fig. 1.16). Structurally, the ECM supports vascular organization as well as plays a mechanosensing role. Vascular networks mature by recruitment of supporting mural cells including pericytes and vascular smooth muscle cells, which occur along the abluminal surface. Vascular stability is further achieved through EC-mural cell interactions and remodeling of the ECM by deposition and cross-linking of ECM components.

Endothelial cells adhere to the ECM through both integrin and non-integrin receptors. These interactions result in activation of a complex set of signaling pathways that include Rho GTPases (including Cdc42 and Rac1), focal adhesion kinase (FAK), protein kinase C$_{\varepsilon}$ (PKC$_{\varepsilon}$) and Src. MAPK signaling is particularly important in the regulation of endothelial cell proliferation, migration, and survival. Although these pathways have diverse targets, one common theme is that most affect directly or indirectly the endothelial cytoskeleton. Regulation of ECM structure and function is primarily achieved through the action of matrix metalloproteases (MMPs), which degrade ECM components and release ECM-bound factors such as cytokines that affect vascular cell behavior (Davis et al., 2011). MMP activities are tightly controlled by specific protein inhibitors such as the tissue inhibitors of metalloproteases (TIMPs), which play a key role in vascular stabilization.

**Transport of Molecules into the Brain**

As an extension of the systemic circulation, the cerebral vasculature delivers oxygen and nutrients into the brain and removes carbon dioxide and other metabolic wastes. Endothelial cells interact with neurons, astrocytes, and microglia, as well as other perivascular cells, including smooth muscle cells and pericytes, to form a neurovascular unit (Lecrux and Hamel, 2011) (Fig. 1.17). This structural organization maintains cerebral homeostasis and also forms a blood–brain barrier (BBB) that restricts exchange of solutes between the systemic circulation and brain. Unlike the fenestrated capillaries most often found in the periphery, the spaces between endothelial cells in brain capillaries are occluded by tight junctions (also known as zonula occludens) that form the physical basis for the BBB by restricting the paracellular movement of molecules.

![FIGURE 1.16 Microvasculature of the adult mouse somatosensory barrel field (S1BF) cortex. Microvessels were stained with antibodies against collagen type IV, a protein component of the extracellular matrix and lightly counter-stained with cresyl violet (Nissl). Cortical layers (I–VI) and the corpus callosum (CC) are indicated. An area of increased vascular density in layer IV, where contralateral somatosensory inputs from the thalamus terminate, is indicated by an arrow. Scale bar = 50 $\mu$m.](image1.png)

![FIGURE 1.17 Ultrastructural analysis of the cerebral microvasculature of a 10-month-old wild type mouse. A transversely sectioned capillary is shown from the prefrontal cortex. An endothelial cell (E) surrounding the lumen, a pericyte (P) and an astrocytic end-foot process (A) are indicated. Scale bar = 1 $\mu$m.](image2.png)
into the brain. The tight junctions between endothelial cells are localized at cholesterol-rich regions along the plasma membrane containing caveolin-1 (Cav-1) and are composed of tetraspan transmembrane proteins (claudins and occludin) and single-span cytoplasmic proteins (junction adhesion molecules or JAM proteins). Adaptor proteins, such as ZO-1, link both classes of proteins to the actin cytoskeleton as well as to other signaling molecules. These junctions seal the paracellular space, although they remain capable of rapid modulation and regulation. Consequently, the only solutes that can passively enter the brain are lipid soluble and able to freely diffuse across endothelial cell membranes.

The presence of a functional BBB means that most substances entering the brain must do so by carrier- or receptor-mediated mechanisms. Some molecules cross the BBB by clathrin-mediated endocytosis. However, in addition to clathrin-mediated transport, the plasma membranes of brain endothelia are enriched in membrane lipid rafts, called caveolae, that participate in endocytosis, a type of endocytosis by which small molecules are transported across the plasma membrane by caveolae rather than clathrin-coated vesicles. Select plasma proteins are, for example, taken up and transported across endothelial cell membranes by both receptor-mediated and receptor-independent transcytosis (transport across the interior of the cell from blood to interstitial fluid or vice versa) involving caveolae. Caveolar membranes, in addition to containing structural proteins including the caveolins (Cav-1 and 2 in endothelial cells and Cav-3 in astrocytes) and cavin (abundant at the cytoplasmic face of caveolae), also contain a variety of signaling molecules (G-protein-coupled receptors, G-proteins, non-receptor tyrosine kinases, non-receptor Ser/Thr kinases, GTPases, and adaptor proteins). Caveolae are also enriched in β-D-galactosyl and β-N-acetylglucosaminyl residues, cholesterol, sphingolipids (sphingomyelin and glycosphingolipids), as well as palmitoleic and stearic acids. Following the BBB breakdown that occurs with acute injuries to the brain, caveolae can fuse to form transendothelial channels that extend from the luminal to the abluminal plasma membrane, which allows passage of macromolecules from the blood to the brain and vice versa, a phenomenon that has not been observed in normal brain endothelium (Nag et al., 2011; Redzic, 2011).

The high metabolic rate within the brain is dependent on a continuous supply of glucose from the circulation, because under normal conditions, glucose is the main source of metabolic energy (see Chapter 3). Because glucose does not readily cross the BBB, mechanisms have evolved for the transcellular transport of glucose from the blood to the brain interstitial fluid. In the brain, the main glucose transporters are glucose transporter 1 (GLUT-1) in microvascular endothelial cells and glia, and GLUT-3 in neurons. These insulin-insensitive glucose transporters are constitutively expressed on the cell surface. In endothelial cells, the concentration of GLUT-1 is higher on the abluminal surface. Capillary densities in different brain regions are directly related to local blood flow, and close correlations exist between local cerebral glucose utilization, local densities of GLUT-1 and GLUT-3, and local capillary density. Moreover, the pericytes, which are contractile cells that wrap around the endothelial cells, regulate the distribution of the capillary blood flow to match the local cerebral metabolic need (Dalkara et al., 2011).

The BBB also limits the diffusion of other circulating solutes, and independent carrier systems exist for neutral, basic, and acidic amino acids, as well as purines, nucleosides, and monooxygenase acids, including lactate and pyruvate. Aromatic amino acids in particular serve as precursors for the synthesis of the neurotransmitters serotonin, dopamine, and histamine, and the synthesis of these neurotransmitters is substrate limited. Cerebral endothelial cells express specific solute carrier proteins (SLCs) that mediate the entry and efflux of amino acids across the luminal and abluminal surfaces of the BBB. Both Na+-dependent and independent systems have been identified. Na+-independent amino acid transport systems that have been identified in brain capillary endothelium are system L (or a high affinity isoform L1) for large neutral amino acids (including L-leucine, L-phenylalanine, L-isoleucine, L-lysine, L-methionine, L-valine); system y+ for the transport of cationic amino acids (L-arginine, L-lysine, L-ornithine), and system x(c) for anionic amino acids (cystine/glutamate exchange transporter system). Na+-dependent transport systems present in brain endothelial cells include systems A and ASC which show preference for small neutral amino acids (L-alanine, L-serine, L-cysteine), system B0,+ for both neutral and basic amino acids, system XAG for L-glutamate and L-aspartate and system β (also Cl-dependent) for β-amino acids β-alanine and taurine. The anionic amino acid transport systems are important in the inactivation of glutamatergic neurotransmission in the brain and for the synthesis of glutathione. The high affinity Na+-dependent transporters are located principally in the abluminal membrane of the cerebral endothelium (Broer and Palacin, 2011; Mann et al., 2003).

The Cerebral Vasculature in Disease States

Many disease states affect the cerebral vasculature. Occlusive cerebral vascular disease can affect both
large and small cerebral vessels, causing ischemia of cerebral tissue and resulting acutely in stroke. By contrast, more chronic cerebral ischemia can result in slow, progressive cognitive impairment and vascular dementia. The integrity of the BBB is also affected in a variety of conditions (Nag et al., 2011). For example, brain tumors often disrupt the BBB, causing fluid to leak into brain tissue. The vasogenic edema that results is often associated with neurological impairment beyond the effects of the tumor itself. A variety of inflammatory and infectious conditions also disrupt the BBB. In multiple sclerosis, inflammatory T lymphocytes cross the BBB and initiate an autoimmune attack on central nervous system (CNS) myelin. Treatment modalities often target the effects of an altered BBB, such as the use of corticosteroids in patients with brain tumors or CNS disorders associated with autoimmunity.

References


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Overview

Cellular Neuroscience Is the Foundation of Modern Neuroscience
Diverse cell types comprising the nervous system interact to create a functioning brain

Neurons: Common Elements and Diversity
The classic image of a neuron includes a perikaryon, multiple dendrites and an axon
Although neurons share common elements with other cells, each component has specialized features
The axon compartment comprises the axon hillock, initial segment, shaft and terminal arbor
Dendrites are the afferent components of neurons
The Synapse is a specialized junctional complex by which axons and dendrites emerging from different neurons intercommunicate

Macroglia: More than Meets the Eye
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The BBB and BCSFB serve a number of key functions critical for brain function
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The Neurovascular Unit Includes Multiple Components
The lumen of the cerebral capillaries that penetrate and course through the brain tissue are enclosed by BECs interconnected by TJ
The basement membrane (BM)/basal lamina is a vital component of the BBB
Astrocytes contribute to the maintenance of the BBB
Pericytes at the BBB are more prevalent than in other capillary types
Brain endothelial cells restrict the transport of many substances while permitting essential molecules access to the brain
There are multiple transporters and transport processes for bidirectional transport at the BBB
Lipid solubility is a key factor in determining the permeability of a substance through the BBB by passive diffusion
The BBB expresses solute carriers to allow access to the brain of molecules essential for metabolism
Receptor-mediated transcytosis (RMT) is the Primary route of transport for some essential peptides and signaling molecules
ATP-binding cassette transporters (ABC) on luminal membranes of the BBB restrict brain entry of many molecules
During development, immune-competent microglia develop and reside in the brain tissue
There is increasing evidence of BBB dysfunction, either as a cause or consequence, in the pathogenesis of many diseases affecting the CNS
The presence of an intact BBB affects the success of potentially beneficial therapies for many CNS disorders

Acknowledgments

Box: Bardet-Biedl Syndrome and the Neuronal Primary Cilium

References

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OVERVIEW

More than 100 years since the idea of a nervous system made of distinct cell populations gained acceptance, we are beginning to understand how these different cells are produced and how they relate to each other. More importantly, many aspects of the molecular and biochemical basis for these relationships, i.e., the basic neurochemistry of the nervous system, have been defined. The molecular specialization of cells in the nervous system defines function and interactions. In this chapter, we begin by considering the cells and microanatomy of the nervous system as a way of providing a foundation for detailed considerations of the cellular, molecular, and biochemical properties of the nervous system.

CELLULAR NEUROSCIENCE IS THE FOUNDATION OF MODERN NEUROSCIENCE

Diverse cell types comprising the nervous system interact to create a functioning brain

Modern neurobiology emerged at the turn of the last century out of the demonstration that the brain represented a complex network of distinct cells interacting in precise ways, rather than a syncytium (Ramon y Cajal, 1967). Cells of the nervous system exhibit an extraordinary diversity in shape, size and number of unique interactions with other cells. In the first fifty years, the focus was on identifying and describing these cells, establishing a rich database of information on the anatomy of the nervous system. As our knowledge of neuroanatomy and histology deepened, scientists began to appreciate the specialized biochemistry of the brain and neurochemistry emerged as a distinct field of investigation. Diverse cell types are organized into assemblies and patterns such that specialized components are integrated into a physiology of the whole organ (Fig. 1-1). Brain development and the origins and differentiation of these diverse cell types are discussed in Chapters 28, 30, and 31.

NEURONS: COMMON ELEMENTS AND DIVERSITY

The classic image of a neuron includes a perikaryon, multiple dendrites and an axon

The stereotypical image of a neuron is that of a stellate cell body, the perikaryon or soma, with broad dendrites emerging from one pole and a single axon emerging from the opposite pole (Fig. 1-2). Although this image is near universal in textbooks, neuroanatomists have long recognized the remarkable diversity of neuronal sizes and morphologies (Ramon y Cajal, 1909). The neuron is the most polymorphic cell in the body and defies formal classification on the basis of shape, location, function, fine structure or transmitter substance. Despite this diversity, homologous neurons are often easily recognized across considerable phylogenetic distance. Thus, a Purkinje cell from lamprey shares many recognizable features with those of humans (Bullock et al., 1977). Before the work of Deiters and Ramón y Cajal more than 100 years ago, neurons and neuroglia were believed to form syncytia, with no intervening membranes. The demonstration of neurons and glia as discrete cells proved to be the foundation of modern neuroscience.

FIGURE 1.1 The major components of the CNS and their interrelationships. Microglia are depicted in light purple. In this simplified schema, the CNS extends from its meningeal surface (M), through the basal lamina (solid black line) overlying the subpial astrocyte layer of the CNS parenchyma, and across the CNS parenchyma proper (containing neurons and glia) and subependymal astrocytes to ciliated ependymal cells lining the ventricular space (V). Note how the astrocyte also invests blood vessels (BV), neurons and cell processes. The pia-astroglia (glia limitans) provides the barrier between the exterior (dura and blood vessels) and the CNS parenchyma. One neuron is seen (center), with synaptic contacts on its soma and dendrites. Its axon emerges to the right and is myelinated by an oligodendrocyte (above). Other axons are shown in transverse section, some of which are myelinated. The ventricles (V) and the subarachnoid space of the meninges (M) contain cerebrospinal fluid.
FIGURE 1.2 Diagram of a motor neuron with myelinated axon. The traditional view of a neuron includes a perikaryon, multiple dendrites and an axon. The perikaryon contains the machinery for transcription and translation of proteins as well as their processing. These proteins must be targeted to somal, dendritic or axonal domains as appropriate. The dendrites typically contain postsynaptic specializations, particularly on spines. Some dendritic proteins are locally translated and processed in response to activity. Axonal domains typically contain presynaptic terminals and machinery for release of neurotransmitters. Large axons are myelinated by glia in both the CNS and PNS. The action potential is initiated at the initial segment and saltatory conduction is possible because of concentration of sodium channels at the nodes of Ranvier. Neuronal processes are maintained through the presence of cytoskeletal structures: neurofilaments (axons) and microtubules (axons and dendrites). However, there may be no neurons with this simple structure.
Nerve cell shapes and sizes range from the small, globular cerebellar granule cells, with a perikaryal diameter of approximately 6–8 µm, to the distinctive, pear-shaped Purkinje cells and star-shaped anterior horn cells, with perikaryons that may reach diameters of 60–80 µm in humans. Perikaryal size is generally a poor index of total cell volume or surface area. The dendritic and axonal processes of a neuron may represent the overwhelming bulk of neuronal volume and surface, approaching 95–99% of the total cell volume in some cases.

Both axons and dendrites typically exhibit extensive branching with a cell type–specific pattern (see Fig. 1-3, for example). The extent of the branching displayed by the dendrites is a useful index of their functional importance. Dendritic trees represent the expression of the receptive fields, and large fields can receive inputs from multiple origins. A cell with a less-developed dendritic ramification, such as the cerebellar granule cell, has synapses with a more homogeneous population of afferent sources.

The axon emerges from a neuron as a slender thread and frequently does not branch until it nears its target. In contrast to the dendrite and the soma, the axon is frequently myelinated, thus increasing its efficiency as a conducting unit. Myelin, a spirally wrapped membrane (see Ch. 4), is laid down in segments, or internodes, by oligodendrocytes in the CNS and by Schwann cells in the PNS. The naked regions of axon between adjacent myelin internodes are known as nodes of Ranvier (Fig. 1-2).

Although neurons share common elements with other cells, each component has specialized features

Neurons contain the morphological features of other cell types, particularly with regard to the cell soma. The major structures are similarly distributed and some of the most common, such as the Golgi apparatus, Nissl substance and mitochondria, for example, were described first in neurons. However, neurons are distinctive for their size, metabolic activity, and unusual degree of polarization.

The large, pale nucleus and prominent nucleolus of neurons helps identify neurons in histological sections and are consistent with the high level of transcription characteristic of neurons (Fig. 1-4). The nucleolus is vesiculated and easily visualized in the background of pale euchromatin with sparse heterochromatin. The nucleolus usually contains two textures: the pars fibrosa, which are fine bundles of filaments composed of newly transcribed ribosomal RNA, and the pars granulosa, with dense granules consisting of ribonuclear proteins that form ribosomes in the cytoplasm. As with other cells, the nucleus is enclosed by the nuclear envelope, made up of nuclear lamins with a cytoplasmic side membrane, which is in continuity with the endoplasmic reticulum and a more regular membrane on the inner, or nuclear, aspect of the envelope. Periodically, the inner and outer membranes of the envelope come together to form a single diaphragm, forming a 70 nm nuclear pore. In some neurons, as in Purkinje cells, that segment of the nuclear envelope that faces the dendritic pole is deeply invaginated.

The perikaryon (i.e., soma or cell body) of the neuron tends to be larger than other cells of the nervous system and is rich in organelles (Fig. 1-4) including components of the translational machinery, mitochondria, endoplasmic reticulum (ER), lysosomes and peroxisomes, Golgi complex, intermediate components, tubulovesicular organelles, endosomes and cytoskeletal structures. Various membranous cisternae are abundant, divisible into rough ER (rER), which forms part of the Nissl substance; smooth ER (sER); subsurface cisternae; and the Golgi apparatus, with some degree of interconnectivity. Despite these

**FIGURE 1.3** Real neurons have much more complex morphologies with elaborate branched arbor for both dendrites and axons. Individual neurons may have thousands of presynaptic terminals on their axons and thousands of postsynaptic specializations on their dendrites. Image is adapted from (*Fisher & Boycott*, 1974) and shows an example of a horizontal cell in the retina of the cat.
structural connections, each possesses distinct protein composition and enzymatic activities. In addition, lipofuscin granules, which also are termed aging pigment, are often seen in mature neurons.

Nissl substance, identified by staining for ribonucleic acid, comprises the various components of the translational machinery, including both rER, where membrane associated proteins are synthesized, and cytoplasmic or free polysomes for cytoplasmic proteins, which are actually anchored to the cytoskeleton (Fig. 1-5). Histologically, Nissl substance is seen as cytoplasmic basophilic masses that ramify loosely throughout the cytoplasm and were first described in the nervous system. The distinctive Nissl staining of neurons reflects the high levels of protein synthesis in neurons needed to supply the large volume and surface area of neurons. Nissl substance appears excluded from axons at the axon hillock, but can be seen at lower levels in dendrites. The abundance and distribution of Nissl substance in certain neurons are characteristic and can be used as criteria for identification. In the electron microscope (EM), Nissl substance appears as arrays of flattened cisternae of the rER surrounded by clouds of free polyribosomes. The membranes of the rER are studded with rows of ribosomes, which produce the granular appearance of the rER. A space of 20–40 nm is maintained within cisternae. The rER in neurons produces some secretory components like neuropeptides, but must also generate the wide range of membrane proteins used throughout the neuron, a feature imposed by the extraordinary functional demands placed on the neuron.

Smooth ER is also abundant in neurons (see Ch. 7), although differentiating sER and rER can be problematic given the proximity and abundance of free polysomes. Ribosomes are not associated with sER, and the cisternae usually assume a meandering, branching course throughout the cytoplasm. In some neurons, i.e., Purkinje cells, the smooth ER is quite prominent. Individual cisternae of the smooth ER extend along axons and dendrites (Ch. 7). The cisternae of sER are metabolically active, representing the site of synthesis for lipids and steroids, as well for processing of proteins by glycosylation, formation and rearrangement of disulfide bonds, and conversion of pro forms of proteins or peptide hormones. The sER is also a site for metabolism of drugs, some carbohydrates, and steroids. Given the diverse functions of sER, there is likely to be regional segregation of specific functions and protein complements within the broad category of sER.

For example, a subsurface cisternal system that is often classified as sER plays a critical role in regulation of cytoplasmic Ca\(^{2+}\) (Ch. 24). These are membrane-bound, flattened...
cisternae that can be found in many neurons, bearing some elements in common with the sarcoplasmic reticulum in muscle. These structures abut the plasmalemma of the neuron and constitute a secondary membranous boundary within the cell. The distance between these cisternae and the plasmalemma is usually 10–12 nm and, in some neurons, such as the Purkinje cells, a mitochondrion may be found in close association with the innermost leaflet. Similar cisternae have been described beneath synaptic complexes, presumably playing a role in the innermost leaflet. Similar cisternae have been described beneath synaptic complexes, presumably playing a role in the innermost leaflet.

Mitochondria are the centers for oxidative phosphorylation and the respiratory centers of all eukaryotic cells (see in Ch. 43). These organelles occur ubiquitously in the neuron and its processes. Their overall shape may change from one type of neuron to another but their basic morphology is identical to that in other cell types. Mitochondria consist morphologically of double-membrane sacs surrounded by protuberances, or cristae, extending from the inner membrane into the matrix space. Mitochondrial membranes have a distinctive lipid composition, including the mitochondrial-specific lipid cardiolipin. Mitochondria are primarily considered as the source of ATP from aerobic metabolism of pyruvate or fatty acids, but may have other functions as well. In particular, they play a critical role in regulation of cell death pathways (Chipuk, et al., 2010) (Ch. 37).

Mitochondria and plant chloroplasts are unique among organelles in containing their own genetic complement and machinery for protein synthesis. There are more than twenty mitochondrial genes encoding polypeptides having a mitochondrial function, along with tRNA and ribosomal RNA genes (Szibor & Holtz, 2003). Multiple copies of these genes serve to protect against DNA damage. Protein synthesis in mitochondria shares many features with prokaryotic protein synthesis, including sensitivity to antibiotics that inhibit bacterial protein synthesis. However, mitochondria age and must be renewed on a regular basis (Szibor & Holtz, 2003). As

FIGURE 1.6 A portion of a Golgi apparatus. The smooth-membranous cisternae appear beaded. The many circular profiles represent tangentially sectioned fenestrations and alveolate vesicles (primary lysosomes). Two of the latter can be seen budding from Golgi saccules (arrows). ×60,000.
nuclear genes encode the majority of mitochondrial proteins, both cellular and mitochondrial protein synthesis are needed for generation of new mitochondria (Ch. 43).

The axon compartment comprises the axon hillock, initial segment, shaft and terminal arbor

These regions differ ultrastructurally in membrane morphology and cytoskeletal organization. The axon hillock may contain fragments of Nissl substance, including abundant ribosomes, which diminish as the hillock continues into the initial segment. Here, the various axoplasmic components begin to align longitudinally. A few ribosomes and the smooth ER persist, and some axoaxonic synapses occur. The axolemma of the initial segment where the action potential originates exhibits a dense granular layer similar to that seen at the nodes of Ranvier, consistent with a specialized membrane cytoskeleton. Also present in this region are microtubules, neurofilaments and mitochondria. The arrangement of the microtubules in the initial segment is distinctive in forming fascicles interconnected by side arms. Beyond the initial segment, the axon maintains a relatively uniform caliber even after branching with little or no diminution until the very terminal arbors (Fig. 1-7). One exception is a reduction of caliber for myelinated axons at the peripheral node of Ranvier (Hsien et al., 1994) (see Fig. 1-2 and below). Myelinated axons show granular densities on the axolemma at nodes of Ranvier (Raine, 1982) that correspond to adhesion molecules and high densities of sodium channels. In myelinated fibers, there is a concentration of sodium channels at the nodal axon, a feature underlying the rapid, saltatory conduction of such fibers (Ch. 4).

Microtubules are a prominent feature of all axons. Axonal microtubules are aligned with the long axis of the axon and have a uniform polarity with plus ends distal to the soma (Ch. 6). Microtubules are present in loose groupings rather than bundles and vary in their spacing (Fig. 1-7A). Vesicles and mitochondria are typically seen in association with these microtubule domains, consistent with their movement in fast axonal transport (Ch. 8). In axons less than a micron in diameter, which are usually unmyelinated, microtubules are the primary structural cytoskeletal elements, with sparse neurofilaments and gaps in the neurofilament cytoskeleton. As axons get larger, the number of neurofilaments increases dramatically, becoming the primary determinant of axonal caliber. For large, myelinated axons, neurofilaments occupy the bulk of an axon cross-section (Ch. 6) with microtubules found in small groups along with membrane profiles.

Although neuroscientists typically draw neurons with a single unbranched axon and one presynaptic terminal, most axons are extensively branched into terminal arbors, often producing hundreds or thousands of presynaptic terminals (Fig. 1-3). In addition, many axons in the CNS have en passant presynaptic specializations (Peters et al., 1991) that allow a single axon to have many presynaptic specializations in series. Parallel fibers in the cerebellar cortex may have thousands of these specializations. When en passant synapses occur on myelinated fibers, these synaptic specializations are seen at the nodes of Ranvier. The terminal portion of the axon arborizes and enlarges to form presynaptic specializations at sites of synaptic contact (Chs. 7 and 12).

Dendrites are the afferent components of neurons

In some neurons, they may arise from a single trunk, while other neurons have multiple dendritic trunks emerging from the cell soma. Unlike the axon, dendritic processes taper

I. CELLULAR NEUROCHEMISTRY AND NEURAL MEMBRANES
distally and each successive branch is reduced in diameter. The extensive branching into a dendritic tree gave rise to the name dendrite. Dendrites are typically rich in microtubules and microfilaments, but largely lack neurofilaments. Unlike in axons, the microtubules in proximal dendrites are distinctive in having a mixed polarity and a distinctive microtubule associated protein, MAP2. Proximal dendrites generally contain Nissl substance and components of the Golgi complex. A subset of neuronal mRNAs is transported into the dendrites, where local synthesis and processing of proteins occur in response to synaptic activity (Martin & Zukin, 2006).

Some difficulty may be encountered in distinguishing small unmyelinated axons or terminal segments of axons from similar-sized dendrites. In the absence of morphologically identifiable synaptic structures, they can often be assessed by the content of neurofilaments, which are more typical of axons. The postsynaptic regions of dendrites occur either along the main stems (Fig. 1-8) or more commonly at small protuberances known as dendritic spines (Luscher et al., 2000). Axon presynaptic terminals abut these spines, whose number and detailed structure may be highly dynamic, changing with activity (Bhatt et al., 2009). Spine dynamics are thought to reflect altered synaptic function and may be a substrate for learning and memory. Considerable insights into spine function have been obtained through imaging of spines in intact brain (Bhatt et al., 2009).

The synapse is a specialized junctional complex by which axons and dendrites emerging from different neurons intercommunicate

This was proposed first in 1897 by Sherrington, who also coined the term ‘synapse’. The existence of synapses was immediately demonstrable by EM and can be recognized today in a dynamic fashion by Nomarski optics (differential interference microscopy), confocal and light microscopy, and scanning EM.

Synaptic structures are diverse in morphology and function (Fig. 1-8). Some are polarized or asymmetrical, due to the unequal distribution of electron-dense material on the apposing membranes of the junctional complex and heavier accumulation of organelles within the presynaptic component. The closely applied membranes constituting the synaptic site are overlaid on the presynaptic and postsynaptic aspects by an electron-dense material similar to that seen in desmosomes and separated by a gap or cleft of 15–20 nm. The classic presynaptic terminal of a chemical synapse contains a collection of clear, 40–50 nm synaptic vesicles. The morphology of synaptic vesicles in the terminal may exhibit subtle differences depending on the neurotransmitter being released (Peters & Palay, 1996).

Synaptic vesicles are important in packaging, transport and release of neurotransmitters and, after their discharge into the synaptic cleft, they are recycled within the axon terminal (Ch. 7). Also present are small mitochondria approximately 0.2–0.5 μm in diameter (Fig. 1-8). Microtubules, coated vesicles and cisternae of the smooth ER may be found in the presynaptic compartment. On the postsynaptic side is a density referred to as the subsynaptic web. Aside from relatively

![FIGURE 1.8 Presynaptic morphologies reflect differences in synaptic function. Top panel: A dendrite (D) is flanked by two axon terminals packed with clear, spherical synaptic vesicles. Details of the synaptic region are clearly shown. ×75,000. Middle panel: An axonal terminal at the surface of a neuron from the dorsal horn of a rabbit spinal cord contains both dense-core and clear, spherical synaptic vesicles. Details of the synaptic region are clearly shown. ×68,000. Bottom panel: An electrotonic synapse is seen at the surface of a motor neuron from the spinal cord of a toadfish. Between the neuronal soma (left) and the axonal termination (right), a gap junction flanked by desmosomes (arrows) is visible. ×80,000. (Photograph courtesy of Drs. G. D. Pappas and J. S. Keeter.)
sparse profiles of smooth ER or subsurface cisternae and Golgi profiles, there are few aggregations of organelles in the dendrite. At the neuromuscular junction, the morphological organization is somewhat different. Here, the axon terminal is greatly enlarged and ensheathed by Schwann cells; the post-synaptic or sarcolemmal membrane displays less density and is infolded extensively.

Today, most neuroanatomists categorize synapses depending on the profiles between which the synapse is formed, such as axodendritic, axosomatic, axoaxonic, dendrodendritic, somatosomatic and somatodendritic synapses. However, such a classification does not specify whether the transmission is chemical or electrical nor does it address the neurotransmitter involved in chemical synapses. Alternatively, physiological typing of synapse defines three groups: excitatory, inhibitory and modulatory. Depending on the methods used, the synaptic vesicles can be distinctive (Peters & Palay, 1996). For example, excitatory synapses may have spherical synaptic vesicles, whereas inhibitory synapses contain a predominance of flattened vesicles (Fig. 1-8). However, some consider that the differences between flat and spherical vesicles may reflect an artifact of aldehyde fixation or a difference in physiological state at the time of sampling. Moreover, this classification does not hold true for all regions of the CNS.

The most extensively studied synapses in situ or in synaptosomes are cholinergic (Ch. 13). However, there is a wide range of chemical synapses that utilize biogenic amines (Chs. 14–16) as neurotransmitter substances, as well as other small molecules such as GABA (Ch. 18) and adenosine (Ch. 19). In addition to clear vesicles, slightly larger dense-core or granular vesicles of variable dimensions can be seen in the presynaptic terminal (Fig. 1-8). These larger, dense core vesicles contain neuropeptides (Ch. 20), whose secretion is regulated independently of classic neurotransmitters. Further, some synapses may be so-called silent synapses, which are observed in CNS tissue both in vitro and in vivo. These synapses are morphologically identical to functional synapses but are physiologically dormant.

Finally, there is the well-characterized electrical synapse, where current can pass from cell to cell across regions of membrane apposition that essentially lack the associated collections of organelles present at the chemical synapse. In the electrical synapse (Fig. 1-8 lower panel), the unit membranes are closely apposed, and the outer leaflets sometimes fuse to form a pentalaminar structure; however, in most places, a gap of approximately 20nm exists, producing a so-called gap junction. Not infrequently, desmosome-like domains separate gap junctions. Sometimes, electrical synapses exist at terminals that also display typical chemical synapses; in such cases, the structure is referred to as a mixed synapse.

**MACROGLIA: MORE THAN MEETS THE EYE**

In 1846, Virchow recognized the existence of a fragile, non-nervous, interstitial component made up of stellate or spindle-shaped cells in the CNS. These cells were morphologically distinct from neurons, and were thought to hold the neurons together, hence the name neuroglia, or ‘nerve glue’ (Peters et al., 1991). Today, we recognize three broad groups of glial cells in the CNS: (a) macroglia, such as astrocytes, radial glia and oligodendrocytes of ectodermal origin, like neurons; (b) microglia, of mesodermal origin; and (c) ependymal cells, also of ectodermal origin. Microglia invade the CNS via the pia mater, the walls of blood vessels and the tela choroidea at the time of vascularization. Glial cells are not electrically excitable and some types, such as astrocytes, retain the ability to proliferate, particularly in response to injury, while others may be replaced by differentiation from progenitors (see Ch. 30). The rough schema represented in Fig. 1-1 illustrates the interrelationships between glia and other CNS components.

**Virtually nothing can enter or leave the central nervous system parenchyma without passing through an astrocytic interphase**

The complex packing achieved by the processes and cell bodies of astrocytes reflects their critical role in brain metabolism (Sofroniew & Vinters, 2010). Astrocytes traditionally have been subdivided into stellate-shaped protoplasmic and fibrous astrocytes as well as the elongate radial and Bergman glia (Kimelberg & Nedergaard, 2010). The astrocyte lineage is increasingly recognized as more complex and dependent on the developmental context than previously recognized (Kimelberg & Nedergaard, 2010).

Although many structural components of fibrous and protoplasmic astrocytes are shared, their functions are diverse. Protoplasmic astrocytes range in size from 10–40µm, are frequently located in gray matter in relation to capillaries and have a clearer cytoplasm than fibrous astrocytes (Fig. 1-9). Fibrous astrocytes are found in white matter and are typically smaller. All astrocytes have intermediate filaments containing glial fibrillary acidic protein (GFAP), which is a standard marker for

**FIGURE 1.9** A protoplasmic astrocyte abuts a blood vessel (lumen at L) in rat cerebral cortex. The nucleus shows a rim of denser chromat in, and the cytoplasm contains many organelles, including Golgi and rough endoplasmic reticulum. ×10,000.
Astrocytic cells, and microtubules (Fig. 1-10), often extending together with loose bundles of filaments along cell processes. They also contain glycogen granules; lysosomes and lipofuscin-like bodies; isolated cisternae of the rough ER; a small Golgi apparatus opposite one pole of the nucleus; and small, elongated mitochondria. Characteristically, the nucleus is ovoid and nucleochromatin homogeneous, except for a narrow, continuous rim of dense chromatin and one or two poorly defined nucleoli, consistent with modest levels of transcription and translation. Another common feature of astrocytes is that they form tight junctions, particularly desmosomes (mediated by cadherins) and gap junctions (mediated by connexins) that occur between adjacent astrocytic processes.

Fibrous astrocyte (Fig. 1-10) processes appear twig-like, with large numbers of tightly bundled GFAP filaments, while GFAP filaments in protoplasmic astrocytes are less tightly bundled. Filaments within these astrocyte processes can be readily distinguished from neurofilaments by their close packing and the absence of side arms (see Ch. 6). GFAP staining is a standard marker for identification of astrocytes and has traditionally been used to estimate the extent of astrocytic processes. However, expression of green fluorescent protein (GFP) under astrocyte-specific promoters indicates that GFAP staining significantly underestimates the size and extent of astrocytic processes. This is important because each astrocyte typically defines a domain based on the soma and processes, with little overlap between adjacent domains through peripheral processes that are largely invisible with GFAP staining (Nedergaard et al., 2003). Remarkably, the number, size, and extent of astrocytes is species-dependent, so human astrocytes are 2.5 times larger than comparable mouse astrocytes (Kimelberg & Nedergaard, 2010) and the number of astrocytes per neuron is 3–4 times greater in human brain (Nedergaard et al., 2003).

In addition to protoplasmic and fibrous forms, a set of elongate cells is also derived from the astrocyte lineage, including Müller glia in the retina (Ch. 51) and Bergman glia in the cerebellum (Kimelberg & Nedergaard, 2010). In addition to providing structural support, these elongated astrocytes may have additional roles to play. For example, Müller cells may serve as light guides, analogous to fiber optics, channeling light to photoreceptors (Franze et al., 2007). Finally, regional specialization occurs among astrocytes. In addition to differences between white (fibrous) and grey matter (protoplasmic) astrocytes, there may be additional subtypes. For example, the outer membranes of astrocytes located in subpial zones and those facing blood vessels possess a specialized thickening, sometimes called hemidesmosomes, and there may be additional functional specializations.

New functions of astrocytes continue to be identified (Kimelberg & Nedergaard, 2010). Astrocytes ensheath synaptic complexes and the soma of some neurons (i.e., Purkinje cells). This places them in a unique position to influence the environment of neurons and to modulate synaptic function. Astrocytes are not excitable cells, but have large negative membrane potentials. This allows them to buffer extracellular K⁺, so astrocytes play a significant role in K⁺ homeostasis in the brain (Leis et al., 2005), particularly after injury (see also Aquaporin 4 in Ch. 3). Astrocytes similarly buffer extracellular pH in the brain and may modulate Na⁺ levels as well (Deitmer & Rose, 2010). Recent studies have established that astrocytes express metabotropic glutamate receptors (Ch. 17) and purinergic receptors (Ch. 19). Activation of purinergic receptors may produce Ca²⁺ waves that affect groups of astrocytes by release of Ca²⁺ from intracellular stores and that may involve communication between astrocytes through gap junctions (Nedergaard et al., 2003). Complementary to these functions, astrocytes may play a role in regulation of cerebral blood flow and availability of both glucose and lactate for maintenance of neuronal metabolism. Further, even the entry of water into the brain may be modulated by the action of aquaporins on astrocytes (Kimelberg & Nedergaard, 2010).

Astrocytes may affect neuronal signaling in a variety of ways. Prolonged elevation of extracellular levels of the excitatory neurotransmitter glutamate can lead to excitotoxicity due to overactivation of glutamate receptors and excessive entry of Ca²⁺ into neurons. Astrocytes express both metabotropic glutamate receptors and glutamate transporters, which are responsible for glutamate uptake and limit the possibility of neuronal damage (Sattler & Rothstein, 2006). The astrocyte enzymatically converts glutamate to glutamine, which can then be recycled to the neuron. Astrocytes similarly provide glutathione to neurons through a uptake and conversion of glutathione to glutathione.
cysteine (McBean, 2011). Finally, GABA transporters on astrocytes may affect the balance between excitatory and inhibitory pathways.

The role of astrocytes in injury and neuropathology is complex (Sofroniew & Vinters, 2010). Subsequent to trauma, astrocytes invariably proliferate, swell, accumulate glycogen and undergo fibrosis by the accumulation of GFAP filaments. This state of gliosis may be total, in which case all other elements are lost, leaving a glial scar, or it may be a generalized response occurring against a background of regenerated or normal CNS parenchyma. With age, both fibrous and protoplasmic astrocytes accumulate filaments. Mutations in GFAP are now known to be the cause of the childhood leukodystrophy called Alexander disease (Johnson, 2002) (Ch. 41).

Oligodendrocytes are myelin-producing cells in the central nervous system

Oligodendrocytes are definable by morphological criteria. The roughly globular cell soma ranges from 10–20 µm and is denser than that of an astrocyte. The margin of the cell is irregular and compressed against the adjacent neuropil. In contrast to astrocytes, few cell processes are seen. Within the cytoplasm, many organelles are found. Parallel cisternae of rough ER and a widely dispersed Golgi apparatus are common. Free ribosomes occur, scattered amid occasional multivesicular bodies, mitochondria and coated vesicles. Distinguishing the oligodendrocyte from the astrocyte is the absence of glial or any other intermediate filament, but abundant microtubules are present (Figs. 1-10 and 11). Microtubules are most common at the margins of the cell, in the occasional cell process and in cytoplasmic loops around myelin sheaths. Lamellar dense bodies, typical of oligodendrocytes, are also present. The nucleus is usually ovoid, but slight lobation is not uncommon. The nucleochromatin stains heavily and contains clumps of denser heterochromatin. Desmosomes and gap junctions occur between interfascicular oligodendrocytes.

Myelinating oligodendrocytes have been studied extensively (see Chs. 10 and 31). Examination of the CNS during myelogenesis (Fig. 1-11) reveals connections between the cell body and the myelin sheath (Chs. 10 and 31). The oligodendrocyte is capable of producing many internodes of myelin simultaneously. It has been estimated that oligodendrocytes in the optic nerve produce between 30 and 50 internodes of myelin. Damage to only a few oligodendrocytes, therefore, can be expected to produce an appreciable area of primary demyelination. Oligodendrocytes are among the most vulnerable elements and the first to degenerate (Ch. 39). Like neurons, they lose their ability to proliferate once differentiated.

Analogous to a neuron, the relatively small oligodendrocyte soma produces and supports many times its own volume of membrane and cytoplasm. For example, an average oligodendrocyte produces 20 internodes of myelin. Each axon has a diameter of 3 µm and is covered by at least six lamellae of myelin, with each lamella representing two fused layers of unit membrane. Calculations based on the length of the myelin internode (which may exceed 500 µm) and the length of the cell processes connecting the sheaths to the cell body indicate that the ratio between the surface area for the cell soma and the myelin it sustains can be 1:1000 or greater.

The schwann cell is the myelin-producing cell of the peripheral nervous system

The schwann cell is the myelin-producing cell of the peripheral nervous system. When axons leave the CNS, they lose their neuroglial interrelationships and traverse a short transitional zone where they are invested by an astroglial sheath enclosed in the basal lamina of the glia limitans. The basal lamina then becomes

FIGURE 1.11 A myelinating oligodendrocyte, nucleus (N), from the spinal cord of a 2-day-old kitten extends cytoplasmic connections to at least two myelin sheaths (arrows). Other myelinated and unmyelinated fibers at various stages of development, as well as glial processes, are seen in the surrounding neuropil. ×12,750.
myelin differs biochemically and antigenically from that of the CNS (see Ch. 10).

Not all PNS fibers are myelinated but all PNS axons interact with Schwann cells. For small axons (<1 μm), nonmyelinating Schwann cells interact with multiple axons (Peters et al., 1991). Nonmyelinated fibers in the PNS are grouped into bundles surrounded by Schwann cell processes, in contrast to the situation in the CNS. Each axon is largely separated from adjacent axons by invaginations of Schwann cell membrane and cytoplasm. However, the axon connects to the extracellular space via a short channel, the mesaxon, formed by the invaginated Schwann cell plasmalemma.

Ultrastructurally, the Schwann cell is unique and distinct from the oligodendrocyte. Each Schwann cell is surrounded by a basal lamina made up of a mucopolysaccharide approximately 20–30 nm thick that does not extend into the mesaxon (Fig. 1-12). The basal lamina of adjacent myelinating Schwann cells at the nodes of Ranvier is continuous, and Schwann cell processes interdigitate so that the PNS myelinated axon is never in direct contact with the extracellular space (Fig. 1-13). These nodal Schwann cell fingers display intimate relationships with the axolemma, and a similar arrangement between the nodal axon and the fingers of astroglial cells is seen in the CNS, but the specific function of these fingers is not well understood. The axon in the peripheral node of Ranvier is significantly restricted (Fig. 1-13) and the neurofilaments are dephosphorylated (Witt & Brady, 2000), which is thought to be related to targeting of proteins to the nodal membrane. However, changes in axon caliber and neurofilament density at CNS nodes of Ranvier are not as dramatic. The Schwann cells of nonmyelinated PNS fibers overlap, so there are no gaps and no nodes of Ranvier.

The cytoplasm of the Schwann cell is rich in organelles (Fig. 1-12). A Golgi apparatus is located near the nucleus, and cisternae of the rough ER occur throughout the cell. Lysosomes, multivesicular bodies, glycogen granules and lipid granules, sometimes termed PI granules, also can be seen. The cell is rich in microtubules and filaments, in contrast to the oligodendrocyte. The plasmalemma frequently shows pinocytic vesicles. Small, round mitochondria are scattered throughout the soma. The nucleus, which stains intensely, is flattened and oriented longitudinally along the nerve fiber. Aggregates of dense heterochromatin are arranged peripherally.

In sharp contrast to the differentiated oligodendrocyte, the Schwann cell responds vigorously to most forms of injury (Ch. 39). An active phase of mitosis occurs following traumatic insult, and the cells are capable of local migration. Studies on their behavior after primary demyelination have shown that they phagocytose damaged myelin. They possess remarkable ability for regeneration and begin to lay down new myelin approximately one week after a fiber loses its myelin sheath. After primary demyelination, PNS fibers are remyelinated efficiently, whereas similarly affected areas in the CNS show relatively little proliferation of new myelin (see Ch. 39). After severe injury leading to transection of the axons, axons degenerate and the Schwann cells form tubes, termed Büngner bands, containing cell bodies and processes surrounded by a single basal lamina. These structures provide channels along which regenerating axons might later grow. The presence and integrity of the Schwann cell basal lamina is essential for

**FIGURE 1.12** A myelinated PNS axon (A) is surrounded by a Schwann cell nucleus (N). Note the fuzzy basal lamina around the cell, the rich cytoplasm, the inner and outer mesaxons (arrows), the close proximity of the cell to its myelin sheath and the 1:1 (cell:myelin internode) relationship. A process of an endoneurial cell is seen (lower left), and unstained collagen (c) lies in the endoneurial space (white dots). ×20,000.
reinnervation, and transplantation of Schwann cells into the CNS environment can facilitate regeneration of CNS axons (Kocsis & Waxman, 2007) (Ch. 32). A number of pathologies have been identified that are associated with mutations in Schwann cell proteins, including many forms of Charcot-Marie-Tooth disease (Ch. 38).

**FIGURE 1.13** The axon is constricted at the peripheral node of Ranvier. **Top panel:** Low-power electron micrograph of a node of Ranvier in longitudinal section. Note the abrupt decrease in axon diameter and the attendant condensation of axoplasmic constituents in the paranodal and nodal regions of the axon. Paranodal myelin is distorted artificially, a common phenomenon in large-diameter fibers. The nodal gap substance (arrows) contains Schwann cell fingers, the nodal axon is bulbous and lysosomes lie beneath the axolemma within the bulge. Beaded smooth endoplasmic reticulum sacs are also seen. ×5,000. **Bottom panel:** A transverse section of the node of Ranvier (7-8nm across) of a large fiber shows a prominent complex of Schwann cell fingers around an axon highlighted by its subaxolemmal densification and closely packed organelles. The Schwann cell fingers arise from an outer collar of flattened cytoplasm and abut the axon at regular intervals of approximately 80nm. The basal lamina of the nerve fiber encircles the entire complex. The nodal gap substance is granular and sometimes linear. Within the axoplasm, note the transversely sectioned sacs of beaded smooth endoplasmic reticulum (ER); mitochondria; dense lamellar bodies, which appear to maintain a peripheral location; flattened smooth ER sacs; dense-core vesicles; cross-bridged neurofilaments; and microtubules, which in places run parallel to the circumference of the axon (above left and lower right), perhaps in a spiral fashion. ×16,000.

**FIGURE 1.14** A microglial cell (M) has elaborated two cytoplasmic arms to encompass a degenerating apoptotic oligodendrocyte (O) in the spinal cord of a 3-day-old kitten. The microglial cell nucleus is difficult to distinguish from the narrow rim of densely staining cytoplasm, which also contains some membranous debris. ×10,000.

The microglial cell plays a role in phagocytosis and inflammatory responses

Of the few remaining types of CNS cells, some of the most interesting and enigmatic cells are the microglia (Graeber, 2010). The microglia are of mesodermal origin, are located in normal brain in a resting state (Fig. 1-14), and convert to a mobile, active brain macrophage during disease or injury (van Rossum & Hanisch, 2004) (Ch. 34). Microglia sense pathological changes in the brain and are the major effector cell in immune-mediated damage in the CNS. However, they also express immunological molecules that have functions in the normal brain. Indeed, microglia in healthy tissue behave very differently from macrophages and should be considered a distinct cell type (Graeber, 2010).

Microglia are pleiotropic in form, being extensively ramified cells in quiescent state and converting to macrophage-like amoeboïd cells with activation. The availability of new selective stains for different stages of activation has expanded our understanding of their number, location and properties (Graeber, 2010). Nonactivated microglia have a thin rim of densely staining cytoplasm that is difficult to distinguish from the nucleus. The nucleochromatin is homogeneously dense and the cytoplasm does not contain an abundance of organelles, although representatives of the usual components can be found. During normal wear and tear, some CNS elements degenerate and microglia phagocytose the debris (Fig. 1-14). Their identification and numbers, as determined by light microscopy, differ from species to species.
species. The CNS of rabbit is richly endowed. In a number of disease instances, such as trauma, microglia are stimulated and migrate to the area of injury, where they phagocytose debris. As our understanding of microglia expands, the number of functions and pathologies in which they play a role increases.

**Ependymal cells line the brain ventricles and the spinal cord central canal**

They typically extend cilia into the ventricular cavity and play numerous roles in development and maintenance of the nervous system (Del Bigio, 2010). One emerging aspect of ependymal cells is their role in supporting neurogenesis in subventricular zones. They express aquaporins and help regulate the fluid balance of the brain. Defects in ependymal cells can produce hydrocephalus, and these cells are particularly vulnerable to viral infections of the nervous system.

The presence of motile cilia is a hallmark of the ependymal cell. The cilia emerge from the apical pole of the cell, where they are attached to a blepharoplast, the basal body (Fig. 1-15), which is anchored in the cytoplasm by means of ciliary rootlets and a basal foot. The basal foot is the contractile component that determines the direction of the ciliary beat. Like all flagellar structures, the cilium contains the common microtubule arrangement of nine peripheral pairs around a central doublet (Fig. 1-15). In the vicinity of the basal body, the arrangement is one of nine triplets; at the tip of each cilium, the pattern is one of haphazardly organized single tubules. Also extending from the free surface of the cell are numerous microvilli containing actin microfilaments (Fig. 1-15). The cytoplasm of ependymal cells stains intensely, having an electron density comparable to oligodendrocyte, whereas the nucleus is more similar in density to that of the astrocyte. Microtubules, large whorls of filaments, coated vesicles, rough ER, Golgi apparatus, lysosomes and abundant small, dense mitochondria are also present in ependymal cells.

The base of the cell is composed of involuted processes that interdigitate with the underlying neuropil (Fig. 1-15). Tight junctions between ependymal cells make them an effective component of the brain–cerebrospinal fluid barrier. The coordinate action of various cells to isolate the brain illustrates multiple elements of cell biology of the brain and will be considered in greater depth.

**BLOOD–BRAIN BARRIERS AND THE NERVOUS SYSTEM**

**Homeostasis of the central nervous system (CNS) is vital to the preservation of neuronal function**

In order to create a stable environment, the CNS milieu is separated from the vasculature by two main interfaces: the blood–brain barrier (BBB) and the blood–cerebral spinal (CSF) fluid barrier (BCSFB) (Fig. 1-16). In addition, the arachnoid epithelium underlying the dura mater is a third interface, though a significantly smaller one than the BBB and BCSFB. The BBB, with an estimated surface area 5000 times larger than the BCSFB, is the largest barrier and forms a primary role in the barrier function of the brain.

The BBB is defined by the restricted permeability of brain capillaries compared to capillaries of other organs, particularly with regard to hydrophilic (water-soluble) molecules. The BBB comprises the largest area of brain-blood contact, with a surface area between 10–20 m². The extent of this vascularization is such that it is estimated that each neuron is perfused by its own blood vessel. Specialized brain endothelial cells (BECs) that line brain capillaries are a key component of the BBB. Neighboring BECs are connected by tight junctions (TJ), also called zonulae occludens. TJ reduce the paracellular (between cells) movement of molecules, creating a
physical barrier. BECs also form metabolic and transport barriers to many molecules through enzymes and transporters, while allowing access of essential nutrients to the brain (see Ch. 3).

The BSCFB occurs at the choroid plexuses. The choroid plexuses are highly vascular, branched structures that project into the ventricles of the brain. Epithelial cells at the choroid plexus produce cerebral spinal fluid (CSF) with a turnover rate of around four times every 24 hours. BECs at the choroid plexus are fenestrated and leaky; however, tight junctions (TJs) of epithelial cells form the BCSFB.

In addition to the choroid plexuses, there are a few other regions in the CNS where BECs are fenestrated. These are collectively called the circumventricular organs (CVOs). CVOs are ventricularly located structures that carry out the two main functions of neuropeptide secretion (e.g., medial eminence, pineal gland) and hormone/neurotransmitter detection (e.g., subfornical organ). At these sites, specialized ependymal cells/tanycytes are linked by TJ to act as a barrier.

**The BBB and BCSFB serve a number of key functions critical for brain function**

First, the BBB regulates ionic gradients. Neuronal action potentials depend on the intra- and extra-neuronal
TABLE 1.1 Concentration of Plasma and CSF Levels for Selected Ions and Molecules in Humans. Notice that K⁺ ions, amino acids like glutamate and selected plasma proteins are all present in the plasma at much higher concentrations than the CSF, and could cause neuronal toxicity if allowed to enter unhindered into the brain.

<table>
<thead>
<tr>
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<td>Na⁺</td>
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<td>140</td>
<td>141</td>
<td>1</td>
</tr>
<tr>
<td>K⁺</td>
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<tr>
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<td>2.4</td>
<td>1.4</td>
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<td>mM</td>
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<td>124</td>
<td>1.23</td>
</tr>
<tr>
<td>Glucose</td>
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Amino Acids

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<th>CSF</th>
<th>Ratio</th>
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<tr>
<td>Glutamate</td>
<td>µM</td>
<td>83</td>
<td>1.8-14.7</td>
<td>0.02-0.18</td>
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<tr>
<td>Glycine</td>
<td>µM</td>
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<td>4.7-8.5</td>
<td>0.012-0.034</td>
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<tr>
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<tr>
<td>Alanine</td>
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<td>330</td>
<td>23.2-37.8</td>
<td>0.07-0.1</td>
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Proteins

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<th>Plasma</th>
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<td>0.0046</td>
<td>0.0015</td>
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</tbody>
</table>

Adapted from Abbott et al., (2010)

concentrations of ions, specifically K⁺ and Na⁺. These ionic concentrations must be maintained within a narrow range to allow efficient neuronal firing and neurotransmitter release. Plasma K⁺ levels are 1.5 times higher than the CSF (CSF levels are a guide for brain concentrations in the absence of interstitial fluid measurements), and K⁺ ions are excluded from the brain by the BBB (Table 1-1).

Second, the BBB controls protein, metabolite and toxin exchange. Blood plasma contains many elements that are relatively nontoxic in the periphery, but interfere with neuronal function and may be neurotoxic if allowed to enter the brain along their concentration gradients (Table 1-1). For example, plasma levels of the excitatory neurotransmitter glutamate are more than five times greater than levels in CSF. Similarly, some high–molecular weight proteins abundant in plasma (e.g., albumin, plasmin and thrombin) can induce neuronal apoptosis, as can certain ingested xenobiotics and by-products of digestion or physiological metabolism.

Third, the BBB allows nutrient exchange. The brain utilizes 20% of the total body oxygen consumption and a comparable fraction of glucose. However, energy reserves in the brain are limited and a constant supply is critical. Since the BBB excludes many hydrophilic molecules, transporters allow nutrients and signaling molecules brain access e.g., glucose transport via glucose transporter-1 (glut-1) (Ch. 3).

Fourth, the BBB and choroid plexus control the composition of extracellular fluid (ECF). CSF produced by the choroid plexuses, in particular at the lateral ventricles, acts to cushion the brain, reduces brain weight by up to a third and provides drainage for the ECF. The ECF supplied by the BBB is often referred to as the interstitial fluid (ISF), although the distinction between CSF and ISF is not always clear because the ISF drains partially into the CSF. The BBB also produces a slow ISF drainage via osmotic and ionic gradients. Overall, the BBB and BCSFB not only control ECF composition but also generate a flow to allow metabolite drainage.

Evolution of the blood–brain barrier concept

The concept of the BBB was first proposed over 100 years ago by Paul Erlich as an explanation for exclusion of hydrophilic aniline dyes from the brain when administered into the peripheral vasculature. Trypan blue injected directly into the CSF stained all brain cell types, whereas intravenous application did not, adding further evidence for a barrier function protecting the brain. These and other findings sparked a search for the cellular basis for the BBB, but it was not until the advent of electron microscopy that BECs emerged as a central component of the BBB.

During the 1960s, horseradish peroxidase (HRP) had been employed as an enzymatic tracer for vascular transport studies due to high sensitivity of detection and low molecular weight (MW 43,000). HRP passes from the circulation to extravascular spaces by diffusion through gaps between ECs and via pinocytotic vesicles in cardiac and skeletal muscle. However, Reese and Karnovsky (1967) demonstrated that HRP administered via the tail veins of mice was undetectable in the brain tissue. These studies established that neighboring BECs overlap and are linked together by TJ (Fig. 1-17), thereby preventing the intercellular/paracellular passage of HRP. Based on this identification of BECs as a key component of the BBB, subsequent experiments yielded a detailed understanding of the BBB.

THE NEUROVASCULAR UNIT INCLUDES MULTIPLE COMPONENTS

The lumen of the cerebral capillaries that penetrate and course through the brain tissue are enclosed by BECs interconnected by TJ

Pericytes are small connective tissue cells containing smooth muscle myosin sparingly attached to the abluminal (brain-side) surface of BECs, with both cells surrounded by a basal lamina or basement membrane. In addition, astrocytic end feet are contiguous with the basal lamina and surround the BECs. The term neurovascular unit has recently been introduced to describe the interactions between BECs,
Astrocytes, pericytes, neurons and neighboring glia (Paolinelli et al., 2011). Astrocytes and pericytes lie closest to BECs. Both cell types have been demonstrated to play a role in the maintenance and induction of the BBB. The key features of the neurovascular unit shall now be discussed in detail.

**The basement membrane (BM)/basal lamina is a vital component of the BBB**

The BM surrounds BECs and pericytes and acts to hold the cells in their place (Ch. 9). The 20–200 nm thick BM is a mixture of different classes of extracellular matrix proteins including structural proteins (collagen type IV); adhesion proteins (laminin, fibronectin); and heparan sulfate proteoglycans (perlecan, agrin) among others. BECs, astrocytes and pericytes all bind to the BM via specific receptors. Integrins are the major class of receptors expressed by all cell types in the neurovascular unit, and exist in different subclasses to allow binding to different components of the BM, e.g., collagen or laminin. In addition, the non-integrin receptor dystroglycan mediates binding to the proteoglycans and laminin. All cells of the neurovascular unit secrete components of the basement membrane. Matrix metalloproteinases (MMP) can actively digest the membrane, and are found up-regulated in inflammatory disease, e.g., multiple sclerosis (Ch. 39).

**Astrocytes contribute to the maintenance of the BBB**

The close proximity of astrocytic foot processes to BECs provided the basis for the view that astrocytes regulate the BBB. Indeed, the loss of astrocytes is associated with a loss of BBB integrity in vivo. Co-culture of isolated BECs with astrocytes or astrocyte-conditioned media modifies BEC functions such as reduced permeability, and increased transporter protein expression (e.g., glut-1 and TJ proteins). A combination of astrocytic secreted factors (e.g., basic fibroblast growth factor, transforming growth factor beta, angiopoietin-1, glia-derived neurotrophic factor, lipoproteins) and the physical presence of astrocytes contribute to the maintenance of the BBB. BECs can also influence astrocytic properties through a two-way communication.

**Pericytes at the BBB are more prevalent than in other capillary types**

At the rat BBB, there is one pericyte to every 5 ECs compared to 1 per 100 in skeletal muscle capillaries. Thus, association of pericytes is correlated with TJ expression and barrier function in endothelia. The presence of pericytes appears critical in BBB development and BM formation. Recruitment of pericytes to BEC during development is in part mediated by platelet-derived growth factor (PDGF), and accordingly PDGF knockout mice develop numerous aneurisms, possibly due to capillary wall instability. An early observation of pericytes’ ultrastructure was that they contain smooth muscle actin. Pericytes have been demonstrated to contract in *in vivo* and *in vitro*, indicating a role in controlling blood flow. During hypoxia and traumatic brain injury, both conditions associated with an increased BBB permeability, pericytes migrate away from BECs, highlighting the importance of pericytes in BBB maintenance.

**Brain endothelial cells restrict the transport of many substances while permitting essential molecules access to the brain**

BECs at the BBB are thin cells (500 nm), but create an effective barrier. The presence of TJ between BECs results in a decreased paracellular permeability of the BBB to many molecules, especially ions. Reduced ion permeability induces a high BBB electrical resistance of 1000–2000 ohms-cm² compared to peripheral vessels (2–20 ohms-cm²). BECs also differ from peripheral EC by high mitochondrial content (~10% of total cytoplasmic volume compared to ~4% in peripheral ECs), consistent with high levels of energy utilization. ATP-dependent transporters act to transport blood-borne harmful agents back into the blood (e.g., P-glycoprotein, see below) and to maintain ionic and nutrient homeostasis. BECs may have lower levels of endocytosis.

BECs are linked together by a complex of proteins, which span the inter-endothelial space and form homo (to the same protein) or hetero (to different proteins) interactions with adjacent cells, sealing the interendothelial cleft. Accessory proteins link these transmembrane proteins to the actin cytoskeleton, providing structural support. Aderhern junctions and TJ are the main types of interendothelial protein complexes present on BECs at the BBB. TJ proteins are regulated both in normal physiology and in pathological states and are thus highly dynamic. In addition to reducing paracellular permeability, TJ help maintain the polarization of BECs by
preventing exchange of transporters between the abluminal (brain-side) membrane and luminal (blood-side) membrane.

A detailed description of TJ components and structure is beyond the scope of this chapter (Abbott et al., 2010). Adheren junctions are found throughout the vasculature and are essential for TJ formation, EC adhesion and paracellular permeability, but TJ differentiates BECs from other ECs. TJ proteins are composed of transmembrane proteins, which span the membrane and bind to those on adjacent cells. Cytoplasmic accessory proteins bind to the transmembrane proteins and mediate their connection to a pericellular ring of actin filaments. Junctional adhesion molecules (JAMs), occludin and claudin family members, form the transmembrane components of TJ, while zonulae occludens 1, 2 and 3 (ZO-1,2,3), cingulin, AF-6 and 7H6 act as cytoplasmic accessory proteins. Signaling molecules associated with the TJ complex affect the TJ permeability.

TJ structures are highly dynamic and can be modulated continually by locally secreted molecules or over longer periods of time (Nag, Kapadia et al., 2011). BEC permeability is regulated via TJ translocation, decreased TJ protein expression and/or phosphorylation of the TJ complex. There are multiple extracellular mediators that can induce a leaky BBB, including cytokines (e.g., TNF-, IL-1); oxidative stress (nitric oxide); inflammatory mediators (e.g., prostaglandins and histamine); and disease-associated cells (HIV virus, bacteria) or proteins (amyloid beta) (see also Chs. 34–36). TJ-altering mediators often act via intracellular signaling pathways including protein kinase family (A, G and C); mitogen-activated protein kinase family (MAPK, including JNK, ERK and p38MAPK); the small G protein family (especially rho and rac); and wnt and eNOS pathways. Intracellular Ca$^{2+}$ ions are also key.

**FIGURE 1.18 Transport mechanisms at the blood–brain barrier.** (a) BECs contain a number of transport mechanisms to allow homeostatic control of nutrients, ions and signaling molecules. (1) Na$^+$ dependent symporters (A, ASC, LNAA, EAAT) eliminate amino acids from the brain, thus preventing excess accumulation. (2) Facilitated diffusion allows essential amino acids (L1, Y$^+$) and glucose (Glut-1) into the brain and elimination of excitatory amino acids (N, XG) into the blood across the luminal and (3) abluminal membrane. (4–5) Ion transporters at the BBB regulate extracellular K$^+$ and Na$^+$ ions and intracellular pH. (6) ABC transporters (P-gp, BCRP, MRP) protect the brain from toxins circulating in the blood. (7) Receptor-mediated transport allows essential proteins and signaling molecules into the brain (e.g., insulin receptor, transferrin receptor). Receptors can also mediate export of materials from brain. (8) Adsorptive mediated transcytosis (AMT). At physiological pH the gycocalyx of the luminal BEC membrane has an overall net negative charge, which allows cationic molecules access to the brain via non-specific transcytosis. AMT also goes in the brain-to-blood direction. (b) Structure of P-gp. P-gp is a transmembrane efflux transporter consisting of two transmembrane (TMD) domains and two nucleotide-binding (NBD) domains. P-gp uses the energy derived from ATP to actively prevent the blood-to-brain transport of many substances.
regulators of TJ protein distribution (see specific discussions in relevant portions of Chs. 21–27).

There are multiple transporters and transport processes for bidirectional transport at the BBB

BBB transport mechanisms are often divided into different categories: passive diffusion, efflux transporters, solute carriers, receptor-mediated transcytosis, and immune cell migration (Fig. 1-18). Further subdivisions are based on their specific transport properties, such as \( \text{Na}^+ \)-dependent facilitated diffusion or macromolecule transport (Ch. 3). **Table 1-2** shows examples of some important receptors of the BBB.

**Lipid solubility** is a key factor in determining the permeability of a substance through the BBB by passive diffusion. Substances with a high oil/water-partitioning coefficient diffuse through plasma membranes and into the brain. Diffusion across the plasma membrane is the primary route of

<table>
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<tr>
<th>TABLE 1.2</th>
<th>Examples of Transporters at the BBB and Their Ligands. Luminal = L, Abluminal = Ab, Intracellular IC. (Deli, 2009; Nag et al., 2011; Pardridge, 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transporter</strong></td>
<td><strong>Symbol</strong></td>
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<tr>
<td><strong>ION TRANSPORTERS</strong></td>
<td></td>
</tr>
<tr>
<td>( \text{Na}^+/\text{K}^- )</td>
<td>–</td>
</tr>
<tr>
<td>( \text{Na}^+/\text{H}^+ )</td>
<td>–</td>
</tr>
<tr>
<td>( \text{Cl}^-/\text{HCO}_3^- )</td>
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<tr>
<td>( \text{Na}^-\text{Cl}^-\text{K}^+ )</td>
<td>–</td>
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<tr>
<td><strong>SOLUTE TRANSPORTERS</strong></td>
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<tr>
<td>Glucose transporter -1</td>
<td>Glut-1</td>
</tr>
<tr>
<td>Essential neutral amino acid system</td>
<td>L1</td>
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<tr>
<td>Cationic amino acid system</td>
<td>( \gamma^+ )</td>
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<tr>
<td>Acidic amino acid system</td>
<td>Xg</td>
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<tr>
<td>Glutamine</td>
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<td>( \text{Na}^+ )-dependent amino acid transporters:</td>
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<tr>
<td>A</td>
<td>Alanine</td>
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<td>ASC</td>
<td>Alanine, cysteine, serine</td>
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<tr>
<td>N</td>
<td>Glutamine, asparagine histidine</td>
</tr>
<tr>
<td>EAAT</td>
<td>Aspartate, glutamate</td>
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<tr>
<td>L</td>
<td>Asparagine, isoleucine</td>
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<tr>
<td><strong>RECEPTOR MEDIATED TRANSCYTOSIS</strong></td>
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<td>Insulin</td>
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<td>Transferrin</td>
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<td>Low density receptor related protein 1</td>
<td>LRP1</td>
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<tr>
<td>Immunoglobulin G</td>
<td>fc( \gamma )-R</td>
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<tr>
<td>Leptin</td>
<td>–</td>
</tr>
<tr>
<td>Receptor for advanced glycosylation end-products</td>
<td>RAGE</td>
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<tr>
<td><strong>ABC TRANSPORTERS</strong></td>
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<tr>
<td>P-glycoprotein</td>
<td>P-gp</td>
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<tr>
<td>Breast cancer resistance protein</td>
<td>BCRP</td>
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</table>
entry for dissolved gases to and from the brain, i.e., O\textsubscript{2} into and CO\textsubscript{2} out. In addition, BECs at the BBB carry an overall negative surface charge on their luminal membrane due to heparan sulfate proteoglycans. Thus cationic molecules have a greater brain access than anionic ones. Such considerations are important in design of therapeutic agents with target sites of action in brain.

The BBB expresses solute carriers to allow access to the brain of molecules essential for metabolism

These include glucose, amino acids and some ions (Table 1-1). Glucose is an essential energy source for neurons in the CNS. Glucose transport at the BBB is mediated by the facilitative diffusion through glucose transporter-1 (glut-1, 55 kDa form). Glut-1 is expressed symmetrically on both luminal and abluminal membranes, with an intracellular pool that may be mobilized in times of increased neuronal demand, e.g., for seizures.

The transport of amino acids at the BBB is complex and mediated by both facilitative diffusion and Na\textsuperscript{+}-dependent transport (Table 1-2). There are four facilitative BBB amino acid carriers: an essential neutral amino acid system (L), a cationic amino acid system (Y\textsuperscript{+}), an acidic amino acid system (X\textsubscript{C}) and a glutamine system (n). System L, expressed on luminal and abluminal sides, functions to transport essential amino acids (e.g., leucine, valine, isoleucine, phenylalanine, tryptophan). Y\textsuperscript{+}, located on abluminal and luminal membranes, is a cationic transporter facilitating lysine, arginine and ornithine transport into brain. Collectively, L1 and Y\textsuperscript{+} allow brain access to all essential amino acids. Systems n and X\textsubscript{C} are expressed only on the luminal membranes and have a high affinity for glutamine and glutamate, respectively.

CSF amino acid concentrations are lower than plasma levels (Table 1-1), much lower than expected if facilitative transport were the only mechanism of amino acid transport. Five Na\textsuperscript{+}-dependent transporters on the abluminal side eliminate non-essential toxic amino acids and maintain homeostatic control over all amino acids. The Na\textsuperscript{+}-dependent transporters are A (non-essential preferring), ASC (alanine, serine, cysteine preferring), N (glutamine, asparagine, histidine), excitatory amino acid transporter family (EAAT, aspartate and glutamate preferring) and L (neutral amino acid system).

Glutamate regulation is an example of the polarized BBB controlling homeostatic levels of a potentially excitotoxic neurotransmitter. The Na\textsuperscript{+}-dependent symporter family, EAAT, transports ISF glutamate into BECs using the Na\textsuperscript{+} gradient. Glutamate then diffuses into the blood via system X\textsubscript{C}. Glutamate transport into the brain from the plasma is virtually impossible due to the lack of a facilitative glutamate transporter and the high expression of EAAT on the abluminal side. Glutamine is regulated in a similar manner, whereby Na\textsuperscript{+} transporters A and N pump glutamine into BECs. Glutamine is then either transported into the blood via system N or converted to glutamate via glutaminase (Ch. 17).

Free movement of ions across the BBB is limited by TJ, but BECs contain a number of ion transporters to maintain the Na\textsuperscript{+} gradient needed for Na\textsuperscript{+}-dependent transporters, to allow fluid movement, and to control ionic gradients. Na\textsuperscript{+}-K\textsuperscript{+} ATPase is located on the abluminal BBB membrane, while a Na\textsuperscript{+}/H\textsuperscript{+} exchanger is located on both abluminal and luminal membranes. Further luminal ion transporters include a Na\textsuperscript{+}/Cl\textsuperscript{−} co-transporter, a Na\textsuperscript{+}/K\textsuperscript{+}/Cl\textsuperscript{−} co-transporter and a Na\textsuperscript{+}/HCO\textsubscript{3} dependent influx transporter.

Receptor-mediated transcytosis (RMT) is the primary route of transport for some essential peptides and signaling molecules

Examples include insulin, leptin and transferrin (Table 1-2). Transcytosis at the BBB requires ligand-receptor binding, followed by coupled endocytosis and exocytosis. During transcytosis, the lysosomal degradation pathway is bypassed. An important example of RMT is transferrin-bound iron. Iron is an essential trace element and virtually all iron in plasma is bound to hydrophilic transferrin. Iron-bound transferrin is taken up in endocytic compartments via the transferrin receptor. Under the relatively acidic environment of the endosome (Ch. 7), iron-transferrin is assumed to dissociate and become available for uptake after exocytosis.

ATP-binding cassette transporters (ABC) on luminal membranes of the BBB restrict brain entry of many molecules

Although lipid solubility is a good indicator of brain penetration, for some compounds, e.g., phenobarbital, brain penetration is much less than expected. ABC transporters use ATP to actively eject a range of substances, including both xenobiotics and endogenous toxic molecules (Ch. 3). At least 48 ABC transporter genes have been identified and classified into seven subfamilies. ABCA to ABCG, ABCB1/MDR1 (P-glycoprotein, P-gp), ABCG2 (breast cancer resistance protein, BCRP), and ABCC (multidrug resistance protein family, MRP) are the most significant at the BBB. A lack of specific inhibitors has hindered study of MRP, but much more is known about P-gp.

P-gp (170 kDa) was first identified in multidrug resistant cell lines, and in the epithelial cells of many tissues, including the gastrointestinal tract. P-gp expression is on the luminal membrane of the BBB. Two types of P-gp exist, termed type I and type II. Type I is located on epithelia of many organs, including gastro-intestinal tract and kidney, and on ECs in the testes and brain. Type II is expressed by canicular hepatocytes and acts as a bile salt transporter and a flippase for certain membrane lipids. P-gp contains two nucleotide-binding domains and 12 transmembrane segments. P-gp is arranged in two halves (as a barrel-like configuration), with a 75-amino acid linker region. Each half contains a transmembrane domain (TMD) made of six transmembrane segments and an intracellular nucleotide-binding domain (NBD). Ligand binding to the transmembrane domain is thought to lead to a conformational change in the NBDs, increasing their affinity for ATP. ATP binding induces a conformational change in the TMD to induce ligand translocation. ATP hydrolysis causes dissolution of the closed NBD dimer, releasing ADP and allowing P-gp to return to a high-affinity ligand binding
state. P-gp has a diverse substrate list including endogenous molecules (e.g., steroid hormones, cytokines) and pharmaceutical drugs, including anticancer drugs (vinblastine, verapamil) and HIV-protease inhibitors. P-gp substrates are in general hydrophobic, but identifying whether a new drug will be a P-gp substrate based on structure is extremely difficult. The specificity of P-gp prevents many therapeutic drugs from reaching their target site, thus preventing their effective use.

During development, immune-competent microglia develop and reside in the brain tissue

After development, under physiological conditions, the brain is considered a relatively immune privileged site. Although leukocytes and immune cells can enter the brain, immune surveillance is much lower than in other organs. For example, leukocyte traffic into the brain has been estimated at 100 fold less than into the spleen or lungs. Decreased CNS immune surveillance is in part mediated by the barrier nature of the cerebral vasculature but, more importantly, is also mediated by the restricted expression of cerebral cell adhesion molecules required for leukocyte capture from the blood. During inflammatory conditions, there is a marked up-regulation of these markers, which can result in neuronal damage in disease states such as multiple sclerosis.

Immune cells cross into the brain at the level of the postcapillary venule, which is arranged slightly differently than other capillaries. BECs at these sites have fewer TJ compared to capillaries and are more subject to modulation. At the postcapillary venule, BECs are intermingled with pericytes and surrounded by a BM. At this site, there is a BM layer of variable size and a perivascular space, which is enclosed by a parenchymal BM and astrocytic end feet. Leukocyte diapedesis occurs in a two-step process across the postcapillary venule (Greenwood et al., 2011). Initially, leukocytes are captured by P-selectin and vascular cell adhesion molecules (VCAM) (expressed on BECs). Chemokines produced locally up-regulate integrins in leukocytes, resulting in a firm interaction between BEC and leukocytes. This tight binding allows leukocyte diapedesis both through the BECs (transcellular transport) and between cells (paracellular transport). Transcellular transport is hypothesized to occur under more physiological conditions to prevent TJ disruption, but opening of TJ under inflammatory conditions increases paracellular transport. After gaining access to the perivascular space, the leukocyte crosses the parenchymal basement membrane mediated by matrix metalloproteinase digestion.

There is increasing evidence of BBB dysfunction, either as a cause or consequence, in the pathogenesis of many diseases affecting the CNS

BBB dysfunction is found in many pathological states including Alzheimer’s disease (AD), multiple sclerosis (MS) and stroke, among others. A wide variety of conditions have the potential to compromise the BM or TJ, including brain tumors, inflammation, MS, infections, trauma and stroke. Many of these conditions are discussed in detail in other chapters. Compromises in the BBB and associated inflammation exacerbate some conditions such as AD and are a primary mechanism in certain pathologies such as MS.

BBB breakdown that occurs in acute brain injury, e.g., infarcts and trauma, can lead to severe brain edema (increase in brain fluid volume), increased intracranial pressure, and, potentially, death (Ch. 35). In the initial stages after brain injury, there is an increase in the number of caveolae vesicles that transport plasma proteins across the BBB, beginning a process of BBB breakdown. The expression of TJ proteins also decreases and the actin cytoskeleton becomes disrupted, as does the BM. Often a repair phase occurs with angiogenesis resulting in a second stage of BBB breakdown. As an example, cerebral ischemia is an event whereby parts of the brain do not receive sufficient blood supply to maintain neuronal function. Ischemic events, e.g., stroke, can lead to increased microvascular permeability and edema both clinically in vivo and in vitro. Reduced occludin expression and increased brain permeability are found under hypoxic conditions in experimental models.

The presence of an intact BBB affects the success of potentially beneficial therapies for many CNS disorders

For example, P-gp and BCRP expressed by BECs hinder access to the brain of many drugs used to treat epilepsy (where increased P-gp expression is also found), brain tumors and depression. Similarly, many drugs effective in peripheral infectious diseases are not effective in CNS infections, due to restricted brain penetration for both low–molecular weight compounds and larger peptide and antibody pharmaceuticals. To address this issue, there is considerable interest in developing methods to overcome the BBB for drug delivery, including temporary barrier disruption and the use of receptor-mediated transport. These may take the form of receptor-mediated drug transport through modifications of the drug (Pardridge, 2008) or of opening the BBB transiently to allow drug brain access (Deli, 2009). This latter method is already in limited therapeutic use for anticancer drugs in the treatment of brain tumors. Mannitol injections cause the plasma to become hyperosmotic compared to the inside of BECs, and water leaves the brain by osmosis. The loss of water causes BECs to shrink and the TJ structures are pulled away from each other, creating a transient opening of the barrier for drug entry. However, the efficacy of these approaches in the clinics remains to be proven.

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BARDET-BIEDEL SYNDROME AND THE NEURONAL PRIMARY CILium

Scott T. Brady

One structure often overlooked in considerations of the cell biology of the brain is the primary cilium, but the importance of cilia in neuronal development and function is becoming apparent (Han & Alvarez-Buylla, 2010). In part, this awareness stems from recognition that specific defects in ciliary structure or function can have profound effects on the nervous system as well as other tissues (Cardenas-Rodriguez & Badano, 2009). Most biologists are familiar with the motile cilium, such as those on ependymal cells that line the ventricular surface (Del Bigio, 2010), but most mammalian cells also have a single primary cilium that is nonmotile. This hair-like extension is typically 200–200 nm in diameter and may be as much as 10µm in length (Han & Alvarez-Buylla, 2010), but is rarely seen in standard histological preparations (Peters et al., 1991). The primary cilium extends from a modified centriole in neurons and neuronal progenitors as well as glia, though the prevalence of these structures in the central nervous system has only come to be appreciated in the last few decades (Louvi & Grove, 2011).

Primary cilia have multiple functions in the central nervous system (Praetorius & Spring, 2005). For example, the photoreceptors of the retina are all modified primary cilia (see Ch. 51). Indeed, primary cilia in the nervous system are best described as sensory organelles that play a key role in mechanosensory and chemosensory functions. The location of specific receptors for signals critical in neurodevelopment on primary cilia is particularly striking (see Ch. 28). Three distinct pathways critical for normal development of the nervous system have been shown to require a primary cilium: Sonic Hedgehog (Shh), platelet-derived growth factor (PDGF) and Wnt signaling (Louvi & Grove, 2011). In mammalian cells, critical components of the Shh pathway are located in the primary cilium and shuttle between cytoplasm and cilium. Shh is critical for ventralization of the neural tube, formation of spinal motor neurons and differentiation of oligodendrocytes, as well as for playing a wide range of other roles in neural development by regulating transcription of specific genes. Similarly, PDGF receptors are localized to the primary cilium and signal through cytoplasmic kinases like Akt and MAP kinases (see Ch. 25). PDGF signaling may be important for cell polarity and regulation of cell migration. Finally, the primary cilium appear to suppress the canonical Wnt pathway mediated by β-catenin and GSK3β. They may activate a noncanonical pathway that orients sheets of neuroepithelial cells and they may influence neuronal migration.

Given these diverse functions, it is not surprising that ciliopathies are pleiotropic and typically affect a wide range of cell types and tissues. Many of the phenotypes reflect alterations in brain structure or function. One example of human disease associated with defects in primary cilia is Bardet-Biedl Syndrome (BBS), a genetically heterogeneous autosomal recessive disease that results from mutations in 1 of 12 genes (Sheffield, 2010). The syndrome was first recognized as a discrete pathology in 1920, but the role of cilia in this disease was not recognized for more than 60 years. These gene products form a complex associated with the basal body (kinetosome, organelle formed from a centriole and a short cylindrical array of microtubules found at the base of the cilium). BBS gene complexes are required for the generation and maintenance of cilia, both primary and motile. BBS patients exhibit a constellation of symptoms that include both neuronal and nonneuronal pathology. Pathologies involving the nervous system include degeneration of photoreceptors, anosmia, mental retardation or developmental delay, posterior encephalocele (a neural tube defect caused by the tube’s failure to completely close), and obesity. Nonneuronal effects may include hypogonadism, kidney defects, polydactyly, diabetes, and situs inversus (randomization of normal organ locations, i.e., heart on right side instead of left side of chest). Although some of these pathologies, such as situs inversus and hypogonadism, are likely due to loss of motile cilia, others are clearly due to a loss of nonmotile, primary cilia. For example, failure to maintain sensory primary cilia is associated with retinal degeneration and renal failure. In turn, loss of signaling through primary cilium is likely to contribute to mental retardation, obesity, and anosmia, among other issues. Until the role of the primary cilium was recognized, the pleiotropic nature of BBS had baffled physicians looking for a common thread through all of these pathologies. Thus, a better understanding of the cell biology of the nervous system has illuminated a baffling and complex set of genetic disorders.

References
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CHAPTER 5

Brain imaging

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LISTENING TO THE BRAIN

Direct electrical recording and stimulation of the left hemisphere of a waking patient. The patient feels no pain because the cortex has no pain receptors, and the incision is protected with local anesthesia. It is important for the patient to be conscious during exploratory surgery to help locate vital functions that must be protected. Notice that the surgeon has labeled electrodes for the mouth (white dots with red stripes) and for picture naming (dark purple stripes). The three graphs show the averaged electrical activity over three seconds after the patient is shown a visual object to name. Gamma activity (80–100 Hz) peaks the fastest in the posterior site, then near the motor cortex (red), and finally in the upper temporal lobe (purple). Can you guess why?

Source: Crone et al., 2006.

1.0 INTRODUCTION

The ability to directly observe the living brain has created a scientific turning point, much like Galileo’s first glimpse of the moons of Jupiter. Humans have studied the sky for many centuries, but when glass lenses and telescopes were invented, the pace of discovery took off. But just as Galileo’s telescope required constant improvement, our “brain scopes” have their limits. We should know their limits as well as their capabilities.

A perfect brain observer would keep track of tens of billions of neurons many times per second. The perfect observer should then be able to track the shifting interplay between groups of...
neurons, with thousands of signals traveling back and forth. By analogy, a perfect spy satellite would see every human being on Earth as well as all the things we say to one another.

Such a perfect observer does not exist. We are more like distant space explorers beginning to observe a new planet. We pick up signals without knowing exactly where they come from, whether they are made by living creatures, what languages they speak, or even whether the signals mean anything.

We know that the brain has major pathways and maplike arrays of neurons and that single spikes as well as waves (oscillations) can travel between brain maps. Brain oscillations vary between 0.5 to 120 Hz (i.e., up to about 120 cycles per second), with occasional faster events. If we add in the neurotransmitters and neuromodulators that shape signal transmission, the potential number of signals is enormous.

Neurons have electrical, magnetic, chemical, and anatomical properties. Each of these can be measured. As you know from Chapter 3, every neuron can send a fast electrical signal down its axon. We can record this activity in the cell or in the surrounding fluid. The brain also has a rich fuel supply, and when a specific region of the brain is working harder, it needs extra fuel. Those facts give us all our brain measurement techniques: neurons, and networks of neurons, generate electrical and magnetic signals. Metabolic processes can be picked up using methods like PET and fMRI. The anatomical shape of brain structures can be detected by CAT scans and MRI. As a result, we can now see functional brain activities for speech, action control, motivation, sensory perception, and more.

1.1 Basics

Brain imaging has been a breakthrough technology for cognitive neuroscience, building on decades of cognitive psychology, behavioral conditioning, psychophysics, and brain science. Before imaging techniques matured, our knowledge came from animal studies and the haphazard injuries incurred by human beings. But brain injuries are extremely imprecise, and to locate the damage, neurologists often had to rely on postmortem examination of patients’ brains—as in the case of Broca’s and Wernicke’s patients. The brain can often compensate for damage, so lesions change over time as cells die and adaptation occurs. Therefore, post-mortem examinations do not necessarily reflect the injury at the time of diagnosis. Animal studies depend on presumed homologies—similarities across species—that were often not convincing. No other animals have language and other human specializations. It was therefore very difficult to understand how brain functions in animals mapped onto human cognition.

Today, we have perhaps a dozen techniques that are rapidly becoming more precise. Medical needs often drive this expensive technology because it applies to many organs in the body. As a result, we now have ways to study the distribution of billions of neurochemical receptors in the brain, the thickness of cortex, the great highway system of white fiber bundles, and, most important for cognitive neuroscience, the functional activity of the brain—the basis of its adaptive capacities. New advances are allowing scientists to investigate not only specific brain regions but also the dynamic pattern of connectivity between them. Some of the massive “wiring” of the brain is shown in the figure, but like the World Wide Web, the wiring
is only part of the story: ever-changing connections are being made that can alter in a fraction of a second.

1.1.1 Accuracy in space and time

Figure 5.1 shows today’s methods and their accuracy in space and time. (See Chapter 1 for the spatial and time magnitudes of the brain.) Techniques like functional magnetic resonance imaging (fMRI), which records metabolic changes like blood oxygenation, have good spatial resolution and relatively poor time resolution. fMRI has a response time of about six seconds because the fMRI signal (called the BOLD signal) reflects a flow of oxygen-rich blood traveling to “hot spots” that are doing extra work. The changes in blood flow take several seconds to catch up with the neuronal activity. fMRI is therefore too slow for tracking single neurons and populations in “real time.”

We do not have a complete census of all the neurons in the brain the way a human society might conduct a census of the whole population. We are always sampling from a very large set of active neurons. For that reason we cannot be sure that we know every single cell type in the brain, down to the smallest level. Brain anatomists are constantly discovering new specialized neurons in some local neighborhood. For example, the light receptors that adjust our body to sunlight and darkness were only discovered in recent years.

fMRI has very good spatial specificity compared to electroencephalography (EEG) and magnetoencephalography (MEG), which use electrical and magnetic signals, respectively.

**FIGURE 5.1** How good are current methods? Pros and cons of imaging techniques: differing imaging modalities have different resolutions. While some approaches have a very high temporal (time-based) resolution but a low spatial (space-based) resolution, other modalities have an opposite relation.
Thus, fMRI is used to localize brain functions. But EEG and MEG have excellent temporal resolution—almost instantaneous—and relatively poor spatial precision. They can track neuron population activity as quickly as tens and hundreds of milliseconds, but it is hard to know which set of neurons is doing it. fMRI is sometimes used in combination with EEG to obtain the best temporal and spatial precision. Combined measures may give us the best of both worlds.

1.1.2 A brain in a shoebox: coordinates

When sailors learned to draw imaginary lines of latitude and longitude to place a coordinate system around the earth, it became possible to specify any place with a precise “x” and “y” number. The earth’s coordinate system became a major advance in navigation.

The shape of the brain is more complex than the earth’s, but the strategy of placing it in a three-dimensional space is the same. Scientists can specify a precise location in the brain by placing it in a virtual shoebox so each point in the brain has a unique address in three orthogonal dimensions. Each point can be specified as $x$, $y$, and $z$. The best example is the Talairach Coordinate System illustrated in Figure 5.2.

![A coordinate system for the brain. The brain is typically imaged in three dimensions: axial, sagittal, and coronal (see Chapter 1). Putting the brain together into a three-dimensional image allows the coordinates to be determined across these three dimensions.](http://neuroinf.imm.dtu.dk/old_brede/WOROI_96.html)
Different people have different brains. Just as our heads have distinctive shapes, so do the organs inside. We begin to lose neurons after birth, so infants have more neurons than older children, teenagers, or adults. On the other hand, brain myelination (the wrapping of long axons by white protective cells) keeps growing until about age 30. Serious illnesses, growth and aging, learning and exercise, brain injury, and even malnutrition can add or subtract tissue. The brain keeps changing.

Individual brains therefore need individual images. MRI and CAT scans are used to take a snapshot of the three-dimensional brain at any particular moment. Figure 5.3 shows the smallest unit imaged using MRI: a “voxel.” Figure 5.4 shows a brain navigation program with a screenshot of the standard coordinate system used in most MRI research.

Brain surgeons need to know where to remove disease-causing tissue and which regions need to be left untouched. Structural images from MRI and CAT scans give us a three-dimensional map of the brain. But maps are also essential for understanding functional measures, like EEG, MEG, fMRI, and deep brain recording. Typically, measures of brain structure and function are combined.

**FIGURE 5.3** A single “voxel” gives the smallest unit of volume. The voxel is a representation of a volume in three-dimensional space. In the brain, the resolution of the MRI scanner determines how small the voxels can be. Higher magnetic field strength increase the spatial resolution and thus the ability to represent separate structures in the brain. Source: Jones et al., 2002.
1.2 Single neurons

Even with fast-improving imaging techniques, the most direct evidence about the living brain still comes from intracranial electrical recordings. One reason is that the electrical voltages in the brain are much greater than on the scalp—on the order of millivolts rather than microvolts. Surface EEG is filtered through a watery medium of brain tissue, skin, bone, and muscle. When you frown, the muscles above your eyes contract, and thin layers of muscle across the scalp stretch and adjust. Even eye movements have large effects on the scalp-recorded EEG. Thus surface EEG recordings mix many electrical sources, as well as being filtered through layers of tissue. About 99.9 percent of the signal strength is lost.

Direct brain recording therefore has a great advantage. The biggest drawback is that it requires invasive surgery. In humans it is never done without medical justification. However, many animal studies use direct brain recording, and these still provide much of the basic evidence.

Wilder Penfield and his colleagues pioneered electrocorticography (ECoG) in humans in the 1950s. Epileptics with uncontrolled seizures can benefit from surgical removal of seizure-causing
scars in the cortex. ECoG recordings can show where such “epileptogenic foci” are located. In addition, the surgeons need to know which areas to avoid damaging because they are vital for perception and language. ECoG exploratory studies are relatively safe and practical. Probing the cortical surface is generally pain-free because the cortex does not have pain receptors. The scientific benefits have been very important. ECoG studies in conscious humans are helping to uncover the neural basis of language, conscious perception, and voluntary control.

### 1.2.1 Recording and stimulating neurons

Single neurons have electrical and magnetic properties, like electrical batteries. We can measure the charge left in a battery and the amount of work it can do for us. We can also measure its magnetic field, as well as its chemistry and overall structure. Hubel and Wiesel (1962) recorded single feature-sensitive cells in the visual cortex of the cat—an achievement for which they received a Nobel Prize in 1981. More recent work has recorded in medial temporal lobes (Figure 5.5). Like every method, electrical recording of axonal firing has its limitations, but it continues to be a major source of information.

**FIGURE 5.5** The voltage and current flow of neurons have been studied using microelectrodes inserted in the cell, compared to a reference electrode placed outside. Large numbers of neurons also generate electrical and magnetic fields, and because many neurons are lined up in parallel arrays, like vast fields of wheat or corn, their overall electrical and magnetic fields “point” in a consistent direction. Source: Squire et al., 2002.
Neurons fire a maximum of 1,000 Hz, but cortical neurons average about 10 Hz. We have no “universal census” of cortical neurons, so we do not know with certainty how representative the small samples we observe really are.

In many countries, deep electrode recordings are allowed in primates, such as the macaque monkey, under suitable ethical constraints. The macaque brain has some striking similarities to human brains. Single-neuron recording in the macaque prefrontal cortex may show working memory. In a typical experiment, a macaque is trained to fixate visually on a cross on a computer screen and to perform a delayed response to a visual stimulus. The animal is trained to wait for a moment before looking in the direction where a stimulus appears or, alternatively, to look in the opposite direction. We can then record the activity of a single prefrontal neuron in the three phases: the presentation of a visual stimulus, the period when the monkey keeps the location of the visual stimulus in working memory, and the response of looking in the opposite direction.

1.2.2 Single neuron recording in humans

Some of the most astonishing findings in the last ten years have come from single cell recordings in humans. This is ethically possible only when there is a medical necessity for the risks of surgery, like infection and brain damage. However, the technology for safe and effective epileptic surgery has been worked out over half a century.

Depth electrodes have been used in humans. Typically, these electrodes are implanted before surgery in a patient who has otherwise untreatable epilepsy. The implants can determine where epileptic foci begin at the onset of a seizure and where critical regions of the brain are located that must not be lesioned.

While a single cell cannot tell us much about human cognition, a recent experiment provided some intriguing results regarding conscious and unconscious visual perception (Figure 5.6). While the spiking neuron is a plausible unit of brain activity, some scientists believe that graded dendritic currents in each neuron may do useful information processing; some argue for subcellular processes inside the cell; others point to nonclassical cells and synapses; and many scientists believe that real brain processes only take place at the level of populations of neurons. Therefore, recording axonal spikes is important, but it may not be the only important thing going on. Obviously, it’s a risky business to jump from a single neuron to more than 10 billion in the vast forest of the brain.

2.0 ELECTRICAL AND MAGNETIC FIELDS

The brain has billions of neurons lined up more or less in parallel. The long pyramidal neurons of the cortex mostly point their axons inward to the thalamus, and later many axons join the great hubs and highways of the inner white matter. However, pyramidal cells have input dendrites that are mostly spread out horizontally, following the curve of the cortex. When pyramidal neurons fire a spike down the axons, their dendrites also change. Those flat and bushy horizontal “arbors” of dendrites are picked up as EEG activity at the scalp because their electrical fields happen to point outward.
All streams of electrons generate both electrical and magnetic fields, which radiate at right angles to each other (Figure 5.7). Therefore, we can also pick up the brain’s magnetic field—at least those components that conveniently point outward from the scalp. Most the field strength in the brain is not measurable from the outside. We just take advantage of radiating fields that can be picked up.

**FIGURE 5.6** Single neurons reflecting conscious perception. A remarkable experiment in which both conscious and unconscious stimuli were shown simultaneously to two different eyes using a variant of binocular rivalry (see Chapter 6). In the upper row (c), the woman’s face is conscious, but the ball is not; in the lower row (d), this is reversed. Peak firing rates, in red, during the time periods marked by the green horizontal bars, when the subject is reporting that the woman’s face can be seen. The brain seems to determine which of the two simultaneous stimuli will become conscious when the signal reaches the object recognition areas in the cortex. The electrode locations are shown on the brain scans on the left (a, b). Source: Rees et al., 2002.

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**FIGURE 5.7** The brain radiates electrical and magnetic fields. Here is an example of a 252-channel EEG recording. The head is shown from above, with the front of the face on the top of the figure and the back of the head on the bottom. Dipoles are shown in black arrows, and their centers are shown in blue. Based on these data, what part of the brain do you think is active? Source: Schwartz Center for Computational Neuroscience (http://sccn.ucsd.edu/eeglab/comp252.html), with permission.
2.1 Electroencephalography: the electrical fields of the brain

Scalp EEG is a standard tool in medicine. When an accident victim comes into an emergency room with a head injury, scalp EEG is one of the first tests. With only 21 electrodes, a great deal can be observed, including gross brain damage, coma and stupor, sleep, waking, epileptic signals, and more. Computer programs for analyzing EEG are constantly improving.

The biggest difficulty in scalp EEG is locating the source of the recorded signal. The brain is a large, wet medium, and recording from the scalp results in a loss of 99.9 percent of the source. This is like trying to understand a watery planet where all the radio signals come from deep in the ocean and most of the signal power is lost before the satellite can pick it up.

The brain’s electrical activity can be recorded through the scalp or on the surface of the cortex. Rather than picking up electrical activity direct from neurons, the electroencephalogram picks up the electrical field. The resulting record is an electroencephalogram (EEG). The EEG was discovered in 1929 by Hans Berger.

EEG is a relatively direct measure of the brain’s electrical activity. But with tens of billions of cortical neurons firing about 10 Hz, we have several trillion electrical events per second. The raw EEG was therefore difficult to interpret before the advent of powerful computerized analysis.

However, when the EEG is averaged over a number of trials and “locked” to a specific zero point, like the onset of a stimulus, the averaged electrical activity yields elegant and regular waveforms. This event-related potential (ERP) is sensitive to large population activity that characterizes visual, auditory, and even semantic processes.

The brain can also be stimulated with electrical and magnetic energy. Today we can stimulate deep brain regions using microelectrodes, which are sometimes useful for treating severe depression and Parkinson’s disease.

2.2 Analyzing the electroencephalogram

While the brain has about 100 billion cells, the cortex is now believed to have about 1,000 specialized regions, originally discovered by Brodmann (1909) and therefore still called Brodmann areas. They are the postal codes of the brain. Other major structures, like the thalamus, have their own regional organization.

Humans communicate by phone, radio, and television. Those techniques use different coding schemes, often with different waveforms (such as AM and FM radio). The brain seems to have many coding schemes. The visual cortex, for example, has “visuotopical maps”: two-dimensional arrays of the visual input that begin with the retina and continue in the visual cortex with area V1. Higher-level maps have more abstract visual representations, like motion, color, faces, houses, and other objects. Since these arrays often correspond to one another, they are said to be “topographically mapped.” A particular point on the retina, like your center of visual gaze, corresponds to the same point in the thalamus, V1, and higher visual maps. This is a spatial coding scheme, like a video camera or a computer screen.
The waveforms in Figure 5.8 show major bands (ranges) of waves in the EEG. These oscillations are believed to play important roles in the brain (See Box 5.1 for an interesting experiment using these EEG measures.). Notice the top oscillations, which represent “raw” (unprocessed) EEG, recorded from any electrode on the scalp. During the waking state, these oscillations look nearly random, as if they are generated by chance events. For that reason EEG was controversial for many years, until the emerging evidence showed that the choppy “ocean” of the brain consists of many regular waveforms (Figure 5.9).

When random snippets of EEG are averaged, the result is a straight line, since random positive and negative oscillations add up to zero. This fact is used in the event-related potential, discussed in the following.

The activity of large populations is another important level of analysis (Freeman, 2004; John, 2001). Spontaneous EEG shows different patterns of activation. For example, during deep sleep, the raw EEG shows large, slow waves. This indicates that large groups of neurons are synchronized on a very large scale throughout the brain. When the subject wakes up, this slow pattern is replaced by small, rapid electrical waves, indicative of rapid and

![Figure 5.8](image-url)

**FIGURE 5.8** Regular rhythms in different parts of the brain are shown. A method called Fourier analysis allows us to decompose the density (or power) of regular waveforms that are buried in noisy EEG recordings. The graphs show the resulting power curves. The colors correspond to different frequency ranges. Source: Zoran Josipovich, with permission.
flexible information patterns of interaction in the brain. It is currently believed that cortical neurons do not fire at different rates during deep sleep compared to waking, but rather that waking EEG allows a far greater amount of interactive processing. Sleep is not a passive state, but a different operating mode of the brain, with its own functions. One of these is believed to be the consolidation of memories based on waking experiences (Hobson & Stickgold, 1995).

EEG has a millisecond time resolution, but its spatial resolution is rather poor. It is very difficult to locate the electrical source of the EEG signal. It helps to increase the number of electrodes and to use sophisticated analytical methods. However, EEG gives us little information about brain regions deep beneath the cortex.

FIGURE 5.9 Some power spectra showing peaks at canonical EEG frequencies. Although any of the frequencies can occur at any electrode site, alpha power is often recorded at posterior sites, theta at frontal sites and gamma over sensory cortex. Source: Ward, 2003.
2.3 Averaging the EEG

It makes sense to get the average annual income in a country. One reason is that there is variability between the poorest and wealthiest people. Another is that it helps to have one number to describe millions of people. The point of statistics is to simplify very large numbers to just a few. A similar approach works with the electrical activity of the brain, using the “event-related potential” (the word potential means “voltage”). Event-related potentials (ERPs) were discovered several decades ago, before high-speed computers were available to analyze the EEG. When a bright flash or a loud sound is presented to an animal or a human being, a wave of activity travels throughout the cortex and thalamus. That brain activity is “evoked” by the flash or the bang, and it is helpful to show the results starting with the moment of the stimulus. The evoked (that is, they occur in response to the event, in this case a flash or bang) potential waveforms are reflected in the EEG, but they tend to be swamped by other brain activity, much like a giant wave in the ocean that is invisible as long as it is swamped by other wave activity in the sea.

As just pointed out, the irregular or “random” component of the EEG drops out when multiple stretches of the signal are added up or averaged. However, the large and distinctive signals of the ERP do not drop to zero. Rather, they add up over each trial, showing up as the event-related potential (ERP). ERP peaks and valleys are quite regular and stable over time. They are believed to reflect large neuronal populations that are triggered by the input.

When we average over a number of half-second stretches of EEG, we obtain the ERP in Figure 5.10. EEG reveals brain patterns during sleep and waking, abnormalities during diseases like epilepsy, and even the brain areas that respond to music. A more recent technique, magnetoencephalography (MEG), is related to EEG and has provided new ways to image the human brain.
BOX 5.1

THE EEG OF MEDITATION

A study by Lutz et al. (2004, see Figure 5.11), using EEG, focused on the brain activity of experienced Buddhist meditators. EEG signals were recorded in expert meditators and control subjects during normal rest and during different stages of meditation. Three stages were used: rest, meditative state, and a pause stage between meditative states. Lutz et al. found that, during meditation, the group of experienced meditators had a dramatically higher level of gamma-band oscillations. The researchers also found long-distance phase synchrony between frontal and parietal areas in the brain. From these results, Lutz et al. speculate that meditative training enhances the integration of distant brain areas. Interestingly, the results showed that brain activation even at rest differed between the expert and naïve groups. This indicates that substantial meditative experience can alter the workings of the brain, although at present we can only speculate at the precise cause and effect relationships.

![Image](a)

![Image](b)

![Image](c)

![Image](d)

**FIGURE 5.11** Measuring the effects of meditation: the study of Lutz and colleagues (2004) shows that gamma power is much higher in practicing meditators (right) compared to non-meditating controls (left). The color scale indicates the percentage of subjects in each group that had an increase of gamma activity during the mental training. Source: Lutz et al., 2004.
2.4 Stimulating the brain

What if you could evoke neural activity in a safe fashion? Such a method would be especially useful to test causal relationships between neural activity and cognitive functions. Early work on direct electrical brain stimulation began with Wilder Penfield, a neurosurgeon at the Montreal Neurological Institute (Figure 5.12). Penfield and his colleagues treated patients with intractable epilepsy. In open brain surgery, patients can remain awake and responsive, since only local anesthetic is needed at the site. There are no pain receptors in the brain itself, so the cortex brain can be operated on without general anesthesia.

2.4.1 A safe way of interfering with brain function: transcranial magnetic stimulation (TMS)

It is now possible to stimulate brain lesions in healthy subjects. Without cutting a person’s brain, we can alter the brain’s level of activity locally. Brief magnetic pulses over the scalp either inhibit or excite a region of cortex. For example, if you stimulate the hand area of the motor cortex, the subject’s hand will move and twist. Applying an inhibitory pulse over the same area will cause subjects to have difficulty moving their hands. This is called transcranial magnetic stimulation (TMS) or, as one leading researcher called it, “zapping the brain” (Cowey & Walsh, 2001). TMS appears to be generally safe. By applying TMS, we can test causal hypotheses about the contribution of specific brain regions to cognitive processes. Since the TMS works at the millisecond scale, it is also possible to study how rapid waves of processing develop. Recent TMS studies emphasize that magnetic pulses rarely have simple, local effects. Rather, like other kinds of brain stimulation, magnetic pulses often trigger off widespread activities, depending on the subject’s expectations and ongoing goals.
2.5 Magnetoencephalography: magnetic fields of the brain

Magnetoencephalography (MEG) measures the magnetic field produced by electrical activity in the brain (Figure 5.13). Its spatial resolution is now approaching a few millimeters, while its temporal resolution is in milliseconds. Because of the physics of magnetism, MEG is highly sensitive to dendritic flow at right angles to the walls of the sulci (the cortical folds). MEG results must be superimposed upon a structural image of the living brain. MEG uses a process called magnetic source imaging (MSI) to coregister the magnetic sources of brain activity onto anatomical pictures provided by MRI. MEG has the advantage of being entirely silent and noninvasive. As we will see, MRI is quite noisy, and, of course, depth electrodes require surgery. Thus, MEG is attractive for use with children and vulnerable people. MEG is easy for young children to tolerate as long as they can stay relatively still.

**FIGURE 5.13** Magnetoencephalography and its analysis. The subject is placed in the scanner that has a large set of shielded sensors. The signals are derived from ionic currents flowing in the dendrites (bottom). Action potentials do not produce an observable field. Upper middle: Magnetic fields following painful (touch) stimulation, where (a) shows the recorded data, and (b) and (c) display residual magnetic fields obtained after filtering the somatosensory processing signals from the recorded data. The bottom two lines show the time course of the source strengths during the painful stimulation. Upper right: Source locations of the MEG data overlaid on MR images. Source: VSM MedTech.
3.0 FUNCTIONAL NEUROIMAGING: A BOLD NEW WORLD

EEG and MEG measure brain activity fairly directly. Other neuroimaging methods use indirect measures, such as blood flow or regional oxygen level. Currently, the most popular method is fMRI (Figure 5.14) and especially the kind that measures the oxygen level of the local blood circulation (called BOLD, for blood-oxygen level dependent activity).

When neurons fire, they consume oxygen and glucose and secrete metabolic waste products. An active brain region consumes its local blood oxygen supply, and as oxygen is consumed, we can see a small drop in the BOLD signal. In a fraction of a second, the loss of regional oxygen triggers a new influx of oxygen-rich blood to that region. Here, we see a recovery of the signal. However, as the compensatory mechanism overshoots, flooding more oxygenated blood into the area than is needed, we also see that the signal rises high above the baseline. Finally, as unused oxygen-rich blood flushes out of the region, we can see a drop in the BOLD signal back to the baseline (Figure 5.15).

Thus, as the oxygen content of blood produces changes, we can measure neural activation indirectly. The BOLD signal comes about six seconds after the onset of neuronal firing. The relationship between neural activation and the BOLD fMRI signal is shown in Figure 5.16. Note that these studies frequently use a block design, where a certain task is cycled on and off and the BOLD signal is measured across the ON and OFF blocks.

Positron emission tomography (PET) was developed much earlier than MRI or fMRI, and it provides a measure of metabolic brain activity (Figure 5.17). PET is used less often for research today because it is very expensive, requiring a cyclotron. It also requires subjects to be injected with a radioactive tracer. For nonmedical investigations, MRI and fMRI have

FIGURE 5.14 An MRI scanner. For both MRI and fMRI recording, the subject is lying down on the narrow bed that is then drawn into the core of the scanner. Source: Sharma & Sharma, 2004.
FIGURE 5.15  MRI and functional MRI. Top: The blood oxygenated-level dependent (BOLD) signal simplified in four steps. Step 1: increased neural activation leads to an increase in the consumption of oxygen from the blood, leading to a lower level of oxygenated blood and more deoxygenated blood, leading to a drop in the BOLD signal. Step 2: the vascular response to the increase in oxygen consumption leads to a dramatic increase in new, oxygenated blood at the same time as the oxygen consumption drops due to the decreased levels of neuronal activation. Step 3: a normalizing of flow and deoxy/oxyhemoglobin levels (not shown). Step 4: a poststimulus undershoot caused by the slow recovery of blood volume. Source: Thomas Ramsoy, 2010, with permission.

FIGURE 5.16  fMRI shows the activity of the brain, using a blocked design, where a stimulus is presented in blocks separated by a resting state, the BOLD signal cycles on and off as neural activity changes. Source: Robinson, 2004.
largely taken over the research field (Figure 5.18). However, PET is still important because different tracers can be linked to different molecules. The distribution of neurochemicals can therefore be determined.

Today, it is not possible to have high spatial and high temporal resolution at the same time using the same recording device. There is a tradeoff. Methods like fMRI and PET tell us where in the brain something is happening. EEG, MEG, and single cell recordings show millisecond changes in the brain. In any study, it is important to ask why the authors chose a particular method and what they observed—or might have missed—by the choices they made. In the best cases we can see different methods showing convergent results.

### 3.1 Regions of interest

Finding your way around the brain is not easy. An even harder task is figuring out which areas play which roles in major cognitive processes such as language, attention, and vision. One way is to define regions of interest (ROIs) ahead of the study and make predictions about expected activity in ROIs.

#### 3.1.1 Co-registration of functional activity on a structural scan

The first step is to define the living brain anatomically to make out different areas, connections, and layers of organization. Structural MRI gives us a tool to map out brain structure, including the axonal (white matter) connections between brain regions. MRI shows structure but not function.

Look again at Figure 5.16, where the BOLD signal for a blocked fMRI design is presented. On the right side of Figure 5.16, we see an image with two yellow “hot spots,” reflecting increased fMRI activity. The color is arbitrarily chosen to indicate the degree of activity. In order to pin down the location of the yellow hot spots, we need to superimpose the functional image
on the structural MRI, which has a better spatial resolution. In a process called coregistration, the functional and structural images are aligned to each other. Coregistration ensures that the two images end up in the same space using the same metric. With higher spatial resolution we can ask questions that are anatomically specific.

Another approach is to mark ROI on the structural image alone to constrain the statistical analysis. Newer MRI machines with higher magnetic field strength now make it possible to look at the cellular organization of the living brain and to compare brains between groups of people (e.g., people with schizophrenia and healthy subjects).

### 3.1.2 Subtraction methods for defining functional activity differences

Different layers of cortex have either local or distant connectivity, so layer information is useful to find out how cortical regions interact. Because the brain is remarkably active at all times, it is still a challenge to isolate fleeting, task-related activity. One common method is

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**FIGURE 5.18** A classical PET finding: visual versus auditory brain activity. Brain metabolic activity is shown as noted on the right side of the figure, with red and yellow colors showing higher activity and blue and green colors showing lower activity. These early PET scans show results for hearing, seeing, speaking, and internally generating words. Notice that the auditory, visual, motoric, and speech production regions appear to be activated for the respective conditions. For example, in the “hearing words” condition, auditory regions in the temporal lobe show a high level of activity, while in the “seeing words” condition, visual regions in the occipital lobe show a high level of activity. Note that in these early studies, the results are shown on a brain outline template. Current studies use fMRI with enhanced ability to show brain function coregistered onto anatomical images. Source: Posner & Raichle, 1997.
subtraction between fMRI conditions—for example, the brain’s response to the task of interpreting Arabic numerals (1, 2, 3, ...) is compared to its response to the same numbers when they are spelled out (one, two, three, ...). Subtraction is used because it tends to remove most of the “irrelevant” brain activity that would otherwise drown out the signal of interest. It is much like comparing a choppy sea before and after a whale swims by. If you want to see the waves generated by the whale alone, you might subtract a record of the waves alone. It is the difference between the two conditions that matters (Figure 5.19).

Subtracting conditions can have unwanted consequences. There might be important things going on in both conditions. In addition, the variance of experimental and comparison conditions might be different, there might be interactions between independent variables, and so

FIGURE 5.19 PET and fMRI subtraction methods. The brain has constant dynamic background activity. To remove this background activity, the PET or BOLD signal for an experimental task is subtracted, point by point, from a closely matched control task. Individual scans of the differences are then averaged and used to find the group average. Source: Posner & Raichle, 1997.
on. Another approach therefore is *parametric variation*, in which the variance for each main variable and their interactions can be separated statistically. For example, if you study working memory (WM), instead of subtracting brain activity during working memory from activity without it, one can study how gradually increasing the WM load leads to changes in neural activation. Since statistical testing must be done for every point of interest in the scan over every unit of time, this is a large data set.

### 3.2 The resting brain: intrinsic brain processes

As neuroimagers began to study different cognitive functions, the main approach was to use a contrastive, subtractive approach. Here, the neural activation during a given cognitive function, such as speech production, was compared to a period where subjects were instructed to relax and “do nothing.” Such active-versus-rest comparisons showed powerful effects.

Yet there is a hidden assumption in these studies. If you are asked just to lie still and “rest,” what will you do? Will your mind be a blank screen? A great deal of evidence shows that people just go back to their everyday thoughts, images, and feelings. You might be thinking about something that happened the day before or that you need to do later, or you might just daydream a bit. You are likely to have inner speech, visual imagery, episodic memories coming to mind, and so forth. For the brain, that is not “rest.” Instead, the experimental comparison is really between two active states of the brain. One is driven by experimental task demands, while the other reflects our own thoughts, hopes, feelings, images, inner speech, and the like. In some ways, spontaneous activity may tell us more about the natural conditions of human cognitive activity than specific experimental tasks. Both are important.

The use of MRI to produce both precise anatomical images and to provide functional maps of brain areas has revolutionized the field.

### 3.3 Structural changes in the brain: taxi drivers

The best science is done by combining imaging techniques with genuine creativity. A lot of creativity goes into the selection of functional variables. What is the best way to understand vision? To understand selective attention and conscious cognition? A great deal of ingenuity has been devoted to those questions. Let us consider a few examples. Taxi drivers are well known for their ability to know their way around a city. They know not only how to get from A to B, but they also know the most efficient way to get there. Such ability to navigate through a complex road system depends on our spatial ability. Studies have shown that the hippocampus, a part of the medial temporal lobe, plays an important part in the navigational memory of places and routes. Rats with lesions to the hippocampus have been known for decades to perform disastrously on spatial tests. Birds and other animals that bury or hide their food at multiple places have larger hippocampi than nonstoring animals. Therefore, one question that arises when we think about taxi drivers is, are the brain regions responsible for spatial navigation more developed in taxi drivers than in other people? Indeed, it has been found that part of the hippocampi of taxi drivers was larger than the same region in a group of people
with a different background. Okay, you might be thinking, but what if people with large hippocampi choose to be taxi drivers and not vice versa?

Here, the study showed that the size of the hippocampus depended on how long people had been working as taxi drivers. In other words, the longer you work as a taxi driver (and use your spatial navigation ability), the bigger your relevant part of the hippocampus will become.

Notice how imaginative the taxi driver example was. It is usually easier to randomly select human subjects (usually undergraduate students!) to stand for the entire human population. But the fact is that there are differences in age, particular abilities and talents, and other cognitive capacities among “average” subjects. One important implication is that the size of brain structures may change with specific experiences (Figure 5.21) (Maguire et al., 2000). That claim has now been supported for other brain regions as well. The taxi driver study is therefore an excellent example of creative selection of comparison conditions.

### 3.4 Connectivity and causality

White matter fiber tracts are the vast internal highway system of the cortex. We can visualize these fiber tracts using an MRI method called **diffusion tensor imaging (DTI)**. DTI uses water flow along the axons surrounded by white myelin to measure the relative direction of white matter tracts (Figure 5.22). DTI helps us to understand brain connectivity patterns.
in the healthy brain, as well as investigate these patterns in individuals with brain diseases that affect white matter, such as multiple sclerosis.

By far the largest fiber bundle in the brain is the corpus callosum (see Chapter 4), which connects the two hemispheres; but there are many other fiber bundles or tracts that connect regions within the hemispheres. The view of the vast array of white matter tracts is shown in Figure 5.23, with a midsagittal (center-line) view of the fiber tracts that extend upward from the spinal cord to the cortex. These fiber tracts make up the vertical “traffic arteries” that flow to and from the cortex and that provide the connective pathways throughout the central nervous system.

A recent study investigated the correspondence between white matter and gray matter, and the results are presented in Figure 5.24. Constructed maps of peripheral white matter tracts are presented at the top of the figure, and their corresponding cortical gray matter regions are shown below. Note that Figure 5.24 shows the same information across three views of the brain: from the front or anterior perspective (a and d), from the same or lateral perspective (b and e), and from the back or posterior perspective (c and f).
4.0 CORRELATION AND CAUSATION

We typically combine brain and behavioral observations. We can present visual images on a screen and have the subject read aloud or meditate. Thus we typically observe a correlation between behavior and brain activity. In methods with high spatial resolution, such as fMRI, different tasks show local increases or decreases in the BOLD signal, indicating that the brain works more in some regions than others (see Figure 5.16).

We can take the example of the Counting Stroop Task, in which subjects are asked simply to count the number of words shown on a screen. On some occasions a word like dog is shown three times. The subject should say “three,” which is not difficult. However, we can introduce a conflict between the words shown and the number of words. If we show the word one four times, there is an automatic tendency for expert readers (like college students) to say the word “one.” But the correct answer is “four.” This is very much like the Color-Naming Stroop Task. Subjects take longer to answer correctly, since they must inhibit their automatic tendency to read the words on the screen.

FIGURE 5.22 Diffusion of water depends on the local environment. (a) In the free and unrestricted medium (i.e., a glass of water), water can diffuse freely. The diffusion is isotropic—that is, it has the potential to move in all directions. If the water molecule is physically restricted, it can no longer move freely in any direction. This diffusion is anisotropic, meaning it cannot move in any direction. In a medium of fibers such as the brain’s white matter (schematically shown in (b)), water molecules are highly restricted by the axonal fibers. In this way, it is possible to visualize the fiber tracts of the brain and to estimate the integrity of white matter within a given region. (c) Such visualization produces the typical colored DTI brain image that displays different trajectory trends in regional white matter. Source: Thomas Ramsoy, 2010, with permission.
Zheng and Rajapakse (2006) reported the BOLD activity during the Stroop task (Figure 5.25). While many brain regions show activation during both conditions, frontal parts of the brain were more active during the conflict condition. This Stroop task result has now been found many times for conflictual tasks. One of the major roles of prefrontal cortex is to resolve conflicting tendencies, like the automatic tendency just to read words, against the tendency to follow the experimental instructions. Thus we have a correlation among (a) frontal activation, (b) longer reaction times, (c) a sense of subjective effort, and (d) a greater number of errors in the conflict condition. These are significant results, since there are many real-life conditions where conflicting tendencies need to be regulated.

However, so far we have no way to test causal hypotheses among the many brain regions that are involved in any complex task. For example, we know that the task requires visual word recognition, response preparation, choosing between two possible answers, perhaps detecting conflict, stopping the wrong answer from being said, selecting the right answer instead, and so on. An approach called dynamic causal modeling (DCM) is used to analyze causal relationships. Zheng and Rajapakse (2006) performed DCM on the brain activation they found in both word counting tasks. As you can see in Figure 5.25, DCM suggested that each task had a different activation pattern. Although many of the same regions of the brain are active during both tasks, their relative connectivity and contribution were altered. Interestingly, the analysis also showed that the interference condition recruits wider activity than the control condition. This is another common finding for mentally effortful conditions (Duncan & Owen, 2000).
4.1 Why we need multiple tests of brain function

According to some media headlines, brain scientists recently discovered the parts of the brain used for deception and lying. This kind of headline comes from studies like the one shown in Figure 5.26. It shows fMRI differences between brain regions that had greater BOLD activity when people were telling the truth (green) and cortical areas when they were made to tell a lie. Such experiments often use playing cards and ask a group of subjects to “lie” by reporting a different card than the one they actually see.

One major purpose of cognitive neuroscience is identifying function with structure—that is, seeing whether specific brain locations do specific things for us. In that process, however, we must be very clear about the kinds of inferences that we can make from the evidence. The popular media may not be quite as careful as scientists need to be. Do you believe that the green areas in Figure 5.26 are really the “truth-telling” areas of the brain?
4.2 Brain damage and causal inferences

Historically, Paul Broca discovered patients who were unable to speak and also showed damage to the left frontal lobe. However, their ability to comprehend language was relatively spared. About the same time, Carl Wernicke made the discovery that damage to different regions of the left hemisphere was associated with the ability to understand language, while their ability to speak was intact. Today, we call this complementary pair of facts a double dissociation.
Brain damage is correlational, since we cannot produce it and test its results in humans. Nevertheless, after a great deal of debate and controversy, there is no serious doubt today that one important function of Broca’s area is the production of speech and likewise that one important function for Wernicke’s region is speech comprehension (Figure 5.27). Lesions near Broca’s area can lead to dysarthria, a condition where the control of mouth and tongue movement is disrupted but language production is still intact. Thus we have three lesions that lead to three different speech problems: one seems to be important for language
comprehension; another is vital for language production; and a third region is important for the motor commands to produce vocal expressions.

Brain injuries in humans happen for a host of reasons, ranging from car accidents to strokes. Accidental lesions do not neatly follow the borders between brain regions. To test brain-mind hypotheses, it is preferable to be much more precise about exactly where in the brain deficits occur. In order to do this, studies have been conducted in experimental animals, typically rats and monkeys. However, language is a species-specific function for humans, and we have no direct parallels in other species. Safer brain interventions for the human brain are being explored, including TMS, electrical field stimulation, and even color lasers.

Very precise lesions have been studied in monkeys and rats for many years. For example, Buckley and Gaffan (2006) made precise lesions in different areas of the medial temporal lobe (MTL) in macaque monkeys. Very specific damage to the perirhinal cortex (meaning “near the smell cortex”) caused monkeys to make more errors on a visual object discrimination task. Lesions to surrounding areas did not produce this deficit. The harder the discrimination task became, the more errors the lesioned monkeys made. Yet the monkeys performed normally in simple visual discriminations among different colors, shapes, and orientations. This suggests that the perirhinal cortex may have a specific causal role in processing complex visual objects, like faces. A variety of human studies now support this finding. Animal studies have often played this pioneering role, allowing us to pick up leads that are later tested in humans.

5.0 SUMMARY

Brain techniques measure single neurons to large cortical regions, brain structure, dynamic brain activity, and connectivity. The advent of brain imaging has transformed the study of human cognition. New and more refined methods are constantly being produced. One recent advance is using multiple methods in the same study to optimize the tradeoffs between direct and indirect measures of brain activity.
6.0 STUDY QUESTIONS AND DRAWING EXERCISES

1. Label the differences between the brain scans in Figure 5.28, and describe the reasoning of the subtraction method for each image.
2. Define the BOLD response. What does BOLD stand for?
3. What is the time lag between neural activity and a BOLD response? Between neural activity and an EEG response?
4. What are the pros and cons of single cell recording in the brain?
5. What problem might arise when brain activity in a cognitive task is compared to a resting baseline?
6. What does Figure 5.26 tell us about lying and the cortex?

FIGURE 5.28 Drawing exercise. A reproduction of Figure 5.19 shown without labels for use in the drawing exercises. Source: Modified from Posner & Raichle, 1997, with permission.