06 - 1 Neural Sciences

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1.1 Introduction

Neural Sciences 1.1 Introduction The human brain is responsible for our cognitive processes, emotions, and behaviors— that is, everything we think, feel, and do. Although the early development and adult function of the brain are shaped by multiple factors (e.g., epigenetic, environmental, and psychosocial experiences), the brain is the final integrator of these influences. Despite the many advances in neural sciences over the last several decades, including the “decade of the brain” in the 1990s, and the wide acceptance of the brain as the biological substrate for normal and abnormal mental functions, there has not been a true transformational advance in the treatment of mental disorders for more than half a century. The most obvious reason for the absence of more progress is the profound complexity of the human brain. A perhaps less obvious reason is the current practice of psychiatric diagnosis, which, for most clinicians, is based on syndrome-based classification systems. The purpose of this chapter is to introduce the neural sciences sections, which describe the anatomy and function of the human brain, and then to discuss how an evolution of thinking toward a brain-based or biologically based diagnostic system for mental illness might facilitate our efforts to advance brain research, to develop better treatments, and to improve patient care. In other fields of medicine, diagnosis is based on physical signs and symptoms, a medical history, and results of laboratory and radiological tests. In psychiatry, a diagnosis is based primarily on the clinician’s impression of the patient’s interpretation of his or her thoughts and feelings. The patient’s symptoms are then cross-referenced to a diagnostic or classification manual (e.g., Diagnostic and Statistical Manual of Mental Disorders [DSM-5], International Statistical Classification of Diseases and Related Health Problems [ICD]) containing hundreds of potential syndromes, and one or more diagnoses are applied to the particular patient. These standard classification systems represent significant improvements in reliability over previous diagnostic systems, but there is little reason to believe that these diagnostic categories are valid, in the sense that they represent discrete, biologically distinct entities. Although a patient with no symptoms or complaints can be diagnosed as having diabetes, cancer, or hypertension on the basis of blood tests, X-rays, or vital signs, a patient with no symptoms cannot be diagnosed with schizophrenia, for example, because there are no currently recognized objective, independent assessments. The goals of clinicians and researchers are to reduce human suffering by increasing our understanding of diseases, developing new treatments to prevent or cure diseases,

and caring for patients in an optimal manner. If the brain is the organ of focus for mental illnesses, then it may be time to be more ambitious in building the classification of patients with mental illnesses directly from our understanding of biology, rather than only from the assessment of a patient’s symptoms. THE HUMAN BRAIN The following neural sciences sections each address a field of brain biology. Each of these fields could be relevant to the pathophysiology and treatment of

mental illnesses. Although the complexity of the human brain is daunting compared with other organs of the body, progress can only be made if one approaches this complexity consistently, methodically, and bravely. The neuronal and glial cells of the human brain are organized in a characteristic manner, which has been increasingly clarified through modern neuroanatomical techniques. In addition, our knowledge of normal human brain development has become more robust in the last decade. The human brain clearly evolved from the brain of lower animal species, allowing inferences to be made about the human brain from animal studies. Neurons communicate with one another through chemical and electrical neurotransmission. The major neurotransmitters are the monoamines, amino acids, and neuropeptides. Other chemical messengers include neurotrophic factors and an array of other molecules, such as nitric oxide. Electrical neurotransmission occurs through a wide range of ion channels. Chemical and electrical signals received by a neuron subsequently initiate various molecular pathways within other neurons that regulate the biology and function of individual neurons, including the expression of individual genes and the production of proteins. In addition to the central nervous system (CNS), the human body contains two other systems that have complex, internal communicative networks: the endocrine system and the immune system. The recognition that these three systems communicate with each other has given birth to the fields of psychoneuroendocrinology and psychoneuroimmunology. Another property shared by the CNS, the endocrine system, and the immune system is the regular changes they undergo with the passage of time (e.g., daily, monthly), which is the basis of the field of chronobiology. PSYCHIATRY AND THE HUMAN BRAIN In the first half of the 20th century, the advances in psychodynamic psychiatry, as well as in social and epidemiological psychiatry, led to a separation of psychiatric research from the study of the human brain. Since the 1950s, the appreciation of the effectiveness of medications in treating mental disorders and the mental effects of illicit drugs, have reestablished a biological view of mental illness, which had already been seeded by the introduction of electroconvulsive therapy (ECT) and James Papez’s description of the limbic circuit in the 1930s. This biological view has been reinforced further by the development of brain imaging techniques that have helped reveal how the brain

performs in normal and abnormal conditions. During this period, countless discoveries have been made in basic neural science research using experimental techniques to assess the development, structure, biology, and function of the CNS of humans and animals. Psychopharmacology The effectiveness of drugs in the treatment of mental illness has been a major feature of the last half-century of psychiatric practice. The first five editions of this textbook divided psychopharmacological treatment into four chapters on antipsychotic, antidepressant, antianxiety, and mood-stabilizing drugs. The prior division of psychiatric drugs into four classes is less valid now than it was in the past for the following reasons: (1) Many drugs of one class are used to treat disorders previously assigned to another class; (2) drugs from all four categories are used to treat disorders not previously treatable by drugs (for example, eating disorders, panic disorders, and impulse control disorders); and (3) drugs such as clonidine (Catapres), propranolol (Inderal), and verapamil (Isoptin) can effectively treat a variety of psychiatric disorders and do not fit easily into the aforementioned classification of drugs. The primary motivation for this change was that the variety and application of the drug treatments no longer clearly fit a division of disorders into psychosis, depression, anxiety, and mania. In other words, the clinical applications of biologically based treatments did not neatly align with our syndrome-based diagnostic system. An implication of this observation could be that drug response might be a better indicator of underlying biological

brain dysfunction than any particular group of symptoms. For example, although the DSM-5 distinguishes major depressive disorder from generalized anxiety disorder, most clinicians are aware that these are often overlapping symptoms and conditions in clinical practice. Moreover, the same drugs are used to treat both conditions. The animal models that are used to identify new drug treatments may also have affected our ability to advance research and treatment. Many major classes of psychiatric drugs were discovered serendipitously. Specifically, the drugs were developed originally for nonpsychiatric indications, but observant clinicians and researchers noted that psychiatric symptoms improved in some patients, which led to focused study of these drugs in psychiatric patients. The availability of these effective drugs, including monoaminergic antidepressants and antipsychotics, led to the development of animal models that could detect the effects of these drugs (e.g., tricyclic antidepressants increase the time mice spend trying to find a submerged platform in a “forced swim” test). These animal models were then used to screen new compounds in an attempt to identify drugs that were active in the same animal models. The potential risk of this overall strategy is that these animal models are merely a method for detecting a particular molecular mechanism of action (e.g., increasing serotonin concentrations), rather than a model for a true behavioral analog of a human mental illness (e.g., behavioral despair in a depressed patient).

Endophenotypes A possible diagnosis-related parallel to how this textbook separated the four classes of psychotropic drugs into approximately 30 different categories is the topic of endophenotypes in psychiatric patients. An endophenotype is an internal phenotype, which is a set of objective characteristics of an individual that are not visible to the unaided eye. Because there are so many steps and variables that separate a particular set of genes from the final functioning of a whole human brain, it may be more tractable to consider intermediate assessments such as endophenotypes. This hypothesis is based on the assumption that the number of genes that are involved in an endophenotype might be fewer than the number of genes involved in causing what we would conceptualize as a disease. The nature of an endophenotype, as considered in psychiatry, is biologically defined on the basis of neuropsychological, cognitive, neurophysiological, neuroanatomical, biochemical, and brain imaging data. Such an endophenotype, for example, might include specific cognitive impairments as just one of its objectively measured features. This endophenotype would not be limited to patients with a diagnosis of schizophrenia because it might also be found in some patients with depression or bipolar disorder. The potential role of an endophenotype can be further clarified by stating what it is not. An endophenotype is not a symptom, and it is not a diagnostic marker. A classification based on the presence or absence of one or more endophenotypes would be based on objective biological and neuropsychological measures with specific relationships to genes and brain function. A classification based on endophenotypes might also be a productive approach toward the development of more relevant animal models of mental illnesses, and thus the development of novel treatments. PSYCHIATRY AND THE HUMAN GENOME Perhaps 70 to 80 percent of the 25,000 human genes are expressed in the brain, and because most genes code for more than one protein, there may be 100,000 different proteins in the brain. Perhaps 10,000 of these are known proteins with somewhat identified functions, and no more than 100 of these are the targets for existing psychotherapeutic drugs. The study of families with the use of population genetic methods over the last 50 years has consistently supported a genetic, heritable component to mental disorders. More recent techniques in molecular biology have revealed that specific chromosomal regions and genes are associated with particular diagnoses. A potentially very powerful application of these techniques has been to study

transgenic models of behavior in animals. These transgenic models can help us understand the effects of individual genes as well as discover completely novel molecular targets for drug development. It may be a natural response to resist “simple” genetic explanations for human features. Nonetheless, research on humans generally has found that approximately 40 to 70 percent of aspects of cognition, temperament, and personality are attributable to

genetic factors. Because these are the very domains that are affected in mentally ill patients, it would not be surprising to discover a similar level of genetic influence on mental illness, especially if we were able to assess this impact at a more discrete level, such as with endophenotypes. Individual Genes and Mental Disorders Several types of data and observations suggest that any single gene is likely to have only a modest effect on the development of a mental disorder, and that when a mental disorder is present in an individual, it represents the effects of multiple genes, speculatively on the order of five to ten genes. This hypothesis is also supported by our failure to find single genes with major effects in mental illnesses. Some researchers, however, still consider it a possibility that genes with major effects will be identified. “Nature” and “Nurture” within the CNS In 1977, George Engel, at the University of Rochester, published a paper that articulated the biopsychosocial model of disease, which stressed an integrated approach to human behavior and disease. The biological system refers to the anatomical, structural, and molecular substrates of disease; the psychological system refers to the effects of psychodynamic factors; and the social system examines cultural, environmental, and familial influences. Engel postulated that each system affects and is affected by the others. The observation that a significant percentage of identical twins are discordant for schizophrenia is one example of the type of data that support the understanding that there are many significant interactions between the genome and the environment (i.e., the biological basis of the biopsychosocial concept). Studies in animals have also demonstrated that many factors—including activity, stress, drug exposure, and environmental toxins—can regulate the expression of genes and the development and functioning of the brain. Mental Disorders Reflect Abnormalities in Neuroanatomical Circuits and Synaptic Regulation Although genes lead to the production of proteins, the actual functioning of the brain needs to be understood at the level of regulation of complex pathways of neurotransmission and intraneuronal signaling, and of networks of neurons within and between brain regions. In other words, the downstream effects of abnormal genes are modifications in discrete attributes such as axonal projections, synaptic integrity, and specific steps in intraneuronal molecular signaling. Why Not a Genetic-Based Diagnostic System? Some researchers have proposed moving psychiatry toward a completely genetic-based

diagnostic system. This proposal, however, seems premature based on the complexity of the genetic factors presumably involved in psychiatric disorders, the current absence of sufficient data to make these genetic connections, and the importance of epigenetic and environmental influences on the final behavioral outcomes resulting from an individual’s genetic information. LESSONS FROM NEUROLOGY Clinical and research neurologists seem to have been able to think more clearly than psychiatrists about their diseases of interest and their causes, perhaps because the symptoms are generally nonbehavioral. Neurologists have biologically grounded differential diagnoses and treatment choices. This clarity of approach has helped lead to significant advances in neurology in the last two decades, for example, clarification of the amyloid precursor protein abnormalities in some patients with Alzheimer’s disease, the presence of trinucleotide repeat mutations in Huntington’s disease and spinocerebellar ataxia, and the appreciation of alpha-synucleinopathies,

such as Parkinson’s disease and Lewy body dementia. The continued separation of psychiatry from neurology is in itself a potential impediment to good patient care and research. Many neurological disorders have psychiatric symptoms (e.g., depression in patients following a stroke or with multiple sclerosis or Parkinson’s disease), and several of the most severe psychiatric disorders have been associated with neurological symptoms (e.g., movement disorders in schizophrenia). This is not surprising given that the brain is the organ shared by psychiatric and neurological diseases, and the division between these two disease areas is arbitrary. For example, patients with Huntington’s disease are at much greater risk for a wide range of psychiatric symptoms and syndromes, and thus many different DSM5 diagnoses. Because we know that Huntington’s disease is an autosomal dominant genetic disorder, the observation that it can manifest with so many different DSM-5 diagnoses does not speak to a very strong biological distinction among the existing DSM-5 categories. EXAMPLES OF COMPLEX HUMAN BEHAVIORS The goal to understand the human brain and its normal and abnormal functioning is truly one of the last frontiers for humans to explore. Trying to explain why a particular individual is the way he or she is, or what causes schizophrenia, for example, will remain too large a challenge for some decades. It is more approachable to consider more discrete aspects of human behavior. It is not the role of textbooks to set policies or to write diagnostic manuals, but rather to share knowledge, generate ideas, and encourage innovation. The authors believe, however, that it is time to reap the insights of decades of neural science and clinical brain research and to build the classification of mental illnesses on fundamental principles of biology and medicine. Regardless of official diagnostic systems, however, clinicians and researchers should fully understand the biological component of the

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biopsychosocial model, and not let research or patient care suffer because of a diagnostic system that is not founded on biological principles. REFERENCES Agit Y, Buzsaki G, Diamond DM, Frackowiak R, Giedd J. How can drug discovery for psychiatric disorders be improved? Nat Rev. 2007;6:189. Cacioppo JT, Decety J. Social neuroscience: Challenges and opportunities in the study of complex behavior. Ann N Y Acad Sci. 2011;1224:162. Gould TD, Gottesman II. Commentary: Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav. 2006;5:113. Grebb JA, Carlsson A. Introduction and considerations for a brain-based diagnostic system in psychiatry. In: Sadock BJ, Sadock VA, Ruiz P, eds. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. Hoef F, McCandliss BD, Black JM, Gantman A, Zakerani N, Hulme C, Lyytinen H, Whitfield-Gabrieli S, Glover GH, Reiss AL, Gabrieli JDE. Neural systems predicting long-term outcome in dyslexia. Proc Natl Acad Sci U S A. 2011;108:361. Krummenacher P, Mohr C, Haker H, Brugger P. Dopamine, paranormal belief, and the detection of meaningful stimuli. J Cogn Neurosci. 2010;22:1670. Müller-Vahl KR, Grosskreutz J, Prell T, Kaufmann J, Bodammer N, Peschel T. Tics are caused by alterations in prefrontal areas, thalamus and putamen, while changes in the cingulate gyrus reflect secondary compensatory mechanisms. BMC Neurosci. 2014;15:6. Niv Y, Edlund JA, Dayan P, O'Doherty JP. Neural prediction errors reveal a risk-sensitive reinforcement-learning process in the human brain. J Neurosci. 2012;32:551. Peltzer-Karpf A. The dynamic matching of neural and cognitive growth cycles. Nonlinear Dynamics Psychol Life Sci. 2012;16:61. 1.2 Functional Neuroanatomy The sensory, behavioral, affective, and cognitive phenomena and attributes experienced by humans are mediated through the brain. It is the organ that perceives and affects the environment and integrates past and present. The brain is the organ of the mind that enables persons to sense, do, feel, and think. Sensory systems create an internal representation of the external world by processing external stimuli into neuronal impulses. A separate map is formed for each sensory modality. Motor systems enable persons to manipulate their environment and to influence the behavior of others through communication. In the brain, sensory input, representing the external world, is integrated with internal drivers, memories, and emotional stimuli in association units, which in turn drive the actions of motor units. Although psychiatry is concerned primarily with the brain's association function, an appreciation of information processing of the sensory and motor

systems is essential for sorting logical thought from the distortions introduced by psychopathology.
BRAIN ORGANIZATION

The human brain contains approximately 1011 neurons (nerve cells) and approximately 1012 glial cells. Neurons most classically consist of a soma, or cell body, which contains the nucleus; usually multiple dendrites, which are processes that extend from the cell body and receive signals from other neurons; and a single axon, which extends from the cell body and transmits signals to other neurons. Connections between neurons are made at axon terminals; there the axons of one neuron generally contact the dendrite or cell body of another neuron. Neurotransmitter release occurs within axon terminals and is one of the major mechanisms for intraneuronal communications, and also for the effects of psychotropic drugs. There are three types of glial cells, and although they have often been thought of as having only a supportive role for neuronal functioning, glia have been increasingly appreciated as potentially involved in brain functions that may contribute more directly to both normal and disease mental conditions. The most common type of glial cell are the astrocytes, which have a number of functions, including nutrition of neurons, deactivation of some neurotransmitters, and integration with the blood–brain barrier. The oligodendrocytes in the central nervous system and the Schwann cells in the peripheral nervous system wrap their processes around neuronal axons, resulting in myelin sheaths that facilitate the conduction of electrical signals. The third type of glial cells, the microglia, which are derived from macrophages, are involved in removing cellular debris following neuronal death. The neurons and glial cells are arranged in regionally distinct patterns within the brain. Neurons and their processes form groupings in many different ways, and these patterns of organization, or architecture, can be evaluated by several approaches. The pattern of distribution of nerve cell bodies, called cytoarchitecture, is revealed by aniline dyes called Nissl stains that stain ribonucleotides in the nuclei and the cytoplasm of neuronal cell bodies. The Nissl stains show the relative size and packing density of the neurons and, consequently, reveal the organization of the neurons into the different layers of the cerebral cortex. SENSORY SYSTEMS The external world offers an infinite amount of potentially relevant information. In this overwhelming volume of sensory information in the environment, the sensory systems must both detect and discriminate stimuli; they winnow relevant information from the mass of confounding input by applying filtration at all levels. Sensory systems first transform external stimuli into neural impulses and then filter out irrelevant information to create an internal image of the environment, which serves as a basis for reasoned thought. Feature extraction is the quintessential role of sensory systems, which achieve this goal with their hierarchical organizations, first by transforming physical stimuli into neural activity in the primary sense organs and then by refining and narrowing the neural activity in a series of higher cortical processing areas. This neural processing eliminates irrelevant data from higher representations and reinforces crucial features. At the highest levels of sensory processing, neural images are transmitted to the association areas to be acted on in the light of emotions, memories, and drives.

Somatosensory System The somatosensory system, an intricate array of parallel point-to-point connections from the body surface to the brain, was the first sensory system to be understood in anatomical detail. The six somatosensory modalities are light touch, pressure, pain, temperature, vibration, and proprioception (position sense). The organization of nerve bundles and synaptic connections in the somatosensory system encodes spatial relationships at all levels, so that the organization is strictly somatotopic (Fig. 1.2-1). FIGURE 1.2-1 Pathway of somatosensory

information processing. (Adapted from Patestas MA, Gartner LP. A Textbook of Neuroanatomy. Malden, MA: Blackwell; 2006:149.) Within a given patch of skin, various receptor nerve terminals act in concert to mediate distinct modalities. The mechanical properties of the skin's mechanoreceptors and thermoreceptors generate neural impulses in response to dynamic variations in the environment while they suppress static input. Nerve endings are either fast or slow responders; their depth in the skin also determines their sensitivity to sharp or blunt stimuli. Thus the representation of the external world is significantly refined at the level of the primary sensory organs.

The receptor organs generate coded neural impulses that travel proximally along the sensory nerve axons to the spinal cord. These far-flung routes are susceptible to varying systemic medical conditions and to pressure palsies. Pain, tingling, and numbness are the typical presenting symptoms of peripheral neuropathies. All somatosensory fibers project to, and synapse in, the thalamus. The thalamic neurons preserve the somatotopic representation by projecting fibers to the somatosensory cortex, located immediately posterior to the sylvian fissure in the parietal lobe. Despite considerable overlap, several bands of cortex roughly parallel to the sylvian fissure are segregated by a somatosensory modality. Within each band is the sensory "homunculus," the culmination of the careful somatotopic segregation of the sensory fibers at the lower levels. The clinical syndrome of tactile agnosia (astereognosis) is defined by the inability to recognize objects based on touch, although the primary somatosensory modalities—light touch, pressure, pain, temperature, vibration, and proprioception—are intact. This syndrome, localized at the border of the somatosensory and association areas in the posterior parietal lobe, appears to represent an isolated failure of only the highest order of feature extraction, with preservation of the more basic levels of the somatosensory pathway. Reciprocal connections are a key anatomical feature of crucial importance to conscious perception—as many fibers project down from the cortex to the thalamus as project up from the thalamus to the cortex. These reciprocal fibers play a critical role in filtering sensory input. In normal states, they facilitate the sharpening of internal representations, but in pathological states, they can generate false signals or inappropriately suppress sensation. Such cortical interference with sensory perception is thought to underlie many psychosomatic syndromes, such as the hemisensory loss that characterizes conversion disorder. The prenatal development of the strict point-to-point pattern that characterizes the somatosensory system remains an area of active study. Patterns of sensory innervation result from a combination of axonal guidance by particular molecular cues and pruning of exuberant synaptogenesis on the basis of an organism's experience. Leading hypotheses weigh contributions from a genetically determined molecular map—in which the arrangement of fiber projections is organized by fixed and diffusible chemical cues— against contributions from the modeling and remodeling of projections on the basis of coordinated neural activity. Thumbnail calculations suggest that the 30,000 to 40,000 genes in human deoxyribonucleic acid (DNA) are far too few to encode completely the position of all the trillions of synapses in the brain. In fact, genetically determined positional cues probably steer growing fibers toward the general target, and the pattern of projections is fine-tuned by activity-dependent mechanisms. Recent data suggest that well-established adult thalamocortical sensory projections can be gradually remodeled as a result of a reorientation of coordinated sensory input or in response to loss of part of the somatosensory cortex, for instance, in stroke. Development of the Somatosensory System A strict somatotopic representation exists at each level of the somatosensory system. During development, neurons extend axons to connect to distant brain regions; after arriving at the destination, a set of axons

must therefore sort itself to preserve the somatotopic organization. A classic experimental paradigm for this developmental

process is the representation of a mouse’s whiskers in the somatosensory cortex. The murine somatosensory cortex contains a barrel field of cortical columns, each of which corresponds to one whisker. When mice are inbred to produce fewer whiskers, fewer somatosensory cortex barrels appear. Each barrel is expanded in area, and the entire barrel field covers the same area of the somatosensory cortex as it does in normal animals. This experiment demonstrates that certain higher cortical structures can form in response to peripheral input and that different input complexities determine different patterns of synaptic connectivity. Although the mechanisms by which peripheral input molds cortical architecture are largely unknown, animal model paradigms are beginning to yield clues. For example, in a mutant mouse that lacks monoamine oxidase A and, thus, has extremely high cortical levels of serotonin, barrels fail to form in the somatosensory cortex. This result indirectly implicates serotonin in the mechanism of barrel field development. In adults, the classic mapping studies of Wilder Penfield suggested the existence of a homunculus, an immutable cortical representation of the body surface. More recent experimental evidence from primate studies and from stroke patients, however, has promoted a more plastic conception than that of Penfield. Minor variations exist in the cortical pattern of normal individuals, yet dramatic shifts in the map can occur in response to loss of cortex from stroke or injury. When a stroke ablates a significant fraction of the somatosensory homunculus, the homuncular representation begins to contract and shift proportionately to fill the remaining intact cortex. Moreover, the cortical map can be rearranged solely in response to a change in the pattern of tactile stimulation of the fingers. The somatotopic representation of the proximal and distal segments of each finger normally forms a contiguous map, presumably because both segments contact surfaces simultaneously. However, under experimental conditions in which the distal segments of all fingers are simultaneously stimulated while contact of the distal and proximal parts of each finger is separated, the cortical map gradually shifts 90 degrees to reflect the new sensory experience. In the revised map, the cortical representation of the proximal segment of each finger is no longer contiguous with that of the distal segment. These data support the notion that the internal representation of the external world, although static in gross structure, can be continuously modified at the level of synaptic connectivity to reflect relevant sensory experiences. The cortical representation also tends to shift to fit entirely into the available amount of cortex. These results also support the notion that cortical representations of sensory input, or of memories, may be holographic rather than spatially fixed: The pattern of activity, rather than the physical structure, may encode information. In sensory systems, this plasticity of cortical representation allows recovery from brain lesions; the phenomenon may also underlie learning. Visual System Visual images are transduced into neural activity within the retina and are processed through a series of brain cells, which respond to increasingly complex features, from the

eye to the higher visual cortex. The neurobiological basis of feature extraction is best understood in finest detail in the visual system. Beginning with classic work in the 1960s, research in the visual pathway has produced two main paradigms for all sensory systems. The first paradigm, mentioned earlier with respect to the somatosensory system, evaluates the contributions of genetics and experience—or nature and nurture— in the formation of the final synaptic arrangement. Transplantation experiments, resulting in an accurate point-to-point pattern of connectivity, even when the eye was surgically inverted, have suggested an innate, genetically determined

mechanism of synaptic pattern formation. The crucial role of early visual experience in establishing the adult pattern of visual connections, on the other hand, crystallized the hypothesis of activity-dependent formation of synaptic connectivity. The final adult pattern is the result of both factors. The second main paradigm, most clearly revealed in the visual system, is that of highly specialized brain cells that respond exclusively to extremely specific stimuli. Recent work, for example, has identified cells in the inferior temporal cortex that respond only to faces viewed at a specific angle. An individual's response to a particular face requires the activity of large neural networks and may not be limited to a single neuron. Nevertheless, the cellular localization of specific feature extraction is of critical importance in defining the boundary between sensory and association systems, but only in the visual system has this significant question been posed experimentally. In the primary visual cortex, columns of cells respond specifically to lines of a specific orientation. The cells of the primary visual cortex project to the secondary visual cortex, where cells respond specifically to particular movements of lines and to angles. In turn, these cells project to two association areas, where additional features are extracted and conscious awareness of images forms. The inferior temporal lobe detects the shape, form, and color of the object—the what questions; the posterior parietal lobe tracks the location, motion, and distance—the where questions. The posterior parietal lobe contains distinct sets of neurons that signal the intention either to look into a certain part of visual space or to reach for a particular object. In the inferior temporal cortices (ITCs), adjacent cortical columns respond to complex forms. Responses to facial features tend to occur in the left ITC, and responses to complex shapes tend to occur in the right ITC. The brain devotes specific cells to the recognition of facial expressions and to the aspect and position of faces of others with respect to the individual. The crucial connections between the feature-specific cells and the association areas involved in memory and conscious thought remain to be delineated. Much elucidation of feature recognition is based on invasive animal studies. In humans, the clinical syndrome of prosopagnosia describes the inability to recognize faces, in the presence of preserved recognition of other environmental objects. On the basis of pathological and radiological examination of individual patients, prosopagnosia is thought to result from disconnection of the left ITC from the visual association area in the left parietal lobe. Such lesional studies are useful in identifying necessary components of a mental pathway, but they may be inadequate to define the entire pathway. One noninvasive

technique that is still being perfected and is beginning to reveal the full anatomical relation of the human visual system to conscious thought and memory is functional neuroimaging. As is true for language, there appears to be a hemispheric asymmetry for certain components of visuospatial orientation. Although both hemispheres cooperate in perceiving and drawing complex images, the right hemisphere, especially the parietal lobe, contributes the overall contour, perspective, and right-left orientation, and the left hemisphere adds internal detail, embellishment, and complexity. The brain can be fooled in optical illusions. Neurological conditions such as strokes and other focal lesions have permitted the definition of several disorders of visual perception. Apperceptive visual agnosia is the inability to identify and draw items using visual cues, with preservation of other sensory modalities. It represents a failure of transmission of information from the higher visual sensory pathway to the association areas and is caused by bilateral lesions in the visual association areas. Associative visual agnosia is the inability to name or use objects despite the ability to draw them. It is caused by bilateral medial occipitotemporal lesions and can occur along with other visual impairments. Color perception may be ablated in lesions of the dominant occipital lobe that include the splenium of the corpus callosum. Color agnosia is the inability to recognize a color

despite being able to match it. Color anomia is the inability to name a color despite being able to point to it. Central achromatopsia is a complete inability to perceive color. Anton's syndrome is a failure to acknowledge blindness, possibly owing to interruption of fibers involved in self-assessment. It is seen with bilateral occipital lobe lesions. The most common causes are hypoxic injury, stroke, metabolic encephalopathy, migraine, herniation resulting from mass lesions, trauma, and leukodystrophy. Balint's syndrome consists of a triad of optic ataxia (the inability to direct optically guided movements), oculomotor apraxia (inability to direct gaze rapidly), and simultanagnosia (inability to integrate a visual scene to perceive it as a whole). Balint's syndrome is seen in bilateral parietooccipital lesions. Gerstmann's syndrome includes agraphia, calculation difficulties (acalculia), right–left disorientation, and finger agnosia. It has been attributed to lesions of the dominant parietal lobe. Development of the Visual System In humans, the initial projections from both eyes intermingle in the cortex. During the development of visual connections in the early postnatal period, there is a window of time during which binocular visual input is required for development of ocular dominance columns in the primary visual cortex. Ocular dominance columns are stripes of cortex that receive input from only one eye, separated by stripes innervated only by fibers from the other eye. Occlusion of one eye during this critical period completely eliminates the persistence of its fibers in the cortex and allows the fibers of the active eye to innervate the entire visual cortex. In contrast, when normal binocular vision is allowed during the critical development window, the usual dominance columns form; occluding one eye after the completion of innervation of the cortex produces no subsequent alteration of the ocular dominance columns. This paradigm crystallizes the importance of early childhood experience on the formation of adult brain circuitry. Auditory System

Sounds are instantaneous, incremental changes in ambient air pressure. The pressure changes cause the ear's tympanic membrane to vibrate; the vibration is then transmitted to the ossicles (malleus, incus, and stapes) and thereby to the endolymph or fluid of the cochlear spiral. Vibrations of the endolymph move cilia on hair cells, which generate neural impulses. The hair cells respond to sounds of different frequency in a tonotopic manner within the cochlea, like a long, spiral piano keyboard. Neural impulses from the hair cells travel in a tonotopic arrangement to the brain in the fibers of the cochlear nerve. They enter the brainstem cochlear nuclei, are relayed through the lateral lemniscus to the inferior colliculi, and then to the medial geniculate nucleus (MGN) of the thalamus. MGN neurons project to the primary auditory cortex in the posterior temporal lobe. Dichotic listening tests, in which different stimuli are presented to each ear simultaneously, demonstrate that most of the input from one ear activates the contralateral auditory cortex and that the left hemisphere tends to be dominant for auditory processing. Sonic features are extracted through a combination of mechanical and neural filters. The representation of sound is roughly tonotopic in the primary auditory cortex, whereas lexical processing (i.e., the extraction of vowels, consonants, and words from the auditory input) occurs in higher language association areas, especially in the left temporal lobe. The syndrome of word deafness, characterized by intact hearing for voices but an inability to recognize speech, may reflect damage to the left parietal cortex. This syndrome is thought to result from disconnection of the auditory cortex from Wernicke's area. A rare, complementary syndrome, auditory sound agnosia, is defined as the inability to recognize nonverbal sounds, such as a horn or a cat's meow, in the presence of intact hearing and speech recognition. Researchers consider this syndrome the right hemisphere correlate of pure word deafness. Development of the Auditory System Certain children are unable to process auditory input clearly and therefore have impaired speech and comprehension of

spoken language. Studies on some of these children have determined that, in fact, they can discriminate speech if the consonants and vowels—the phonemes—are slowed twofold to fivefold by a computer. Based on this observation, a tutorial computer program was designed that initially asked questions in a slowed voice and, as subjects answered questions correctly, gradually increased the rate of phoneme presentation to approximate normal rates of speech. Subjects gained some ability to discriminate routine speech over a period of 2 to 6 weeks and appeared to retain these skills after the tutoring period was completed. This finding probably has therapeutic applicability to 5 to 8 percent of children with speech delay, but ongoing studies may expand the eligible group of students. This finding, moreover, suggests that neuronal circuits required for auditory processing can be recruited and be made more efficient long after language is normally learned, provided that the circuits are allowed to finish their task properly, even if this requires slowing the rate of input. Circuits thus functioning with high fidelity can then be trained to speed their processing. A recent report has extended the age at which language acquisition may be acquired for the first time.

A boy who had intractable epilepsy of one hemisphere was mute because the uncontrolled seizure activity precluded the development of organized language functions. At the age of 9 years he had the abnormal hemisphere removed to cure the epilepsy. Although up to that point in his life he had not spoken, he initiated an accelerated acquisition of language milestones beginning at that age and ultimately gained language abilities only a few years delayed relative to his chronological age. Researchers cannot place an absolute upper limit on the age at which language abilities can be learned, although acquisition at ages beyond the usual childhood period is usually incomplete. Anecdotal reports document acquisition of reading skills after the age of 80 years. Olfaction Odorants, or volatile chemical cues, enter the nose, are solubilized in the nasal mucus, and bind to odorant receptors displayed on the surface of the sensory neurons of the olfactory epithelium. Each neuron in the epithelium displays a unique odorant receptor, and cells displaying a given receptor are arranged randomly within the olfactory epithelium. Humans possess several hundred distinct receptor molecules that bind the huge variety of environmental odorants; researchers estimate that humans can discriminate 10,000 different odors. Odorant binding generates neural impulses, which travel along the axons of the sensory nerves through the cribriform plate to the olfactory bulb. Within the bulb, all axons corresponding to a given receptor converge onto only 1 or 2 of 3,000 processing units called glomeruli. Because each odorant activates several receptors that activate a characteristic pattern of glomeruli, the identity of external chemical molecules is represented internally by a spatial pattern of neural activity in the olfactory bulb. Each glomerulus projects to a unique set of 20 to 50 separate columns in the olfactory cortex. In turn, each olfactory cortical column receives projections from a unique combination of glomeruli. The connectivity of the olfactory system is genetically determined. Because each odorant activates a unique set of several receptors and thus a unique set of olfactory bulb glomeruli, each olfactory cortical column is tuned to detect a different odorant of some evolutionary significance to the species. Unlike the signals of the somatosensory, visual, and auditory systems, olfactory signals do not pass through the thalamus but project directly to the frontal lobe and the limbic system, especially the pyriform cortex. The connections to the limbic system (amygdala, hippocampus, and pyriform cortex) are significant. Olfactory cues stimulate strong emotional responses and can evoke powerful memories. Olfaction, the most ancient sense in evolutionary terms, is tightly associated with sexual and reproductive responses. A related chemosensory structure, the vomeronasal organ, is thought to detect pheromones, chemical cues that trigger unconscious, stereotyped responses. In some

animals, ablation of the vomeronasal organ in early life may prevent the onset of puberty. Recent studies have suggested that humans also respond to pheromones in a manner that varies according to the menstrual cycle. The structures of higher olfactory processing in phylogenetically more primitive animals have evolved in humans into the limbic system, the center of the emotional brain and the gate through which experience is admitted into memory according to emotional significance. The elusive basic animal drives with which clinical psychiatry

constantly grapples may therefore, in fact, originate from the ancient centers of higher olfactory processing. Development of the Olfactory System During normal development, axons from the nasal olfactory epithelium project to the olfactory bulb and segregate into about 3,000 equivalent glomeruli. If an animal is exposed to a single dominant scent in the early postnatal period, then one glomerulus expands massively within the bulb at the expense of the surrounding glomeruli. Thus, as discussed earlier with reference to the barrel fields of the somatosensory cortex, the size of brain structures may reflect the environmental input. Taste Soluble chemical cues in the mouth bind to receptors in the tongue and stimulate the gustatory nerves, which project to the nucleus solitarius in the brainstem. The sense of taste is believed to discriminate only broad classes of stimuli: sweet, sour, bitter, and salty. Each modality is mediated through a unique set of cellular receptors and channels, of which several may be expressed in each taste neuron. The detection and the discrimination of foods, for example, involve a combination of the senses of taste, olfaction, touch, vision, and hearing. Taste fibers activate the medial temporal lobe, but the higher cortical localization of taste is only poorly understood. Autonomic Sensory System The autonomic nervous system (ANS) monitors the basic functions necessary for life. The activity of visceral organs, blood pressure, cardiac output, blood glucose levels, and body temperature are all transmitted to the brain by autonomic fibers. Most autonomic sensory information remains unconscious; if such information rises to conscious levels, it is only as a vague sensation, in contrast to the capacity of the primary senses to transmit sensations rapidly and exactly. Alteration of Conscious Sensory Perception through Hypnosis Hypnosis is a state of heightened suggestibility attainable by a certain proportion of the population. Under a state of hypnosis, gross distortions of perception in any sensory modality and changes in the ANS can be achieved instantaneously. The anatomy of the sensory system does not change, yet the same specific stimuli may be perceived with diametrically opposed emotional value before and after induction of the hypnotic state. For example, under hypnosis a person may savor an onion as if it were a luscious chocolate truffle, only to reject the onion as abhorrently pungent seconds later, when the hypnotic suggestion is reversed. The localization of the hypnotic switch has not been determined, but it presumably involves both sensory and association areas of the brain. Experiments tracing neural pathways in human volunteers via functional neuroimaging have demonstrated that shifts in attention in an environmental setting determine changes in the regions of the brain that are activated, on an instantaneous time scale.

Thus the organizing centers of the brain may route conscious and unconscious thoughts through different sequences of neural processing centers, depending on a person’s ultimate goals and emotional state. These attention-mediated variations in synaptic utilization can occur instantaneously, much like the alteration in the routing of associational processing that may occur in hypnotic states. MOTOR SYSTEMS Body muscle movements are controlled by the lower motor neurons, which extend axons —some as long as 1 meter—to the muscle fibers. Lower motor neuron firing is regulated by the summation of upper motor neuron activity. In the brainstem, primitive

systems produce gross coordinated movements of the entire body. Activation of the rubrospinal tract stimulates flexion of all limbs, whereas activation of the vestibulospinal tract causes all limbs to extend. Newborn infants, for example, have all limbs tightly flexed, presumably through the dominance of the rubrospinal system. In fact, the movements of an anencephalic infant, who completely lacks a cerebral cortex, may be indistinguishable from the movements of a normal newborn. In the first few months of life, the flexor spasticity is gradually mitigated by the opposite actions of the vestibulospinal fibers, and more limb mobility occurs. At the top of the motor hierarchy is the corticospinal tract, which controls fine movements and which eventually dominates the brainstem system during the first years of life. The upper motor neurons of the corticospinal tract reside in the posterior frontal lobe, in a section of cortex known as the motor strip. Planned movements are conceived in the association areas of the brain, and in consultation with the basal ganglia and cerebellum, the motor cortex directs their smooth execution. The importance of the corticospinal system becomes immediately evident in strokes, in which spasticity returns as the cortical influence is ablated and the actions of the brainstem motor systems are released from cortical modulation. Basal Ganglia The basal ganglia, a subcortical group of gray matter nuclei, appear to mediate postural tone. The four functionally distinct ganglia are the striatum, the pallidum, the substantia nigra, and the subthalamic nucleus. Collectively known as the corpus striatum, the caudate and putamen harbor components of both motor and association systems. The caudate nucleus plays an important role in the modulation of motor acts. Anatomical and functional neuroimaging studies have correlated decreased activation of the caudate with obsessive-compulsive behavior. When functioning properly, the caudate nucleus acts as a gatekeeper to allow the motor system to perform only those acts that are goal directed. When it fails to perform its gatekeeper function, extraneous acts are performed, as in obsessive-compulsive disorder or in the tic disorders, such as Tourette’s disorder. Overactivity of the striatum owing to lack of dopaminergic inhibition (e.g., in parkinsonian conditions) results in bradykinesia, an inability to initiate movements. The caudate, in particular, shrinks dramatically in Huntington’s disease. This disorder is characterized by rigidity, on which is gradually superimposed choreiform, or “dancing,”

movements. Psychosis may be a prominent feature of Huntington’s disease, and suicide is not uncommon. The caudate is also thought to influence associative, or cognitive, processes. The globus pallidus contains two parts linked in series. In a cross section of the brain, the internal and external parts of the globus pallidus are nested within the concavity of the putamen. The globus pallidus receives input from the corpus striatum and projects fibers to the thalamus. This structure may be severely damaged in Wilson’s disease and in carbon monoxide poisoning, which are characterized by dystonic posturing and flapping movements of the arms and legs. The substantia nigra is named the black substance because the presence of melanin pigment causes it to appear black to the naked eye. It has two parts, one of which is functionally equivalent to the globus pallidus interna. The other part degenerates in Parkinson’s disease. Parkinsonism is characterized by rigidity and tremor and is associated with depression in more than 30 percent of cases. Finally, lesions in the subthalamic nucleus yield ballistic movements, sudden limb jerks of such velocity that they are compared to projectile movement. Together, the nuclei of the basal ganglia appear capable of initiating and maintaining the full range of useful movements. Investigators have speculated that the nuclei serve to configure the activity of the overlying motor cortex to fit the purpose of the association areas. In addition, they appear to integrate proprioceptive feedback to maintain an intended movement. Cerebellum The cerebellum consists of a simple six-cell pattern of circuitry that is replicated roughly 10 million times. Simultaneous recordings of the cerebral

cortex and the cerebellum have shown that the cerebellum is activated several milliseconds before a planned movement. Moreover, ablation of the cerebellum renders intentional movements coarse and tremulous. These data suggest that the cerebellum carefully modulates the tone of agonistic and antagonistic muscles by predicting the relative contraction needed for smooth motion. This prepared motor plan is used to ensure that exactly the right amount of flexor and extensor stimuli is sent to the muscles. Recent functional imaging data have shown that the cerebellum is active, even during the mere imagination of motor acts when no movements ultimately result from its calculations. The cerebellum harbors two, and possibly more, distinct “homunculi” or cortical representations of the body plan. Motor Cortex Penfield’s groundbreaking work defined a motor homunculus in the precentral gyrus, Brodmann’s area 4 (Fig. 1.2-2), where a somatotopic map of the motor neurons is found. Individual cells within the motor strip cause contraction of single muscles. The brain region immediately anterior to the motor strip is called the supplementary motor area, Brodmann’s area 6. This region contains cells that when individually stimulated

can trigger more complex movements by influencing a firing sequence of motor strip cells. Recent studies have demonstrated wide representation of motor movements in the brain. FIGURE 1.2-2 Drawing of the lateral view (A) and medial view (B) of the cytoarchitectonic subdivisions of the human brain as determined by Brodmann. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009.) The skillful use of the hands is called praxis, and deficits in skilled movements are termed apraxias. The three levels of apraxia are limb-kinetic, ideomotor, and ideational. Limb-kinetic apraxia is the inability to use the contralateral hand in the presence of preserved strength; it results from isolated lesions in the supplementary motor area, which contains neurons that stimulate functional sequences of neurons in the motor strip. Ideomotor apraxia is the inability to perform an isolated motor act on command, despite preserved comprehension, strength, and spontaneous performance of the same act. Ideomotor apraxia simultaneously affects both limbs and involves functions so specialized that they are localized to only one hemisphere. Conditions in two separate areas can produce this apraxia. Disconnection of the language comprehension area, Wernicke’s area, from the motor regions causes an inability to follow spoken commands, and lesions to the left premotor area may impair the actual motor program as it is generated by the higher-order motor neurons. This program is transmitted across the corpus callosum to the right premotor area, which directs the movements of the left hand. A lesion in this callosal projection can also cause an isolated ideomotor apraxia in the left hand. This syndrome implies the representation of specific motor acts within discrete sections of the left premotor cortex. Thus just as some cells respond selectively to specific environmental features in the higher sensory cortices, some cells in the premotor cortex direct specific complex motor tasks. Ideational apraxia occurs when the individual components of a sequence of skilled acts can be performed in isolation, but the entire series cannot be organized and executed as a whole. For example, the sequence of opening an envelope, removing the letter, unfolding it, and placing it on the table cannot be performed in order, even though the individual acts can be performed in isolation. The representation of the concept of a motor sequence may involve several areas, specifically the left parietal cortex, but it likely also relies on the sequencing and executive functions of the prefrontal cortex. This apraxia is a typical finding of diffuse cortical degeneration, such as Alzheimer’s disease.

Autonomic Motor System The autonomic system is divided into a sensory component (described earlier) and a motor component. The autonomic motor system is divided into two branches: the

sympathetic and the parasympathetic. As a rule, organs are innervated by both types of fibers, which often serve antagonistic roles. The parasympathetic system slows the heart rate and begins the process of digestion. In contrast, the sympathetic system mediates the fight or flight response, with increased heart rate, shunting of blood away from the viscera, and increased respiration. The sympathetic system is highly activated by sympathomimetic drugs, such as amphetamine and cocaine, and may also be activated by withdrawal from sedating drugs such as alcohol, benzodiazepines, and opioids. Investigators who have found an increased risk of heart attacks in persons with high levels of hostility have suggested that chronic activation of the sympathetic fight or flight response, with elevated secretion of adrenaline, may underlie this association. The brain center that drives the autonomic motor system is the hypothalamus, which houses a set of paired nuclei that appear to control appetite, rage, temperature, blood pressure, perspiration, and sexual drive. For example, lesions to the ventromedial nucleus, the satiety center, produce a voracious appetite and rage. In contrast, lesions to the upper region of the lateral nucleus, the hunger center, produce a profound loss of appetite. Numerous research groups are making intense efforts to define the biochemical regulation of appetite and obesity and frequently target the role of the hypothalamus. In the regulation of sexual attraction, the role of the hypothalamus has also become an area of active research. In the 1990s, three groups independently reported neuroanatomical differences between certain of the hypothalamic nuclei of heterosexual and homosexual men. Researchers interpreted this finding to suggest that human sexual orientation has a neuroanatomical basis, and this result has stimulated several follow-up studies of the biological basis of sexual orientation. At present, however, these controversial findings are not accepted without question, and no clear consensus has emerged about whether the structure of the hypothalamus consistently correlates with sexual orientation. In animal studies, early nurturing and sexual experiences consistently alter the size of specific hypothalamic nuclei. Primitive Reflex Circuit Sensory pathways function as extractors of specific features from the overwhelming multitude of environmental stimuli, whereas motor pathways carry out the wishes of the organism. These pathways may be linked directly, for example, in the spinal cord, where a primitive reflex arc may mediate the brisk withdrawal of a limb from a painful stimulus, without immediate conscious awareness. In this loop, the peripheral stimulus activates the sensory nerve, the sensory neuron synapses on and directly activates the motor neuron, and the motor neuron drives the muscle to contract. This response is strictly local and all-or-none. Such primitive reflex arcs, however, rarely generate an organism’s behaviors. In most behaviors, sensory systems project to association areas, where sensory information is interpreted in terms of internally determined memories, motivations, and drives. The exhibited behavior results from a plan of action determined by the association components and carried out by the motor systems. Localization of Brain Functions

Many theorists have subdivided the brain into functional systems. Brodmann defined 47 areas on the basis of cytoarchitectonic distinctions, a cataloging that has been remarkably durable as the functional anatomy of the brain has been elucidated. A separate function, based on data from lesion studies and from functional neuroimaging, has been assigned to nearly all Brodmann’s areas. At the other extreme, certain experts have distinguished only three processing blocks: The brainstem and the thalamic reticular activating system provide arousal and set up attention; the posterior cortex integrates perceptions and generates language; and, at the highest level, the frontal cortex generates programs and executes plans like an orchestra conductor. Hemispheric lateralization of function is a key feature of higher cortical processing. The primary sensory cortices for touch, vision, hearing, smell, and taste are represented bilaterally, and the first level of

abstraction for these modalities is also usually represented bilaterally. The highest levels of feature extraction, however, are generally unified in one brain hemisphere only. For example, recognition of familiar and unfamiliar faces seems localized to the left inferior temporal cortex, and cortical processing of olfaction occurs in the right frontal lobe. Hypotheses about the flow of thought in the brain are based on few experimental data, although this scarcity of findings has not impeded numerous theoreticians from speculating about functional neuroanatomy. Several roles have been tentatively assigned to specific lobes of the brain, on the basis of the functional deficits resulting from localized injury. These data indicate that certain regions of cortex may be necessary for a specific function, but they do not define the complete set of structures that suffices for a complex task. Anecdotal evidence from surface electrocorticography for the study of epilepsy, for example, suggests that a right parietal seizure impulse may shoot immediately to the left frontal lobe and then to the right temporal lobe before spreading locally to the remainder of the parietal lobe. This evidence illustrates the limitations of naively assigning a mental function to a single brain region. Functional neuroimaging studies frequently reveal simultaneous activation of disparate brain regions during the performance of even a simple cognitive task. Nevertheless, particularly in the processing of vision and language, fairly well-defined lobar syndromes have been confirmed. Language The clearest known example of hemispheric lateralization is the localization of language functions to the left hemisphere. Starting with the work of Pierre Broca and Karl Wernicke in the 19th century, researchers have drawn a detailed map of language comprehension and expression. At least eight types of aphasias in which one or more components of the language pathway are inured have been defined. Prosody, the emotional and affective components of language, or “body language,” appears to be localized in a mirror set of brain units in the right hemisphere. Because of the major role of verbal and written language in human communication, the neuroanatomical basis of language is the most completely understood association function. Language disorders, also called aphasias, are readily diagnosed in routine conversation, whereas perceptual disorders may escape notice, except during detailed

neuropsychological testing, although these disorders may be caused by injury of an equal volume of cortex. Among the earliest models of cortical localization of function were Broca’s 1865 description of a loss of fluent speech caused by a lesion in the left inferior frontal lobe and Wernicke’s 1874 localization of language comprehension to the left superior temporal lobe. Subsequent analyses of patients rendered aphasic by strokes, trauma, or tumors have led to the definition of the entire language association pathway from sensory input through the motor output. Language most clearly demonstrates hemispheric localization of function. In most persons, the hemisphere dominant for language also directs the dominant hand. Ninety percent of the population is right-handed, and 99 percent of right-handers have left hemispheric dominance for language. Of the 10 percent who are left-handers, 67 percent also have left hemispheric language dominance; the other 33 percent have either mixed or right hemispheric language dominance. This innate tendency to lateralization of language in the left hemisphere is highly associated with an asymmetry of the planum temporale, a triangular cortical patch on the superior surface of the temporal lobe that appears to harbor Wernicke’s area. Patients with mixed hemispheric dominance for language lack the expected asymmetry of the planum temporale. That asymmetry has been observed in prenatal brains suggests a genetic determinant. Indeed, the absence of asymmetry runs in families, although both genetic and intrauterine influences probably contribute to the final pattern. Language comprehension is processed at three levels. First, in phonological processing, individual sounds, such as vowels or consonants, are recognized in the inferior gyrus of the frontal

lobes. Phonological processing improves if lip reading is allowed, if speech is slowed, or if contextual clues are provided. Second, lexical processing matches the phonological input with recognized words or sounds in the individual's memory. Lexical processing determines whether a sound is a word. Recent evidence has localized lexical processing to the left temporal lobe, where the representations of lexical data are organized according to semantic category. Third, semantic processing connects the words to their meaning. Persons with an isolated defect in semantic processing may retain the ability to repeat words in the absence of an ability to understand or spontaneously generate speech. Semantic processing activates the middle and superior gyri of the left temporal lobe, whereas the representation of the conceptual content of words is widely distributed in the cortex. Language production proceeds in the opposite direction, from the cortical semantic representations through the left temporal lexical nodes to either the oromotor phonological processing area (for speech) or the graphomotor system (for writing). Each of these areas can be independently or simultaneously damaged by stroke, trauma, infection, or tumor, resulting in a specific type of aphasia. The garbled word salad or illogical utterances of an aphasic patient leave little uncertainty about the diagnosis of left-sided cortical injury, but the right hemisphere contributes a somewhat more subtle, but equally important, affective quality to language. For example, the phrase "I feel good" may be spoken with an infinite variety of shadings, each of which is understood differently. The perception of prosody and the appreciation of the associated gestures, or "body language," appear to require an intact

right hemisphere. Behavioral neurologists have mapped an entire pathway for prosody association in the right hemisphere that mirrors the language pathway of the left hemisphere. Patients with right hemisphere lesions, who have impaired comprehension or expression of prosody, may find it difficult to function in society despite their intact language skills. Developmental dyslexia is defined as an unexpected difficulty with learning in the context of adequate intelligence, motivation, and education. Whereas speech consists of the logical combination of 44 basic phonemes of sounds, reading requires a broader set of brain functions and, thus, is more susceptible to disruption. The awareness of specific phonemes develops at about the age of 4 to 6 years and appears to be prerequisite to acquisition of reading skills. Inability to recognize distinct phonemes is the best predictor of a reading disability. Functional neuroimaging studies have localized the identification of letters to the occipital lobe adjacent to the primary visual cortex. Phonological processing occurs in the inferior frontal lobe, and semantic processing requires the superior and middle gyri of the left temporal lobe. A recent finding of uncertain significance is that phonological processing in men activates only the left inferior frontal gyrus, whereas phonological processing in women activates the inferior frontal gyrus bilaterally. Careful analysis of an individual's particular reading deficits can guide remedial tutoring efforts that can focus on weaknesses and thus attempt to bring the reading skills up to the general level of intelligence and verbal skills. In children, developmental nonverbal learning disorder is postulated to result from right hemisphere dysfunction. Nonverbal learning disorder is characterized by poor finemotor control in the left hand, deficits in visuoperceptual organization, problems with mathematics, and incomplete or disturbed socialization. Patients with nonfluent aphasia, who cannot complete a simple sentence, may be able to sing an entire song, apparently because many aspects of music production are localized to the right hemisphere. Music is represented predominantly in the right hemisphere, but the full complexity of musical ability seems to involve both hemispheres. Trained musicians appear to transfer many musical skills from the right hemisphere to the left as they gain proficiency in musical analysis and performance. Arousal and Attention Arousal, or the establishment and

maintenance of an awake state, appears to require at least three brain regions. Within the brainstem, the ascending reticular activating system (ARAS), a diffuse set of neurons, appears to set the level of consciousness. The ARAS projects to the intralaminar nuclei of the thalamus, and these nuclei in turn project widely throughout the cortex. Electrophysiological studies show that both the thalamus and the cortex fire rhythmical bursts of neuronal activity at rates of 20 to 40 cycles per second. During sleep, these bursts are not synchronized. During wakefulness, the ARAS stimulates the thalamic intralaminar nuclei, which in turn coordinate the oscillations of different cortical regions. The greater the synchronization, the higher the level of wakefulness. The absence of arousal produces stupor and coma. In general,

small discrete lesions of the ARAS can produce a stuporous state, whereas at the hemispheric level, large bilateral lesions are required to cause the same depression in alertness. One particularly unfortunate but instructive condition involving extensive, permanent, bilateral cortical dysfunction is the persistent vegetative state. Sleep–wake cycles may be preserved, and the eyes may appear to gaze; but the external world does not register and no evidence of conscious thought exists. This condition represents the expression of the isolated actions of the ARAS and the thalamus. The maintenance of attention appears to require an intact right frontal lobe. For example, a widely used test of persistence requires scanning and identifying only the letter A from a long list of random letters. Healthy persons can usually maintain performance of such a task for several minutes, but in patients with right frontal lobe dysfunction, this capacity is severely curtailed. Lesions of similar size in other regions of the cortex usually do not affect persistence tasks. In contrast, the more generally adaptive skill of maintaining a coherent line of thought is diffusely distributed throughout the cortex. Many medical conditions can affect this skill and may produce acute confusion or delirium. One widely diagnosed disorder of attention is attention-deficit/hyperactivity disorder (ADHD). No pathological findings have been consistently associated with this disorder. Functional neuroimaging studies, however, have variously documented either frontal lobe or right hemisphere hypometabolism in patients with ADHD, compared with normal controls. These findings strengthen the notion that the frontal lobes—especially the right frontal lobe—are essential to the maintenance of attention. Memory The clinical assessment of memory should test three periods, which have distinct anatomical correlates. Immediate memory functions over a period of seconds; recent memory applies on a scale of minutes to days; and remote memory encompasses months to years. Immediate memory is implicit in the concept of attention and the ability to follow a train of thought. This ability has been divided into phonological and visuospatial components, and functional imaging has localized them to the left and right hemispheres, respectively. A related concept, incorporating immediate and recent memory, is working memory, which is the ability to store information for several seconds, whereas other, related cognitive operations take place on this information. Recent studies have shown that single neurons in the dorsolateral prefrontal cortex not only record features necessary for working memory, but also record the certainty with which the information is known and the degree of expectation assigned to the permanence of a particular environmental feature. Some neurons fire rapidly for an item that is eagerly awaited, but may cease firing if hopes are dashed unexpectedly. The encoding of the emotional value of an item contained in the working memory may be of great usefulness in determining goal-directed behavior. Some researchers localize working memory predominantly to the left frontal cortex. Clinically, however, bilateral prefrontal cortex lesions are required for severe impairment of working memory. Other types of memory have been described: episodic, semantic, and procedural. Three brain structures are critical to the formation of

memories: the medial temporal lobe, certain diencephalic nuclei, and the basal forebrain. The medial temporal lobe

houses the hippocampus, an elongated, highly repetitive network. The amygdala is adjacent to the anterior end of the hippocampus. The amygdala has been suggested to rate the emotional importance of an experience and to activate the level of hippocampal activity accordingly. Thus an emotionally intense experience is indelibly etched in memory, but indifferent stimuli are quickly disregarded. Animal studies have defined a hippocampal place code, a pattern of cellular activation in the hippocampus that corresponds to the animal's location in space. When the animal is introduced to a novel environment, the hippocampus is broadly activated. As the animal explores and roams, the firing of certain hippocampal regions begins to correspond to specific locations in the environment. In about 1 hour, a highly detailed internal representation of the external space (a "cognitive map") appears in the form of specific firing patterns of the hippocampal cells. These patterns of neuronal firing may bear little spatial resemblance to the environment they represent; rather, they may seem randomly arranged in the hippocampus. If the animal is manually placed in a certain part of a familiar space, only the corresponding hippocampal regions show intense neural activity. When recording continues into sleep periods, firing sequences of hippocampal cells outlining a coherent path of navigation through the environment are registered, even though the animal is motionless. If the animal is removed from the environment for several days and then returned, the previously registered hippocampal place code is immediately reactivated. A series of animal experiments have dissociated the formation of the hippocampal place code from either visual, auditory, or olfactory cues, although each of these modalities may contribute to place code generation. Other factors may include internal calculations of distances based on counting footsteps or other proprioceptive information. Data from targeted genetic mutations in mice have implicated both the N-methyl-D-aspartate (NMDA) glutamate receptors and the calciumcalmodulin kinase II (CaMKII) in the proper formation of hippocampal place fields. These data suggest that the hippocampus is a significant site for formation and storage of immediate and recent memories. Although no data yet support the notion, it is conceivable that the hippocampal cognitive map is inappropriately reactivated during a déjà vu experience. The most famous human subject in the study of memory is H. M., a man with intractable epilepsy, who had both his hippocampi and amygdalae surgically removed to alleviate his condition. The epilepsy was controlled, but he was left with a complete inability to form and recall memories of facts. H. M.'s learning and memory skills were relatively preserved, which led to the suggestion that declarative or factual memory may be separate within the brain from procedural or skill-related memory. A complementary deficit in procedural memory with preservation of declarative memory may be seen in persons with Parkinson's disease, in whom dopaminergic neurons of the nigrostriatal tract degenerate. Because this deficit in procedural memory can be ameliorated with treatment with levodopa (Larodopa), which is thought to potentiate dopaminergic neurotransmission in the nigrostriatal pathway, a

role has been postulated for dopamine in procedural memory. Additional case reports have further implicated the amygdala and the afferent and efferent fiber tracts of the hippocampus as essential to the formation of memories. In addition, lesional studies have suggested a mild lateralization of hippocampal function in which the left hippocampus is more efficient at forming verbal memories and the right hippocampus tends to form nonverbal memories. After unilateral lesions in humans, however, the remaining hippocampus may compensate to a large extent. Medical causes of amnesia include alcoholism, seizures, migraine, drugs, vitamin deficiencies, trauma, strokes,

tumors, infections, and degenerative diseases. The motor system within the cortex receives directives from the association areas. The performance of a novel act requires constant feedback from the sensory and association areas for completion, and functional neuroimaging studies have demonstrated widespread activation of the cortex during unskilled acts. Memorized motor acts initially require activation of the medial temporal lobe. With practice, however, the performance of ever-larger segments of an act necessary to achieve a goal become encoded within discrete areas of the premotor and parietal cortices, particularly the left parietal cortex, with the result that a much more limited activation of the cortex is seen during highly skilled acts, and the medial temporal lobe is bypassed. This process is called the corticalization of motor commands. In lay terms, the process suggests a neuroanatomical basis for the adage “practice makes perfect.” Within the diencephalon, the dorsal medial nucleus of the thalamus and the mammillary bodies appear necessary for memory formation. These two structures are damaged in thiamine deficiency states usually seen in chronic alcoholics, and their inactivation is associated with Korsakoff’s syndrome. This syndrome is characterized by severe inability to form new memories and a variable inability to recall remote memories. The most common clinical disorder of memory is Alzheimer’s disease. Alzheimer’s disease is characterized pathologically by the degeneration of neurons and their replacement by senile plaques and neurofibrillary tangles. Clinicopathological studies have suggested that the cognitive decline is best correlated with the loss of synapses. Initially, the parietal and temporal lobes are affected, with relative sparing of the frontal lobes. This pattern of degeneration correlates with the early loss of memory, which is largely a temporal lobe function. Also, syntactical language comprehension and visuospatial organization, functions that rely heavily on the parietal lobe, are impaired early in the course of Alzheimer’s disease. In contrast, personality changes, which reflect frontal lobe function, are relatively late consequences of Alzheimer’s disease. A rarer, complementary cortical degeneration syndrome, Pick’s disease, first affects the frontal lobes while sparing the temporal and parietal lobes. In Pick’s disease, disinhibition and impaired language expression, which are signs of frontal dysfunction, appear early, with relatively preserved language comprehension and memory. Memory loss can also result from disorders of the subcortical gray matter structures, specifically the basal ganglia and the brainstem nuclei, from disease of the white matter, or from disorders that affect both gray and white matter.

Emotion Individual emotional experiences occupy the attention of all mental health professionals. Emotion derives from basic drives, such as feeding, sex, reproduction, pleasure, pain, fear, and aggression, which all animals share. The neuroanatomical basis for these drives appears to be centered in the limbic system. Distinctly human emotions, such as affection, pride, guilt, pity, envy, and resentment, are largely learned and most likely are represented in the cortex (see Color Plate 1.2-3). The regulation of drives appears to require an intact frontal cortex. The complex interplay of the emotions, however, is far beyond the understanding of functional neuroanatomists. Where, for example, are the representations of the id, the ego, and the superego? Through what pathway are ethical and moral judgments shepherded? What processes allow beauty to be in the eye of the beholder? These philosophical questions represent a true frontier of human discovery. Several studies have suggested that within the cortex exists a hemispheric dichotomy of emotional representation. The left hemisphere houses the analytical mind but may have a limited emotional repertoire. For example, lesions to the right hemisphere, which cause profound functional deficits, may be noted with indifference by the intact left hemisphere. The denial of illness and of the inability to move the left hand in cases of right hemisphere injury is called anosognosia. In contrast,

left hemisphere lesions, which cause profound aphasia, can trigger a catastrophic depression, as the intact right hemisphere struggles with the realization of the loss. The right hemisphere also appears dominant for affect, socialization, and body image. Damage to the left hemisphere produces intellectual disorder and loss of the narrative aspect of dreams. Damage to the right hemisphere produces affective disorders, loss of the visual aspects of dreams, and a failure to respond to humor, shadings of metaphor, and connotations. In dichotic vision experiments, two scenes of varied emotional content were displayed simultaneously to each half of the visual field and were perceived separately by each hemisphere. A more intense emotional response attended the scenes displayed to the left visual field that were processed by the right hemisphere. Moreover, hemisensory changes representing conversion disorders have been repeatedly noted to involve the left half of the body more often than the right, an observation that suggests an origin in the right hemisphere. Within the hemispheres, the temporal and frontal lobes play a prominent role in emotion. The temporal lobe exhibits a high frequency of epileptic foci, and temporal lobe epilepsy (TLE) presents an interesting model for the role of the temporal lobe in behavior. In studies of epilepsy, abnormal brain activation is analyzed, rather than the deficits in activity analyzed in classic lesional studies. TLE is of particular interest in psychiatry because patients with temporal lobe seizures often manifest bizarre behavior without the classic grand mal shaking movements caused by seizures in the motor cortex. A proposed TLE personality is characterized by hyposexuality, emotional intensity, and a perseverative approach to interactions, termed viscosity. Patients with left TLE may generate references to personal destiny and philosophical themes and display a humorless approach to life. In contrast, patients with right TLE may display excessive emotionality, ranging from elation to sadness. Although patients with TLE may display excessive aggression between seizures, the seizure itself

may evoke fear. The inverse of a TLE personality appears in persons with bilateral injury to the temporal lobes after head trauma, cardiac arrest, herpes simplex encephalitis, or Pick’s disease. This lesion resembles the one described in the Klüver-Bucy syndrome, an experimental model of temporal lobe ablation in monkeys. Behavior in this syndrome is characterized by hypersexuality, placidity, a tendency to explore the environment with the mouth, inability to recognize the emotional significance of visual stimuli, and constantly shifting attention, called hypermetamorphosis. In contrast to the aggression– fear spectrum sometimes seen in patients with TLE, complete experimental ablation of the temporal lobes appears to produce a uniform, bland reaction to the environment, possibly because of an inability to access memories. The prefrontal cortices influence mood in a complementary way. Whereas activation of the left prefrontal cortex appears to lift the mood, activation of the right prefrontal cortex causes depression. A lesion to the left prefrontal area, at either the cortical or the subcortical level, abolishes the normal mood-elevating influences and produces depression and uncontrollable crying. In contrast, a comparable lesion to the right prefrontal area may produce laughter, euphoria, and witzelsucht, a tendency to joke and make puns. Effects opposite to those caused by lesions appear during seizures, in which occurs abnormal, excessive activation of either prefrontal cortex. A seizure focus within the left prefrontal cortex can cause gelastic seizures, for example, in which the ictal event is laughter. Functional neuroimaging has documented left prefrontal hypoperfusion during depressive states, which normalized after the depression was treated successfully. Limbic System Function The limbic system was delineated by James Papez in 1937. The Papez circuit consists of the hippocampus, the fornix, the mammillary bodies, the anterior nucleus of the thalamus, and the cingulate gyrus (Fig. 1.2-4). The boundaries of the limbic system were subsequently expanded to include the amygdala,

septum, basal forebrain, nucleus accumbens, and orbitofrontal cortex.

FIGURE 1.2-4 Schematic drawing of the major anatomic structures of the limbic system. The cingulate and parahippocampal gyri form the “limbic lobe,” a rim of tissue located along the junction of the diencephalon and the cerebral hemispheres. (Adapted from Hendelman WJ. Student’s Atlas of Neuroanatomy. Philadelphia: WB Saunders; 1994:179.) Although this schema creates an anatomical loop for emotional processing, the specific contributions of the individual components other than the hippocampus or even whether a given train of neural impulses actually travels along the entire pathway is unknown. The amygdala appears to be a critically important gate through which internal and external stimuli are integrated. Information from the primary senses is interwoven with internal drives, such as hunger and thirst, to assign emotional significance to sensory experiences. The amygdala may mediate learned fear responses, such as anxiety and panic, and may direct the expression of certain emotions by producing a particular affect. Neuroanatomical data suggest that the amygdala exerts a more powerful influence on the cortex, to stimulate or suppress cortical activity, than the cortex exerts on the amygdala. Pathways from the sensory thalamic relay stations separately send sensory data to the amygdala and the cortex, but the subsequent effect of the amygdala on the cortex is the more potent of the two reciprocal connections. In contrast, damage to the amygdala has been reported to ablate the ability to distinguish fear and anger in other persons’ voices and facial expressions. Persons with such injuries may have a preserved ability to recognize happiness, sadness, or disgust. The limbic system appears

to house the emotional association areas, which direct the hypothalamus to express the motor and endocrine components of the emotional state. Fear and Aggression Electrical stimulation of animals throughout the subcortical area involving the limbic system produces rage reactions (e.g., growling, spitting, and arching of the back). Whether the animal flees or attacks depends on the intensity of the stimulation. Limbic System and Schizophrenia The limbic system has been particularly implicated in neuropathological studies of schizophrenia. Eugen Bleuler’s well-known four A’s of schizophrenia—affect, associations, ambivalence, and autism—refer to brain functions served in part by limbic structures. Several clinicopathological studies have found a reduction in the brain weight of the gray matter but not of the white matter in persons with schizophrenia. In pathological as well as in magnetic resonance imaging (MRI) reports, persons with schizophrenia may have reduced volume of the hippocampus, amygdala, and parahippocampal gyrus. Schizophrenia may be a late sequela of a temporal epileptic focus, with some studies reporting an association in 7 percent of patients with TLE. Functional neuroimaging studies have demonstrated decreased activation of the frontal lobes in many patients with schizophrenia, particularly during tasks requiring willed action. A reciprocal increase in activation of the temporal lobe can occur during willed actions, such as finger movements or speaking, in persons with schizophrenia. Neuropathological studies have shown a decreased density of neuropil, the intertwined axons and dendrites of the neurons, in the frontal lobes of these patients. During development, the density of neuropil is highest around age 1 year and then is reduced somewhat through synaptic pruning; the density plateaus throughout childhood and is further reduced to adult levels in adolescence. One hypothesis of the appearance of schizophrenia in the late teenage years is that excessive adolescent synaptic pruning occurs and results in too little frontolimbic activity. Some experts have suggested that hypometabolism and paucity of interneuronal connections in the prefrontal cortex may reflect inefficiencies in working memory, which permits the disjointed discourse and loosening

of associations that characterize schizophrenia. At present, the molecular basis for the regulation of the density of synapses within the neuropil is unknown. Other lines of investigation aimed at understanding the biological basis of schizophrenia have documented inefficiencies in the formation of cortical synaptic connections in the middle of the second trimester of gestation, which may result from a viral infection or malnutrition. Neurodevelopmental surveys administered during childhood have found an increased incidence of subtle neurological abnormalities before the appearance of the thought disorder in persons who subsequently exhibited signs of schizophrenia. In one intriguing study, positron emission tomography (PET) scanning was used to identify the brain regions that are activated when a person hears spoken language. A consistent set of cortical and subcortical structures demonstrated increased metabolism

when speech was processed. The researchers then studied a group of patients with schizophrenia who were experiencing active auditory hallucinations. During the hallucinations, the same cortical and subcortical structures were activated as were activated by the actual sounds, including the primary auditory cortex. At the same time, decreased activation was seen of areas thought to monitor speech, including the left middle temporal gyrus and the supplementary motor area. This study raises the questions of what brain structure is activating the hallucinations and by what mechanism do neuroleptic drugs suppress the hallucinations. Clearly, functional imaging has much to tell about the neuroanatomical basis of schizophrenia. Frontal Lobe Function The frontal lobes, the region that determines how the brain acts on its knowledge, constitute a category unto themselves. In comparative neuroanatomical studies, the massive size of the frontal lobes is the main feature that distinguishes the human brain from that of other primates and that lends it uniquely human qualities. There are four subdivisions of the frontal lobes. The first three—the motor strip, the supplemental motor area, and Broca’s area—are mentioned in the preceding discussion of the motor system and language. The fourth, most anterior, division is the prefrontal cortex. The prefrontal cortex contains three regions in which lesions produce distinct syndromes: the orbitofrontal, the dorsolateral, and the medial. Dye-tracing studies have defined dense reciprocal connections between the prefrontal cortex and all other brain regions. Therefore, to the extent that anatomy can predict function, the prefrontal cortex is ideally connected to allow sequential use of the entire palette of brain functions in executing goal-directed activity. Indeed, frontal lobe injury usually impairs the executive functions: motivation, attention, and sequencing of actions. Bilateral lesions of the frontal lobes are characterized by changes in personality—how persons interact with the world. The frontal lobe syndrome, which is most commonly produced by trauma, infarcts, tumors, lobotomy, multiple sclerosis, or Pick’s disease, consists of slowed thinking, poor judgment, decreased curiosity, social withdrawal, and irritability. Patients typically display apathetic indifference to experience that can suddenly explode into impulsive disinhibition. Unilateral frontal lobe lesions may be largely unnoticed because the intact lobe can compensate with high efficiency. Frontal lobe dysfunction may be difficult to detect by means of highly structured, formal neuropsychological tests. Intelligence, as reflected in the intelligence quotient (IQ), may be normal, and functional neuroimaging studies have shown that the IQ seems to require mostly parietal lobe activation. For example, during administration of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), the highest levels of increased metabolic activity during verbal tasks occurred in the left parietal lobe, whereas the highest levels of increased metabolic activity during performance skills occurred in the right parietal lobe. In contrast, frontal lobe pathology may become apparent only under unstructured, stressful, real-life situations.

A famous case illustrating the result of frontal lobe damage involves Phineas Gage, a 25-year-old railroad worker. While he was working with explosives, an accident drove an iron rod through Gage's head. He survived, but both frontal lobes were severely damaged. After the accident, his behavior changed dramatically. The case was written up by J. M. Harlow, M.D., in 1868, as follows: [George] is fitfull, irreverent, indulging at times in the grossest profanity (which was not previously his custom), manifesting but little deference for his fellows, impatient of restraint or advice when it conflicts with his desires...His mind was radically changed, so decidedly that his friends and acquaintances said he was "no longer Gage." (see Fig. 1.2-5) FIGURE 1.2-5 The life mask and skull of Phineas Gage. Note damage to the frontal region. "A famous case illustrating the result of frontal lobe damage involves Phineas Gage, a 25-year-old railroad worker. While he was working with explosives, an accident drove an iron rod through Gage's head. He survived, but both frontal lobes were severely damaged. After the accident, his behavior changed dramatically. The case was written up by J.M. Harlow, M.D., in 1868, as follows: [Gage] is fitful, irreverent, indulging at times in the grossest profanity (which was not previously his custom), manifesting but little deference for his fellows, impatient of restraint or advice when it conflicts his desires… His mind was radically changed, so decidedly that his friends and acquaintances said he was 'no longer Gage.'" (Courtesy of Anthony A. Walsh, Ph.D.) In one study of right-handed males, lesions of the right prefrontal cortex eliminated the tendency to use internal, associative memory cues and led to an extreme tendency to interpret the task at hand in terms of its immediate context. In contrast, right-handed males who had lesions of the left prefrontal cortex produced no context-dependent interpretations and interpreted the tasks entirely in terms of their own internal drives. A mirror image of the functional lateralization appeared in left-handed subjects. This test thus revealed the clearest known association of higher cortical functional

lateralization with the subjects' dominant hand. Future experiments in this vein will attempt to reproduce these findings with functional neuroimaging. If corroborated, these studies suggest a remarkable complexity of functional localization within the prefrontal cortex and may also have implications for the understanding of psychiatric diseases in which prefrontal pathology has been postulated, such as schizophrenia and mood disorders. The heavy innervation of the frontal lobes by dopamine-containing nerve fibers is of interest because of the action of antipsychotic medications. At the clinical level, antipsychotic medications may help to organize the rambling associations of a patient with schizophrenia. At the neurochemical level, most typical antipsychotic medications block the actions of dopamine at the D2 receptors. The frontal lobes, therefore, may be a major therapeutic site of action for antipsychotic medications. DEVELOPMENT The nervous system is divided into the central and peripheral nervous systems (CNS and PNS). The CNS consists of the brain and spinal cord; the PNS refers to all the sensory, motor, and autonomic fibers and ganglia outside the CNS. During development, both divisions arise from a common precursor, the neural tube, which in turn is formed through folding of the neural plate, a specialization of the ectoderm, the outermost of the three layers of the primitive embryo. During embryonic development, the neural tube itself becomes the CNS; the ectoderm immediately superficial to the neural tube becomes the neural crest, which gives rise to the PNS. The formation of these structures requires chemical communication between the neighboring tissues in the form of cell surface molecules and diffusible chemical signals. In many cases, an earlier-formed structure, such as the notochord, is said to induce the surrounding ectoderm to form a later structure, in this case the neural plate (see Color Plate 1.2-6). Identification of the chemical mediators of tissue induction is an active area of research. Investigators have begun to examine whether failures of the

interactions of these mediators and their receptors could underlie errors in brain development that cause psychopathology. Neuronal Migration and Connections The life cycle of a neuron consists of cell birth, migration to the adult position, extension of an axon, elaboration of dendrites, synaptogenesis, and, finally, the onset of chemical neurotransmission. Individual neurons are born in proliferative zones generally located along the inner surface of the neural tube. At the peak of neuronal proliferation in the middle of the second trimester, 250,000 neurons are born each minute. Postmitotic neurons migrate outward to their adult locations in the cortex, guided by radially oriented astrocytic glial fibers. Glia-guided neuronal migration in the cerebral cortex occupies much of the first 6 months of gestation. For some neurons in the prefrontal cortex, migration occurs over a distance 5,000 times the diameter of the neuronal cell body. Neuronal migration requires a complex set of cell–cell interactions and is susceptible to errors in which neurons fail to reach the cortex and instead reside in ectopic positions. A group of such incorrectly placed neurons is called a heterotopia. Neuronal heterotopias have been shown to cause epilepsy and are highly associated with mental retardation. In a neuropathological study of the planum temporale of four

consecutive patients with dyslexia, heterotopias were a common finding. Recently, heterotopic neurons within the frontal lobe have been postulated to play a causal role in some cases of schizophrenia. Many neurons lay down an axon as they migrate, whereas others do not initiate axon outgrowth until they have reached their cortical targets. Thalamic axons that project to the cortex initially synapse on a transient layer of neurons called the subplate neurons. In normal development, the axons subsequently detach from the subplate neurons and proceed superficially to synapse on the true cortical cells. The subplate neurons then degenerate. Some brains from persons with schizophrenia reveal an abnormal persistence of subplate neurons, suggesting a failure to complete axonal pathfinding in the brains of these persons. This finding does not correlate with the presence of schizophrenia in every case, however. A characteristic branched dendritic tree elaborates once the neuron has completed migration. Synaptogenesis occurs at a furious rate from the second trimester through the first 10 years or so of life. The peak of synaptogenesis occurs within the first 2 postnatal years, when as many as 30 million synapses form each second. Ensheathment of axons by myelin begins prenatally; it is largely complete in early childhood, but does not reach its full extent until late in the third decade of life. Myelination of the brain is also sequential. Neuroscientists are tremendously interested in the effect of experience on the formation of brain circuitry in the first years of life. As noted earlier, many examples are seen of the impact of early sensory experience on the wiring of cortical sensory processing areas. Similarly, early movement patterns are known to reinforce neural connections in the supplemental motor area that drive specific motor acts. Neurons rapidly form a fivefold excess of synaptic connections; then, through a Darwinian process of elimination, only those synapses that serve a relevant function persist. This synaptic pruning appears to preserve input in which the presynaptic cell fires in synchrony with the postsynaptic cell, a process that reinforces repeatedly activated neural circuits. One molecular component that is thought to mediate synaptic reinforcement is the postsynaptic NMDA glutamate receptor. This receptor allows the influx of calcium ions only when activated by glutamate at the same time as the membrane in which it sits is depolarized. Thus, glutamate binding without membrane depolarization or membrane depolarization without glutamate binding fails to trigger calcium influx. NMDA receptors open in dendrites that are exposed to repeated activation, and their activation stimulates stabilization of the synapse. Calcium is a crucial intracellular messenger that initiates a cascade of events, including gene

regulation and the release of trophic factors that strengthen particular synaptic connections. Although less experimental evidence exists for the role of experience in modulating synaptic connectivity of association areas than has been demonstrated in sensory and motor areas, neuroscientists assume that similar activity-dependent mechanisms may apply in all areas of the brain. Adult Neurogenesis A remarkable recent discovery has been that new neurons can be generated in certain brain regions (particularly the dentate gyrus of the hippocampus) in adult animals, including humans. This is in marked contrast to the previous belief that no neurons were produced after birth in most species. This discovery has a potentially profound impact on our understanding of normal development, incorporation of experiences, as well as the ability of the brain to repair itself after various types of injuries (see Color Plates

1.2-7 and 1.2-8). Neurological Basis of Development Theories In the realm of emotion, early childhood experiences have been suspected to be at the root of psychopathology since the earliest theories of Sigmund Freud. Freud's psychoanalytic method aimed at tracing the threads of a patient's earliest childhood memories. Franz Alexander added the goal of allowing the patient to relive these memories in a less pathological environment, a process known as a corrective emotional experience. Although neuroscientists have no data demonstrating that this method operates at the level of neurons and circuits, emerging results reveal a profound effect of early caregivers on an adult individual's emotional repertoire. For example, the concept of attunement is defined as the process by which caregivers "play back a child's inner feelings." If a baby's emotional expressions are reciprocated in a consistent and sensitive manner, certain emotional circuits are reinforced. These circuits likely include the limbic system, in particular, the amygdala, which serves as a gate to the hippocampal memory circuits for emotional stimuli. In one anecdote, for example, a baby whose mother repeatedly failed to mirror her level of excitement emerged from childhood an extremely passive girl, who was unable to experience a thrill or a feeling of joy. The relative contributions of nature and nurture are perhaps nowhere more indistinct than in the maturation of emotional responses, partly because the localization of emotion within the adult brain is only poorly understood. It is reasonable to assume, however, that the reactions of caregivers during a child's first 2 years of life are eventually internalized as distinct neural circuits, which may be only incompletely subject to modification through subsequent experience. For example, axonal connections between the prefrontal cortex and the limbic system, which probably play a role in modulating basic drives, are established between the ages of 10 and 18 months. Recent work suggests that a pattern of terrifying experiences in infancy may flood the amygdala and drive memory circuits to be specifically alert to threatening stimuli, at the expense of circuits for language and other academic skills. Thus infants raised in a chaotic and frightening home may be neurologically disadvantaged for the acquisition of complex cognitive skills in school. An adult correlate to this cascade of detrimental overactivity of the fear response is found in posttraumatic stress disorder (PTSD), in which persons exposed to an intense trauma involving death or injury may have feelings of fear and helplessness for years after the event. A PET scanning study of patients with PTSD revealed abnormally high activity in the right amygdala while the patients were reliving their traumatic memories. The researchers hypothesized that the stressful hormonal milieu present during the registration of the memories may have served to burn the memories into the brain and to prevent their erasure by the usual memory modulation circuits. As a result, the traumatic memories exerted a pervasive influence and led to a state of constant vigilance, even in safe, familiar settings.

Workers in the related realms of mathematics have produced results documenting the organizing effects of early experiences on internal representations of the external world. Since the time of Pythagoras, music has been considered a branch of mathematics. A series of recent studies has shown that groups of children who were given 8 months of intensive classical music lessons during preschool years later had significantly better spatial and mathematical reasoning in school than a control group. Nonmusical tasks, such as navigating mazes, drawing geometric figures, and copying patterns of twocolor blocks, were performed significantly more skillfully by the musical children. Early exposure to music, thus, may be ideal preparation for later acquisition of complex mathematical and engineering skills. These tantalizing observations suggest a neurological basis for the developmental theories of Jean Piaget, Erik Erikson, Margaret Mahler, John Bowlby, Sigmund Freud, and others. Erikson's epigenetic theory states that normal adult behavior results from the successful, sequential completion of each of several infantile and childhood stages. According to the epigenetic model, failure to complete an early stage is reflected in subsequent physical, cognitive, social, or emotional maladjustment. By analogy, the experimental data just discussed suggest that early experience, particularly during the critical window of opportunity for establishing neural connections, primes the basic circuitry for language, emotions, and other advanced behaviors. Clearly, miswiring of an infant's brain may lead to severe handicaps later when the person attempts to relate to the world as an adult. These findings support the vital need for adequate public financing of Early Intervention and Head Start programs, programs that may be the most cost-effective means of improving persons' mental health. REFERENCES Björklund A, Dunnett SB. Dopamine neuron systems in the brain: An update. Trends Neurosci. 2007;30:194. Blond BN, Fredericks CA, Blumberg HP. Functional neuroanatomy of bipolar disorder: Structure, function, and connectivity in an amygdala-anterior paralimbic neural system. Bipolar Disord. 2012;14(4):340. Green S, Lambon Ralph MA, Moll J, Deakin JF, Zahn R. Guilt-selective functional disconnection of anterior temporal and subgenual cortices in major depressive disorder. Arch Gen Psychiatry. 2012;69(10):1014. Katschnig P, Schwingenschuh P, Jehna M, Svehlík M, Petrovic K, Ropele S, Zwick EB, Ott E, Fazekas F, Schmidt R, Enzinger C. Altered functional organization of the motor system related to ankle movements in Parkinson's disease: Insights from functional MRI. J Neural Transm. 2011;118:783. Kringelbach ML, Berridge KC. The functional neuroanatomy of pleasure and happiness. Discov Med. 2010;9:579. Melchitzky DS, Lewis DA. Functional Neuroanatomy. In: Sadock BJ, Sadock VA, Ruiz P, eds. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. Morris CA. The behavioral phenotype of Williams syndrome: A recognizable pattern of neurodevelopment. Am J Med Genet C Semin Med Genet. 2010;154C:427. Nguyen AD, Shenton ME, Levitt JJ. Olfactory dysfunction in schizophrenia: A review of neuroanatomy and psychophysiological measurements. Harv Rev Psychiatry. 2010;18:279. Prats-Galino A, Soria G, de Notaris M, Puig J, Pedraza S. Functional anatomy of subcortical circuits issuing from or integrating at the human brainstem. Clin Neurophysiol. 2012;123:4. Sapara A, Birchwood M, Cooke MA, Fannon D, Williams SC, Kuipers E, Kumari V. Preservation and compensation: The functional neuroanatomy of insight and working memory in schizophrenia. Schizophr Res. 2014;152:201–209. Vago DR, Epstein J, Catenaccio E, Stern E. Identification of neural targets for the treatment of psychiatric disorders: The role of functional neuroimaging. Neurosurg Clin N Am. 2011;22:279. Watson CE, Chatterjee A. The functional neuroanatomy of actions. Neurology. 2011;76:1428. Weis S, Leube D, Erb M, Heun R, Grodd W, Kircher T. Functional neuroanatomy of sustained memory encoding performance in healthy aging and in Alzheimer's disease. Int J Neurosci. 2011;121:384.

03 - 1.3 Neural Development and Neurogenesis

1.3 Neural Development and Neurogenesis

Zilles K, Amunts K, Smaers JB. Three brain collections for comparative neuroanatomy and neuroimaging. Ann N Y Acad Sci. 2011;1225:E94. 1.3 Neural Development and Neurogenesis The human brain is a structurally and functionally complex system that exhibits ongoing modification in response to both experience and disease. The anatomical and neurochemical systems that underlie the cognitive, social, emotional, and sensorimotor functions of the mature nervous system emerge from neuronal and glial cell populations that arise during the earliest periods of development. An understanding of molecular and cellular mechanisms mediating nervous system development is critical in psychiatry because abnormalities of developmental processes contribute to many brain disorders. Although a developmental basis may not be surprising in early childhood disorders, such as autism, fragile X mental retardation, and Rett syndrome, even mature diseases including schizophrenia and depression reflect ontogenetic factors. For example, evidence from brain pathology and neuroimaging indicates that there are reductions in forebrain region volumes, neuron and glial cell numbers, and some classes of interneurons in schizophrenia that are apparent at the time of diagnosis. Similarly, in autism, early brain growth is abnormally increased, and abnormalities of cellular organization are observed that reflect disturbances in the basic processes of cell proliferation and migration. When there is abnormal regulation of early brain development, a foundation of altered neuron populations that may differ in cell types, numbers, and positions is laid down, or abnormal connections, with consequences for interacting glial populations, may be elaborated. With progressive postnatal development, the maturing brain systems call upon component neurons to achieve increasing levels of complex information processing, which may be deficient should initial conditions be disturbed. New neural properties emerge during maturation as neuron populations elaborate additional functional networks based on and modified by ongoing experience. Given the brain’s dynamic character, we may expect that developmental abnormalities in neural populations and systems, caused by genetic as well as environmental factors, will manifest at diverse times in a person’s life. OVERVIEW OF NERVOUS SYSTEM MORPHOLOGICAL DEVELOPMENT In considering brain development, several overarching principles need to be considered. First, different brain regions and neuron populations are generated at distinct times of development and exhibit specific temporal schedules. This has implications for the consequences of specific developmental insults, such as the production of autism following fetal exposure to the

drug thalidomide only during days 20 to 24 of gestation. Second, the sequence of cellular processes comprising ontogeny predicts that abnormalities in early events necessarily lead to differences in subsequent stages, although not all abnormalities may be accessible to our clinical tools. For example, a deficit in the number of neurons will likely lead to reductions in axonal processes and

ensheathing white matter in the mature brain. However, at the clinical level, since glial cells outnumber neurons 8 to 1, the glial cell population, the oligodendrocytes, and their myelin appear as altered white matter on neuroimaging with little evidence of a neuronal disturbance. Third, it is clear that specific molecular signals, such as extracellular growth factors and cognate receptors or transcription factors, play roles at multiple developmental stages of the cell. For example, both insulin-like growth factor I (IGF-I) and brain-derived neurotrophic factor (BDNF) regulate multiple cellular processes during the developmental generation and mature function of neurons, including cell proliferation, survival promotion, neuron migration, process outgrowth, and the momentary synaptic modifications (plasticity) underlying learning and memory. Thus changes in expression or regulation of a ligand or its receptor, by experience, environmental insults, or genetic mechanisms, will have effects on multiple developmental and mature processes. The Neural Plate and Neurulation The nervous system of the human embryo first appears between 2½ and 4 weeks of gestation. During development, emergence of new cell types, including neurons, results from interactions between neighboring layers of cells. On gestational day 13, the embryo consists of a sheet of cells. Following gastrulation (days 14 to 15), which forms a two-cell-layered embryo consisting of ectoderm and endoderm, the neural plate region of the ectoderm is delineated by the underlying mesoderm, which appears on day 16. The mesoderm forms by cells entering a midline cleft in the ectoderm called the primitive streak. After migration, the mesodermal layer lies between ectoderm and endoderm and induces overlying ectoderm to become neural plate. Induction usually involves release of soluble growth factors from one group of cells, which in turn bind receptors on neighboring cells, eliciting changes in nuclear transcription factors that control downstream gene expression. In some cases, cell–cell contact-mediated mechanisms are involved. In the gene-patterning section below, the important roles of soluble growth factors and transcription factor expression are described. The neural plate, the induction of which is complete by 18 days, is a sheet of columnar epithelium and is surrounded by ectodermal epithelium. After formation, the edges of the neural plate elevate, forming the neural ridges. Subsequently, changes in intracellular cytoskeleton and cell–extracellular matrix attachment cause the ridges to merge in the midline and fuse, a process termed neurulation, forming the neural tube, with a central cavity presaging the ventricular system (Fig. 1.3-1). Fusion begins in the cervical region at the hindbrain level (medulla and pons) and continues rostrally and caudally. Neurulation occurs at 3 to 4 weeks of gestation in humans, and its failure results in anencephaly rostrally and spina bifida caudally. Neurulation defects are well known following exposure to retinoic acid in dermatological preparations and anticonvulsants, especially valproic acid, as well as diets deficient in folic acid.

FIGURE 1.3-1 Mechanisms of neurulation. Neurulation begins with the formation of a neural plate in response to soluble growth factors released by the underlying notochord. The neural plate originates as a thickening of the ectoderm that results from cuboidal epithelial cells becoming columnar in shape. With further changes in cell shape and adhesion, the edges of the plate fold and rise, meeting in the midline to form a tube. Cells at the tips of the neural folds come to lie between the neural tube and overlying epidermis, forming the neural crest that gives rise to the

peripheral nervous system and other structures. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:44.) Another product of neurulation is the neural crest, the cells of which derive from the edges of the neural plate and dorsal neural tube. From this position, neural crest cells migrate dorsolaterally under the skin to form melanocytes and ventromedially to form dorsal root sensory ganglia and sympathetic chains of the peripheral nervous system and ganglia of the enteric nervous system. However, neural crest gives rise to diverse tissues including cells of neuroendocrine, cardiac, mesenchymal, and skeletal systems, forming the basis of many congenital syndromes involving brain and other organs. The neural crest origin at the border of neural and epidermal ectoderm and its generation of melanocytes forms the basis of the neurocutaneous disorders, including tuberous sclerosis and neurofibromatosis. Finally, another nonneuronal structure of mesodermal origin formed during neurulation is the notochord found on the ventral side of the neural tube. As seen in subsequent text of this section, the notochord plays a critical role during neural tube differentiation, since it is a signaling source of soluble growth factors, such as sonic hedgehog (Shh), which affect gene patterning and cell determination. Regional Differentiation of the Embryonic Nervous System After closure, the neural tube expands differentially to form major morphological subdivisions that precede the major functional divisions of the brain. These subdivisions are important developmentally, because different regions are generated according to specific schedules of proliferation and subsequent migration and differentiation. The

neural tube can be described in three dimensions, including longitudinal, circumferential, and radial. The longitudinal dimension reflects the rostrocaudal (anterior–posterior) organization, which most simply consists of brain and spinal cord. Organization in the circumferential dimension, tangential to the surface, represents two major axes: In the dorsoventral axis, cell groups are uniquely positioned from top to bottom. On the other hand, in the medial to lateral axis, there is mirror image symmetry, consistent with right–left symmetry of the body. Finally, the radial dimension represents organization from the innermost cell layer adjacent to the ventricles to the outermost surface and exhibits region-specific cell layering. At 4 weeks, the human brain is divided longitudinally into the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain). These three subdivisions or "vesicles" divide further into five divisions by 5 weeks, consisting of the prosencephalon, which forms the telencephalon (including cortex, hippocampus, and basal ganglia) and diencephalon (thalamus and hypothalamus), the mesencephalon, (midbrain), and the rhombencephalon, yielding metencephalon (pons and cerebellum) and myelencephalon (medulla). Morphological transformation into five vesicles depends on region-specific proliferation of precursor cells adjacent to the ventricles, the so-called ventricular zones (VZs). As discussed later, proliferation intimately depends on soluble growth factors made by proliferating cells themselves or released from regional signaling centers. In turn, growth factor production and cognate receptor expression also depend on regionspecific patterning genes. We now know that VZ precursors, which appear morphologically homogeneous, express a checkerboard array of molecular genetic determinants that control the generation of specific types of neurons in each domain (Fig. 1.3-2).

FIGURE 1.3-2 Progression of brain regional differentiation. Early after neurulation, the neural tube differentiates into four regions (forebrain, midbrain, hindbrain, and spinal cord) that give rise following later divisions and maturation to the different brain structures. (From Sadock BJ, Sadock

VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:45.) In the circumferential dimension, organization begins very early and extends over many rostrocaudal subdivisions. In the spinal cord, the majority of tissue comprises the lateral plates, which later divide into dorsal or alar plates, composed of sensory interneurons, and motor or basal plates, consisting of ventral motor neurons. Two other diminutive plates, termed the roof plate and floor plate, are virtually absent in maturity; however, they play critical regulatory roles as growth factor signaling centers in the embryo. Indeed, the floor plate, in response to Shh from the ventrally located notochord, produces its own Shh, which in turn induces neighboring cells in ventral spinal cord and brainstem to express region-specific transcription factors that specify cell phenotype and function. For example, in combination with other factors, floor plate Shh induces midbrain precursors to differentiate into dopamine-secreting neurons of the substantia nigra. Similarly, the roof plate secretes growth factors, such as bone morphogenetic proteins (BMPs), which induce dorsal neuron cell fate in

spinal cord. In the absence of roof plate, dorsal structures fail to form, such as cerebellum, and midline hippocampal structures are missing. Finally, in the radial dimension, the organization of layers is subdivision specific, produced by differential proliferation of VZ precursors and cell migration, as described later. The Ventricular and Subventricular Proliferative Zones The distinct patterns of precursor proliferation and migration in different regions generate the radial organization of the nervous system. In each longitudinal subdivision, the final population size of a brain region depends on the interplay of regulated neurogenesis with programmed cell death. Traditional concepts had suggested that there was excess cell production everywhere and that final cell number regulation was achieved primarily after neurogenesis through selective cell death mediated by targetderived survival (trophic) factors. We now know that the patterning genes discussed later play major roles in directing regional precursor proliferation that is coordinated with final structural requirements, and that programmed cell death occurs at multiple stages. Consequently, in diseases characterized by brain regions smaller than normal, such as schizophrenia, there may be a failure to generate neurons initially, as opposed to normal generation with subsequent cell loss. Radial and Tangential Patterns of Neurogenesis and Migration Of interest to psychiatry, the cerebral cortex is the paradigmatic model of inside-tooutside neurogenesis. A large number of studies now relate specific genetic mutations to distinct cortical malformations that alter neurogenesis, migration, and cellular organization, thereby increasing our knowledge of both normal and pathophysiologic cortical development. Derived from the embryonic forebrain telencephalic vesicles, the characteristic six-cell layers represent a common cytoarchitectural and physiological basis for neocortical function. Within each layer, neurons exhibit related axodendritic morphologies, use common neurotransmitters, and establish similar afferent and efferent connections. In general, pyramidal neurons in layer 3 establish synapses within and between cortical hemispheres, whereas deeper layer 5/6 neurons project primarily to subcortical nuclei, including thalamus, brainstem, and spinal cord. The majority of cortical neurons originate from the forebrain VZ. At the earliest stages, the first postmitotic cells migrate outward from the VZ to establish a superficial layer termed the preplate. Two important cell types comprise the preplate—Cajal–Retzius cells, which form outermost layer 1 or marginal zone, and subplate neurons, which lay beneath future layer 6. These distinct regions form when later-born cortical plate neurons migrate within and divide the preplate in two (Fig. 1.3-3).

FIGURE 1.3-3 Schematic drawing of radial and tangential migration during cerebral cortex development. A. A coronal section of one half of the developing rat forebrain. The dorsal forebrain gives rise to the cerebral cortex. Medial ganglionic eminences (MGEs) and lateral ganglionic eminences (LGEs) of the ventral forebrain generate neurons of the basal ganglia and the cortical interneurons. The arrows indicate the tangential migration route for γ-aminobutyric acid (GABA) interneurons to the cortex. The boxed area (enlarged in B and C) shows the developing cortex at early and late stages. B. In the dorsal forebrain, the first cohort of postmitotic neurons migrate out from the ventricular zone (VZ) and create a preplate (PP) below the pial surface. C. Subsequent postmitotic neurons will migrate along radial glia through the intermediate zone (IZ) and take position in the middle of the preplate, creating a cortical plate (CP) between the outer marginal zone (MZ) and inner subplate (SP). Ultimately, the CP will be composed of six layers that are born sequentially, migrating in an inside-to-outside pattern. Horizontal processes in the IZ represent axon terminals of thalamic afferents. (From Nadarajah B, Parnavelas JG. Modes of neuronal migration in the developing cerebral cortex. Nat Neurosci. 2002;3:423, with permission.) A recent discovery, postulated for years, has changed the view of the origins of cortical neuron populations involved in human brain disease. Neuron tracing experiments in culture and in vivo demonstrate that the neocortex, a dorsal forebrain derivative, is also populated by neurons generated in the ventral forebrain (see Fig. 1.33). Molecular studies of patterning genes, especially Dlx, strongly support this model

(see below). In contrast to excitatory pyramidal neurons, the overwhelming majority of inhibitory γ-aminobutyric acid (GABA)–secreting interneurons originate from mitotic precursors of the ganglionic eminences that generate the neurons of the basal ganglia. Subsets of interneurons also secrete neuropeptides, such as neuropeptide Y (NPY) and somatostatin, and express nitrous oxide (NOS)-generating enzyme. Not associated with cortical VZ radial glia, these GABA interneurons reach the cortical plate by migrating tangentially, in either the superficial marginal zone or a deep position above the VZ, the subplate region where thalamic afferents are also growing. Significantly, in brains from patients with schizophrenia, the prefrontal cortex exhibits a reduced density of interneurons in layer 2. In addition, there is upregulation of GABAA-receptor binding, a potential functional compensation, as well as a relative deficiency of NOS-expressing neurons. These observations have led to the hypothesis that schizophrenia is due to reduced GABAergic activity. The origin of GABA interneurons from the ganglionic eminences and their association with specific patterning genes raises new genetic models of disease causation and possible strategies for disease intervention. Thus, more broadly, normal cortical development depends on a balance of two principal patterns of neurogenesis and migration, consisting of radial migration of excitatory neurons from the dorsal forebrain VZ and tangential migration of inhibitory neurons from the ventral forebrain. In contrast to inside-to-outside neurogenesis observed in cortex, phylogenetically older regions, such as hypothalamus, spinal cord, and hippocampal dentate gyrus, exhibit the reverse order of cell generation. First-formed postmitotic neurons lie superficially, and last-generated cells localize toward the center. Although this outside-to-inside pattern might reflect passive cell displacement, radial glia and specific migration signaling molecules clearly are involved. Furthermore, cells do not always lie in direct extension from their locus of VZ generation. Rather, some groups of cells migrate to specific locations, as observed for neurons of the inferior olivary nuclei. Of prime importance in psychiatry, the hippocampus demonstrates both radial and nonradial patterns of neurogenesis and migration. The pyramidal cell layer, Ammon’s horn Cornu Ammonis (CA) 1 to 3 neurons, is generated in a typical outside-to-inside fashion in the dorsomedial

forebrain for a discrete period, from 7 to 15 weeks of gestation, and exhibits complex migration patterns. In contrast, the other major population, dentate gyrus granule neurons, starts appearing at 18 weeks and exhibits prolonged postnatal neurogenesis, originating from several migrating secondary proliferative zones. In rats, for instance, granule neurogenesis starts at embryonic day 16 (E16) with proliferation in the forebrain VZ. At E18, an aggregate of precursors migrates along a subpial route into the dentate gyrus itself where they generate granule neurons in situ. After birth, there is another migration, localizing proliferative precursors to the dentate hilus, which persists until 1 month of life. Thereafter, granule precursors move to a layer just under the dentate gyrus, termed the subgranular zone (SGZ), which produces neurons throughout life in adult rats, primates, and humans. In rodents, SGZ precursors proliferate in response to cerebral ischemia, tissue injury, and seizures, as well as growth factors. Finally, the diminished hippocampal volume reported in schizophrenia raises the possibility that disordered neurogenesis plays a role in pathogenesis, as either a basis for dysfunction or a consequence of brain injuries, consistent with associations of gestational infections with disease manifestation. Finally, a different combination of radial and nonradial migration is observed in cerebellum, a brain region recently

recognized to play important functions in nonmotor tasks, with particular significance for autism spectrum disorders. Except for granule cells, the other major neurons, including Purkinje and deep nuclei, originate from the primary VZ of the fourth ventricle, coincident with other brainstem neurons. In rats, this occurs at E13 to E15, and in humans, at 5 to 7 weeks of gestation. The granule neurons, as well as basket and stellate interneurons, originate in the secondary proliferative zone, the external germinal cell layer (EGL), which covers newborn cerebellum at birth. EGL precursors originate in the fourth ventricle VZ and migrate dorsally through the brainstem to reach this superficial position. The rat EGL proliferates for 3 weeks, generating more neurons than in any other structure, whereas in humans, EGL precursors exist for at least 7 weeks and up to 2 years. When an EGL precursor stops proliferating, the cell body sinks below the surface and grows bilateral processes that extend transversely in the molecular layer, and then the soma migrates further down into the internal granule layer (IGL). Cells reach the IGL along specialized Bergmann glia, which serve guidance functions similar to those of the radial glia. However, in this case, cells originate from a secondary proliferative zone that generates neurons exclusively of the granule cell lineage, indicating a restricted neural fate. Clinically, this postnatal population in infants makes cerebellar granule neurogenesis vulnerable to infectious insults of early childhood and an undesirable target of several therapeutic drugs, such as steroids, well known to inhibit cell proliferation. In addition, proliferative control of this stem cell population is lost in the common childhood brain tumor, medulloblastoma (see Fig. 1.3-4). FIGURE 1.3-4 Neurogenesis, migration, and differentiation of granule cells during cerebellar development. Granule cell precursors proliferate in the external germinal layer. After exiting the cell cycle, they migrate through the molecular layer and past the Purkinje neurons to reach the internal granule layer where they differentiate and make synapses. Neurons that do not migrate properly or that do not establish proper synaptic connections undergo apoptosis. EGL, external germinal cell layer; Mol, molecular layer; P, Purkinje cell layer; IGL, internal granule cell layer; Wm, white matter. (From Sadock

BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:48.) Developmental Cell Death During nervous system development, cell elimination is apparently required to coordinate the proportions of interacting neural cells. Developmental cell death is a reproducible, spatially and temporally

restricted death of cells that occurs during the organism's development. Three types of developmental cell death have been described: (1) phylogenetic cell death that removes structures in one species that served evolutionarily earlier ones, such as the tail or the vomeronasal nerves; (2) morphogenetic cell death, which sculpts the fingers from the embryonic paddle and is required to form the optic vesicles, as well as the caudal neural tube; and (3) histogenetic cell death, a widespread process that allows the removal of select cells during development of specific brain regions. Numerous studies have focused on histogenetic cell death, the impact of which varies among brain regions but can affect 20 to 80 percent of neurons in some populations. A major role for developmental cell death was proposed in the 1980s based on the paradigm of nerve growth factor, suggesting that following neurogenesis, neurons compete for trophic factors. In this model, survival of differentiating neurons depended absolutely on establishing axonal connections to the correct targets in order to obtain survival-promoting (trophic) growth factors, such as the neurotrophins. Otherwise, they would be eliminated by programmed cell death. This competitive process was thought to ensure proper matching of new neuronal populations with the size of its target field. Although such interactions are involved in controlling cell degeneration, this model is overly simplistic: Developmental cell death also occurs in neural precursors and immature neurons, before any synaptic contacts are established. Apoptosis. Apoptotic cell death, or apoptosis, is the major type of developmental cell degeneration. Apoptosis or "programmed cell death" involves specific molecules that possess enzymatic activities such as cysteine-containing aspartate-specific proteases, also called "caspases," which participate in complex intracellular mechanisms. A large number of signals (both proapoptotic and antiapoptotic) converge to regulate common signaling pathways. Of importance for psychiatry, both developmental as well as pathological cell death involve many of the same signaling cascades. A failure to inhibit apoptosis is involved in cancers and autoimmune diseases (multiple sclerosis), whereas excess stimulation of apoptosis is observed in neurodegenerative diseases during both development (Huntington's disease, lysosomal diseases, and leukodystrophy) and aging (Alzheimer's and Parkinson's diseases). Massive apoptotic cell death is also observed during acquired developmental brain injuries such as hypoxia-ischemia, fetal alcohol syndrome, and exposure to ionizing radiations and neurotoxicants. Thus dysregulation of apoptotic cell death during development can lead to severe brain abnormalities, which may only manifest later as

mature functional impairments. Programmed cell death is a necessary process during neurodevelopment, as genetic deletion of caspases in embryonic mice produces enlarged and disorganized brains with marked regional specificity. Programmed cell death occurs at multiple stages of nervous system development, interacting with neurogenesis and differentiation with precise and complex mechanisms. As many neuropathologies also involve dysregulation of apoptosis, future studies hold promise for elucidation and treatment of neurological diseases. THE CONCEPT OF NEURAL PATTERNING Principles of Function The morphological conversion of the nervous system through the embryonic stages, from neural plate through neural tube to brain vesicles, is controlled by interactions between extracellular factors and intrinsic genetic programs. In many cases, extracellular signals are soluble growth factors secreted from regional signaling centers, such as the notochord, floor, or roof plates, or surrounding mesenchymal tissues. The precursor's ability to respond (competence) depends on cognate receptor expression, which is determined by patterning genes whose proteins regulate gene transcription. The remarkable new observation is that the subdivisions of the embryonic telencephalon that were initially based on mature differences in morphology, connectivity, and neurochemical profiles are also distinguished

embryonically by distinct patterns of gene expression. Classical models had suggested that the cerebral cortex was generated as a fairly homogeneous structure, unlike most epithelia, with individual functional areas specified relatively late, after cortical layer formation, by the ingrowth of afferent axons from thalamus. In marked contrast, recent studies indicate that proliferative VZ precursors themselves display regional molecular determinants, a “protomap,” which the postmitotic neurons carry with them as they migrate along radial glia to the cortical plate. Consequently, innervating thalamic afferents may serve to modulate only intrinsic molecular determinants of the protomap. Indeed, in two different genetic mutants, Gbx2 and Mash1, in which thalamocortical innervation is disrupted, expression of cortical patterning genes proceeds unaltered. On the other hand, thalamic afferent growth may be directed by patterning genes and subsequently play roles in modulating regional expression patterns. Thus experience-dependent processes may contribute less to cortical specialization than originally postulated. The term patterning genes connotes families of proteins that serve primarily to control transcription of other genes, the products of which include other transcription factors or proteins involved in cellular processes, such as proliferation, migration, or differentiation. Characteristically, transcription factor proteins contain two principal domains, one that binds DNA promoter regions of genes and the other that interacts with other proteins, either transcription factors or components of intracellular second messengers. It is notable that transcription factors form multimeric protein complexes to control gene activation. Therefore, a single transcription factor will play diverse roles in

multiple cell types and processes, according to what other factors are present, the socalled cellular environment. The combinatorial nature of gene promoter regulation leads to a diversity of functional outcomes when a single patterning gene is altered. Furthermore, because protein interactions depend on protein–protein affinities, there may be complex changes as a single factor’s expression level is altered. This may be one important mechanism of human variation and disease susceptibility, since polymorphisms in gene promoters, known to be associated with human disease, can alter levels of gene protein products. A transcription factor may associate primarily with one partner at a low concentration but with another at a higher titer. The multimeric nature of regulatory complexes allows a single factor to stimulate one process while simultaneously inhibiting another. During development, a patterning gene may thus promote one event, say generation of neurons, by stimulating one gene promoter, while simultaneously sequestering another factor from a different promoter whose activity is required for an alternative phenotype, such as glial cell fate. Finally, the factors frequently exhibit cross-regulatory functions, where one factor negatively regulates expression of another. This activity leads to the establishment of tissue boundaries, allowing the formation of regional subdivisions, such as basal ganglia and cerebral cortex in the forebrain. In addition to combinatorial interactions, patterning genes exhibit distinct temporal sequences of expression and function, acting in hierarchical fashion. Functional hierarchies were established experimentally by using genetic approaches, either deleting a gene (loss of function) or over-/ectopically expressing it (gain of function), and defining developmental consequences. At the most general level, genetic analyses indicate that regionally restricted patterning genes participate in specifying the identity, and therefore function, of cells in which they are expressed. Subdivisions of the brain, and of cerebral cortex specifically, are identified by regionalized gene expression in the proliferative VZ of the neural tube, leading to subsequent differentiation of distinct types of neurons in each mature (postmitotic) region. Thus the protomap of the embryonic VZ apparently predicts the cortical regions it will generate and may instruct the hierarchical temporal sequence of patterning gene expression. It appears that the different genes

underlie multiple stages of brain development including the following: (1) determining that ectoderm will give rise to nervous system (as opposed to skin); (2) defining the dimensional character of a region, such as positional identity in dorsoventral or rostrocaudal axes; (3) specifying cell class, such as neuron or glia; (4) defining when proliferation ceases and differentiation begins, (5) determining specific cell subtype, such as GABA interneuron, as well as projection pattern; and (6) defining laminar position in the region, such as cerebral cortex. Although investigations are ongoing, studies indicate that these many steps depend on interactions of transcription factors from multiple families. Furthermore, a single transcription factor plays regulatory roles at multiple stages in the developmental life of a cell, yielding complex outcomes, for instance, in genetic loss of function studies and human disease. Recent advances in molecular biology have led to identification of another principle of nervous system organization,

which if sustained by further studies, may provide a molecular basis for brain system diseases, such as Parkinson’s disease and autism. Using molecular techniques to permanently identify cells that had expressed during development of a specific gene, in this case the soluble growth factor, Wnt3a, investigators were able to determine where cells originated embryonically and could trace their path of migration along the neuraxis during development. These genetic-fate mapping studies indicate that cells that expressed Wnt3a migrated widely from the dorsal midline into the dorsal regions of the brain and spinal cord, thereby contributing to diverse adult structures in the diencephalon, midbrain, and brainstem and rostral spinal cord. Of interest, most of these structures were linked into a functional neural network, specifically the auditory system. The observation that a single functional system emerges from a specific group of fated cells would allow for restricted neurological-system–based disorders, such as deficits in dopamine or catecholamine neurons, or for the dysfunction of inter-related brain regions that subserve social cognition and interaction, a core symptom of the autism spectrum disorders. Other adult system degenerations may also be considered. This new observation may change the way that we consider temporal changes in patterning gene expression of specific brain regions during development. Finally, patterning gene expression in nervous system subdivisions is not insensitive to environmental factors. To the contrary, expression is intimately regulated by growth factors released from regional signaling centers. Indeed, although a century of classical experimental embryology described morphologically the induction of new tissues between neighboring cell layers, we have only recently defined molecular identities of soluble protein morphogens and cell response genes underlying development. Signaling molecules from discrete centers establish tissue gradients that provide positional information (dorsal or ventral), impart cell specification, and/or control regional growth. Signals include the BMPs, the Wingless-Int proteins (Wnts), Shh, fibroblast growth factors (FGFs), and epidermal growth factors (EGFs), to name a few. These signals set up developmental domains characterized by expression of specific transcription factors, which in turn control further regional gene transcription and developmental processes. The importance of these mechanisms for cerebral cortical development is only now emerging, altering our concepts of the roles of subsequent thalamic innervation and experience-dependent processes. In light of the temporal and combinatorial principles discussed earlier, brain development can be viewed as a complex and evolving interaction of extrinsic and intrinsic information. SPECIFIC INDUCTIVE SIGNALS AND PATTERNING GENES IN DEVELOPMENT Induction of the central nervous system (CNS) begins at the neural plate stage when the notochord, underlying mesenchyme, and surrounding epidermal ectoderm produce signaling molecules that affect the identity of neighboring cells. Specifically, the ectoderm produces BMPs that prevent neural fate determination by promoting and maintaining

epidermal differentiation. In other words, neural differentiation is a default state that manifests unless it is inhibited. In turn, neural induction proceeds when BMP's epidermis-inducing activity is blocked by inhibitory proteins, such as noggin, follistatin, and chordin, which are secreted by Hensen's node (homologous to the amphibian Spemann organizer), a signaling center at the rostral end of the primitive streak. Once

the neural tube closes, the roof plate and floor plate become new signaling centers, organizing dorsal and ventral neural tube, respectively. The same ligand/receptor system is used sequentially for multiple functions during development. BMPs are a case in point, since they prevent neural development at the neural plate stage, whereas after neurulation the factors are produced by the dorsal neural tube itself to induce sensory neuron fates. The Spinal Cord The spinal cord is a prime example of the interaction of soluble signaling factors with intrinsic patterning gene expression and function. The synthesis, release, and diffusion of inductive signals from signaling sources produce concentration gradients that impose distinct neural fates in the spinal cord (Fig. 1.3-5). The notochord and floor plate secrete Shh, which induces motoneurons and interneurons ventrally, whereas the epidermal ectoderm and roof plate release several BMPs that impart neural crest and sensory relay interneuron fates dorsally. Growth factor inductive signals act to initiate discrete regions of transcription factor gene expression. For instance, high concentrations of Shh induce winged helix transcription factor Hnf3β gene in floor plate cells and Nkx6.1 and Nkx2.2 in ventral neural tube, whereas the expression of more dorsal genes, Pax6, Dbx1/2, Irx3, and Pax7, is repressed. In response to Shh, ventral motoneurons express transcription factor gene Isl1, whose protein product is essential for neuron differentiation. Subsequently, ventral interneurons differentiate, expressing En1 or Lim1/2 independent of Shh signaling. In contrast, the release of BMPs by dorsal cord and roof plate induces a distinct cascade of patterning genes to elicit sensory interneuron differentiation. In aggregate, the coordinated actions of Shh and BMPs induce the dorsoventral dimension of the spinal cord. Similarly, other inductive signals determine rostrocaudal organization of the CNS, such as retinoic acid, an upstream regulator of hox patterning genes, anteriorly, and the FGFs posteriorly. The overlapping and unique expression of the many hox gene family members are important for establishing the segmental pattern in the anterior–posterior axis of the hindbrain and spinal cord, now classic models well described in previous reviews.

FIGURE 1.3-5 Patterning genes in the spinal cord. A. Diagram illustrating the localization of gene expression in the developing "trunk." Rhombomere boundaries are specified by specific combinations of transcription factors. (Modified from Darnell, 2005.) B. Morphogen induction of spinal cord cell fate. Dorsoventral gradients of sonic hedgehog (Shh) and bone morphogenetic protein (BMP) induce expression of several position identity genes. Combinatorial effects of these factors establish progenitor domains and result in the expression of specific downstream molecular markers. D, dorsal neurons; V, ventral neurons. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:51.) Recent advances in spinal cord transcription factor expression and function support the principle that these factors play roles at multiple stages of a cell's development, likely due to their participation in diverse protein regulatory complexes: The transcription factors Pax6, Olig2, and Nkx2.2, which define the positional identity of multipotent progenitors early in development, also play crucial roles in controlling the timing of neurogenesis and gliogenesis in the developing ventral spinal cord. The Cerebral Cortex Recent evidence suggests that forebrain development also depends on inductive signals and patterning genes as observed in more caudal

neural structures. In the embryo, the dorsal forebrain structures include the hippocampus medially, the cerebral cortex

dorsolaterally, and the entorhinal cortex ventrolaterally, whereas in basal forebrain, the globus pallidus lies medially and the striatum laterally. On the basis of gene expression and morphological criteria, it has been hypothesized that the forebrain is divided into a checkerboard-like grid pattern of domains generated by the intersection of longitudinal columns and transverse segments, perpendicular to the longitudinal axis. The columns and segments (prosomeres) exhibit restricted expression of patterning genes, allowing for unique combinations of factors within each embryonic subdivision. Many of these genes, including Hnf3β, Emx2, Pax6, and Dlx2, are first expressed even before neurulation in the neural plate and are then maintained, providing the “protomap” determinants of the VZ described earlier. As in spinal cord, initial forebrain gene expression is influenced by a similar array of signaling center soluble factors—Shh, BMP, and retinoic acid. As the telencephalic vesicles form, signaling centers localize to the edges of the cortex. In the dorsal midline there is the anterior neural ridge, an anterior cranial mesenchyme secreting FGF8, the roof plate, and, at the junction of the roof plate with the telencephalic vesicle, the cortical hem (Fig. 1.3-6). Other factors originate laterally from the dorsal–ventral forebrain junction, as well as from basal forebrain structures themselves. FIGURE 1.3-6 Patterning genes and signaling centers in the developing cerebral cortex. This schematic diagram shows a lateral–superior view of the two cerebral hemispheres of the embryonic mouse, sitting above the midbrain and hindbrain (broken lines). The anterior–lateral extent of Pax6 gene expression is indicated by circles. The posterior–medial domain of Emx2 expression is indicated by stripes. The genes exhibit continuous gradients of

expression that decrease as they extend to opposite poles. The signaling factor fibroblast growth factor 8 (FGF8) is produced by and released from mesenchymal tissue in the anterior neural ridge, which regulates Pax6 and Emx2 expression. In the midline, bone morphogenetic proteins (BMPs) and Wingless-Int proteins (Wnts) are secreted from other signaling centers, including the roof plate and the cortical hems. (Courtesy of E. DiCicco-Bloom and K. Forgash.) Do molecular studies identify how different cortical regions interact with thalamic neurons to establish specific functional modalities, such as vision and sensation? And once regional identity is established, can it be modified by later developmental events? It has been proposed that initially there are no functional distinctions in the cortex but that they are induced by the ingrowth of extrinsic thalamic axons, which convey positional and functional specifications, the so-called “protocortex model.” However, in contrast, the abundant molecular evidence provided earlier suggests that intrinsic differences are established early in the neuroepithelium by molecular determinants that regulate areal specification, including the targeting of thalamic axons, termed the “protomap” model. The foregoing mutants now provide experimental tests of these two alternative models and indicate that neither model is completely correct. Although there is early molecular regionalization of the cortex, the initial targeting of thalamic axons to the cortex is independent of these molecular differences. In the rodent, thalamic afferents first target to their usual cortical regions prenatally in the late embryo. However, once thalamic afferents reach the cortex, which occurs several days after birth, interactions of thalamic axon branches with local regional cues leads to modifications of initial outgrowth and the establishment of connections that conform to areal molecular identities. Furthermore, the developing cortex exhibits a remarkable and unexpected level of flexibility in mediating modality-specific functions: In the ferret, surgical elimination of visual pathway (lateral

geniculate nucleus) in postnatal pups results in the transfer of visual signaling to the auditory cortex, which successfully mediates vision! Thus the animal's visual information is effectively processed by their auditory cortex. The Hippocampus The hippocampus is a region of major importance in schizophrenia, depression, autism, and other disorders, and defining mechanisms regulating hippocampal formation may provide clues to the developmental bases of these disorders. In mice, the hippocampus is located in the medial wall of the telencephalic vesicle. Where it joins the roof plate, the future roof of the third ventricle, there is a newly defined signaling center, the cortical hem, which secretes BMPs, Wnts, and FGFs (see Fig. 1.3-6). Genetic experiments have defined patterning genes localized to the cortical hem and hippocampal primordia, whose deletions result in a variety of morphogenetic defects. In mice lacking Wnt3a, which is expressed in the cortical hem, the hippocampus is either completely missing or greatly reduced, whereas neighboring cerebral cortex is mainly preserved. A similar phenotype is produced by deleting an intracellular factor downstream to Wnt receptor activation, the Lef1 gene, suggesting that the Wnt3a–Lef1 pathway is required for hippocampal cell specification and/or proliferation, issues remaining to be defined. When another cortical hem gene, Lhx5, is deleted, mice lack both the hem and neighboring choroid plexus, both sources of growth factors. However, in this case, the cortical hem cells may in fact proliferate in excess, and the hippocampal primordia may be present but disorganized, exhibiting abnormalities in cell proliferation, migration,

and differentiation. A related abnormality is observed with Lhx2 mutation. Finally, a sequence of bHLH transcription factors plays roles in hippocampal neurogenesis: Dentate gyrus differentiation is defective in NeuroD and Mash1 mutants. Significantly, expression of all these hippocampal patterning genes is regulated by factors secreted by anterior neural ridge, roof plate, and the cortical hem, including FGF8, Shh, BMPs, and Wnts. Moreover, the basal forebrain region secretes an EGF-related protein, transforming growth factor α (TGF-α), which can stimulate expression of the classical limbic marker protein, lysosomal-associated membrane protein (LAMP). These various signals and genes now serve as candidates for disruption in human diseases of the hippocampus. The Basal Ganglia In addition to motor and cognitive functions, the basal ganglia take on new importance in neocortical function, since they appear to be the embryonic origin of virtually all adult GABA interneurons, reaching the neocortex through tangential migration. Gene expression studies have identified several transcription factors that appear in precursors originating in the ventral forebrain ganglionic eminences, allowing interneurons to be followed as they migrate dorsally into the cortical layers. Conversely, genetic deletion mutants exhibit diminished or absent interneurons, yielding results consistent with other tracing techniques. These transcription factors, including Pax6, Gsh2, and Nkx2.1, establish boundaries between different precursor zones in the ventral forebrain VZ, through mechanisms involving mutual repression. As a simplified model, the medial ganglionic eminence (MGE) expresses primarily Nkx2.1 and gives rise to most GABA interneurons of the cortex and hippocampus, whereas the lateral ganglionic eminence (LGE) expresses Gsh2 and generates GABA interneurons of the SVZ and olfactory bulb. The boundary between ventral and dorsal forebrain then depends on LGE interaction with the dorsal neocortex, which expresses Pax6. When Nkx2.1 is deleted, LGE transcription factor expression spreads ventrally into the MGE territory, and there is a 50 percent reduction in neocortical and striatal GABA interneurons. In contrast, deletion of Gsh2 leads to ventral expansion of the dorsal cortical molecular markers and concomitant decreases in olfactory interneurons. Finally, Pax6 mutation causes both MGE and LGE to spread laterally and into dorsal cortical areas, yielding increased interneuron migration. The final phenotypic changes are complex, as these factors exhibit unique

and overlapping expression and interact to control cell fate. Neuronal Specification As indicated for basal ganglia, throughout the nervous system transcription factors participate in decisions at multiple levels, including determining the generic neural cell, such as neuron or glial cell, as well as neuron subtypes. Mash1 can promote a neuronal fate over a glial fate as well as induce the GABA interneuron phenotype. However, another bHLH factor, Olig1/2, can promote oligodendrocyte development, whereas it promotes motor neuron differentiation elsewhere, indicating that the variety of factors

expressed in a specific cell leads to combinatorial effects and thus diverse outcomes for cell differentiation. The bHLH inhibitory factor, Id, is expressed at the transition from somatosensory to motor cortex, implying roles of family members in areal characteristics. In the hippocampus, granule neuron fate is dependent on NeuroD and Math1, with deficient cell numbers when either one is deleted. The role of specific factors in cortical cell layer determination remains an area of active investigation but likely includes Tbr1, Otx1, and Pax6. A NEW MECHANISM FOR REGULATING GENE EXPRESSION: MIRNAS Over the last decade a new mechanism for regulating messenger ribonucleic acid (mRNA) has been explored in simple to complex organisms that involves microRNAs (miRNAs). We now know that miRNAs contribute not only to normal development and brain function but also to brain disorders, such as Parkinson’s and Alzheimer’s disease, tauopathies, and brain cancer. miRNAs can affect the regulation of RNA transcription, alternative splicing, molecular modifications, or RNA translation. miRNAs are 21- to 23nucleotide-long single-strand RNA molecules. Unlike mRNAs that encode the instructions for ribosome complex translation into proteins, miRNAs are noncoding RNAs that are not translated but are instead processed to form loop structures. miRNAs exhibit a sequence that is partially complementary to one or several other cellular mRNAs. By binding to target mRNA transcripts, the miRNAs serve to interfere with their function, thereby downregulating expression of these gene products. This gene silencing involves a complex mechanism: The larger miRNA primary transcript is first processed by the Microprocessor, an enzymatic complex consisting of the nuclease Drosha and the doublestranded RNA binding protein Pasha. The mature miRNA binds to its complementary RNA and then interacts with the endonuclease Dicer that is part of the RNA-induced silencing complex (RISC), resulting in the cleavage of the target mRNA and gene silencing (Fig. 1.3-7).

FIGURE 1.3-7 Processing and function of micro RNA (miRNA). After transcription, the primary miRNA forms a hairpin conformation. This structure allows the enzyme Drosha to cleave the transcript, producing a pre-miRNA that then exits the nucleus through nuclear pores. In the cytoplasm, Dicer cleaves the pre-miRNA stem loop, resulting in the formation of two complementary short RNA molecules. Only one of these molecules is integrated in the RNA-induced silencing complex (RISC) and serves as a guide strand that allows recognition and specificity for target RNA due to its sequence complementarity. After integration into the RISC complex, the miRNA matches with the complementary mRNA strand and induces mRNA duplex degradation by the argonaute protein, the catalytic enzyme of the RISC complex. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:55.) Currently, 475 miRNAs have been identified in humans, and their total number is estimated to be between 600 and 3,441. Potentially, up to 30 percent of all genes might be regulated by miRNAs, a whole new layer of molecular complexity. A connection between miRNAs and several brain diseases has already been made. For example, miR-133b, which is specifically expressed in midbrain dopaminergic neurons, is deficient in midbrain tissue from

patients with Parkinson’s disease. Furthermore, the miRNAs encoding miR-9, miR-124a, miR-125b, miR-128, miR-132, and miR-219 are abundantly represented in fetal hippocampus, are differentially regulated in the aged brain, and are altered in Alzheimer’s

disease hippocampus. Similar RNA species termed short-interfering RNAs (siRNAs) have been discovered in plants where they prevent the transcription of viral RNA. The mechanisms involved in these effects are closely related to those of miRNA. Thus siRNAs are now being used in both basic and clinical research to downregulate specific cellular gene products, thereby advancing the study of pathways involved in neurodevelopment and providing new selective tools to regulate disease-causing genes or therapeutic molecular targets. REGULATION OF NEURODEVELOPMENT BY EXTRACELLULAR FACTORS The interaction of extracellular factors with intrinsic genetic determinants controlling region-specific neurogenesis includes signals that regulate cell proliferation, migration, differentiation, and survival (Table 1.3-1). Patterning genes control the expression of growth factor receptors and the molecular machinery of the cell division cycle. Extracellular factors are known to stimulate or inhibit proliferation of VZ precursors and originate from the cells themselves, termed autocrine, neighboring cells/tissues, or paracrine, or from the general circulation, as in endocrine, all sources known to affect proliferation in prenatal and postnatal developing brain. Although defined initially in cell culture, a number of mitogenic growth factors are now well-characterized in vivo, including those stimulating proliferation, such as basic FGF (bFGF), EGF, IGF-I, Shh, and signals inhibiting cell division, such as pituitary adenylate-cyclase-activating polypeptide (PACAP), GABA and glutamate, and members of the TGF-β superfamily. However, in addition to stimulating re-entry of cells into the cell cycle, termed a mitogenic effect, extracellular signals also enhance proliferation by promoting survival of the mitotic population, a trophic action. Stimulation of both pathways is necessary to produce maximal cell numbers. These mitogenic and trophic mechanisms during development parallel those identified in carcinogenesis, reflecting roles of c-myc and bcl-2, respectively. Several of the neurotrophins, especially BDNF and neurotrophin-3 (NT3), promote survival of mitotic precursors as well as the newly generated progeny. Table 1.3-1 Regulation of Neurodevelopment by Extracellular Factors

The developmental significance of extracellular mitogens is demonstrated by the expression of the factors and their receptors in regions of neurogenesis, and by the profound and permanent consequences of altering their activities during development. For example, by administering growth factors to developing embryos or pups, one can induce changes in proliferation in prenatal cortical VZ, postnatal cerebellar EGL, and hippocampal dentate gyrus that produce lifelong modifications in brain region population size and cell composition. Such changes may be relevant to structural differences observed in neuropsychiatric disorders, such as depression, schizophrenia, and autism. Specifically, in the cerebral cortex VZ of the embryonic rat, proliferation is controlled by promitogenic bFGF and antimitogenic PACAP, which are expressed as autocrine/paracrine signals. Positive and negative effects were shown in living embryos in utero by performing intracerebroventricular (ICV) injections of the factors or antagonists. ICV injection of bFGF produced a larger adult cortex composed of 87 percent more neurons, which employed glutamate, thus increasing the ratio of excitatory pyramidal neurons to GABA inhibitory neurons, which were unchanged. Conversely, embryonic PACAP injection inhibited proliferation of cortical precursors by 26 percent, reducing the number of labeled layer 5/6 neurons in the cortical plate 5 days later. A similar reduction was accomplished by genetically deleting promitogenic bFGF or leukocyte inhibitory factor (LIF)/ciliary neurotrophic factor (CNTF)/gp130 signaling, diminishing cortical size.

Furthermore, effects of mitogenic signals depended critically on the stage-specific program of regional development, since bFGF injection at later ages when gliogenesis predominates affected glial numbers selectively. Thus developmental dysregulation of mitogenic pathways due to genetic or environmental factors (hypoxia, maternal/fetal infection, or drug or toxicant exposure) will likely produce subtle changes in the size and composition of the developing cortex. Other signals likely to play proliferative roles include Wnt's, TGF-α, IGF-I, and BMPs. Although interactions between intrinsic cortical programs and extrinsic factors remain to be defined, a remarkable new study of mouse embryonic stem cells suggests that embryonic mammalian forebrain specification may be a developmentally ancestral intrinsic program that emerges in the absence of extrinsic signals. In

specific culture conditions that block endogenous Shh signaling, mouse embryonic stem cells can sequentially generate the various types of neurons that display most salient features of genuine cortical pyramidal neurons. When grafted into the cerebral cortex, these cells differentiate into neurons that project to select cortical (visual and limbic regions) and subcortical targets, corresponding to a wide range of pyramidal layer neurons. Insight into precision control of neuronal differentiation will open new avenues to perform neuronal grafts in humans for cellular replacement in various acquired and neurodegenerative diseases. Similar to cerebral cortex, later generated populations of granule neurons, such as in cerebellum and hippocampal dentate gyrus, are also sensitive to growth factor manipulation, which is especially relevant to therapies administered intravenously to premature and newborn infants in the neonatal nursery. Like in humans, cerebellar granule neurons are produced postnatally in rats, but for only 3 weeks, whereas in both species dentate gyrus neurons are produced throughout life. Remarkably, a single peripheral injection of bFGF into newborn rat pups rapidly crossed into the cerebrospinal fluid (CSF) and stimulated proliferation in the cerebellar EGL by 30 percent as well as hippocampal dentate gyrus by twofold by 8 hours, consistent with an endocrine mechanism of action. The consequence of mitogenic stimulation in cerebellum was a 33 percent increase in the number of internal granule layer neurons and a 22 percent larger cerebellum. In hippocampus, mitotic stimulation elicited by a single bFGF injection increased the absolute number of dentate gyrus granule neurons by 33 percent at 3 weeks, defined stereologically, producing a 25 percent larger hippocampus containing more neurons and astrocytes, a change that persisted lifelong. Conversely, genetic deletion of bFGF resulted in smaller cerebellum and hippocampus at birth and throughout life, indicating that levels of the growth factor were critical for normal brain region formation. Other proliferative signals regulating cerebellar granule neurogenesis include Shh and PACAP, the disruption of which contributes to human medulloblastoma, whereas in hippocampus the Wnt family may be involved. Clinical Implications There are several clinical implications of these surprising growth factor effects observed in newborns. First, we may need to investigate possible neurogenetic effects of therapeutic agents we administer in the newborn nursery for long-term consequences. Second, because bFGF is as effective in stimulating adult neurogenesis (see subsequent text) as in newborns because of specific transport across the mature blood–brain barrier (BBB), there is the possibility that other protein growth factors are also preferentially transported into the brain and alter ongoing neurogenesis. Indeed, in rats, IGF-I also stimulates mature hippocampal dentate gyrus neurogenesis. Third, other therapeutics cross the BBB efficiently due to their lipid solubility, such as steroids, which inhibit neurogenesis across the age spectrum. Steroids are frequently used perinatally to promote lung maturation and treat infections and trauma, but effects on human brain formation have not been examined. Fourth, it is well known that neurological development may be delayed in children who experience serious systemic illness that is

associated with numerous inflammatory cytokines, and one may wonder to what degree this reflects interference with neurogenesis and concomitant processes, potentially producing long-term differences in cognitive and motor functional development. Finally, maternal infection during pregnancy is a known risk factor for schizophrenia, and cytokines that cross the placental barrier may directly affect fetal brain cell proliferation and differentiation as well as cell migration, target selection, and synapse maturation, as shown in animal models, eventually leading to multiple brain and behavioral abnormalities in the adult offspring. CELL MIGRATION Throughout the nervous system, newly generated neurons normally migrate away from proliferative zones to achieve final destinations. If this process is disrupted, abnormal cell localization and function result. In humans, more than 25 syndromes with disturbed neuronal migration have been described. As noted earlier, neurons migrate in both radial and tangential fashions during development and may establish cell layers that are inside-to-outside, or the reverse, according to region. In developing cerebral cortex, the most well-characterized mechanism is radial migration from underlying VZ to appropriate cortical layers in an inside-to-outside fashion. In addition, however, the inhibitory GABA interneurons that are generated in ventrally located medial ganglionic eminences reach the cortex through tangential migration in the intermediate zone along axonal processes or other neurons. The neurons in developing cerebellum also exhibit both radial and tangential migration. Purkinje cells leave the fourth ventricle VZ and exhibit radial migration, whereas other precursors from the rhombic lip migrate tangentially to cover the cerebellar surface, establishing the EGL, a secondary proliferative zone. From EGL, newly generated granule cells migrate radially inward to create the internal granule cell layer. Finally, granule interneurons of the olfactory bulb exhibit a different kind of migration, originating in the SVZ of the lateral ventricles overlying the striatum. These neuroblasts divide and migrate simultaneously in the rostral migratory stream in transit to the bulb, on a path comprising chains of cells that support forward movements. The most commonly recognized disorders of human neuronal migration are the extensive lissencephalies (see subsequent text), although incomplete migration of more restricted neuron aggregates (heterotopias) frequently underlies focal seizure disorders. Animal models have defined molecular pathways involved in neuronal migration. Cell movement requires signals to start and stop migration, adhesion molecules to guide migration, and functional cytoskeleton to mediate cell translocation. The best-characterized mouse model of aberrant neuronal migration is reeler, a spontaneous mutant in which cortical neuron laminar position is inverted, being generated in outside-to-inside fashion. Reelin is a large, secreted extracellular glycoprotein produced embryonically by the earliest neurons in the cortical preplate, Cajal–Retzius cells, and hippocampus and cerebellum. Molecular and genetic analysis has established a signaling sequence in reelin activity that includes at least two receptors, the very low-density lipoprotein receptor (VLDLR) and the apoprotein E receptor 2 (ApoER2), and the intracellular adapter protein, disabled 1 (Dab1), initially identified in the scrambler mutant mouse, a

reelin phenocopy. Current thoughts consider the reelin system as one mediator of radial glia-guided neuron migration, although its specific functions in starting or stopping migration remain controversial. The roles of the VLDL and ApoE2 receptors are intriguing for their possible contributions to Alzheimer’s disease risk. Recent studies have found human reelin gene (RELN) mutations associated with autosomal recessive lissencephaly with cerebellar hypoplasia, exhibiting a markedly thickened cortex with pachygyria, abnormal hippocampal formations, and severe cerebellar hypoplasia with absent folia. Additional studies suggest that reelin polymorphisms may contribute to autism spectrum disorder (ASD) risk as well. With regard to cytoskeletal proteins,

studies of the filamentous fungus Aspergillus nidulans surprisingly provided insight into the molecular machinery underlying the human migration disorder, Miller-Dieker syndrome, a lissencephaly associated with abnormal chromosome 17q13.3. Lissencephaly is a diverse disorder characterized by a smooth cortical surface lacking in gyri and sulci, with markedly reduced brain surface area. The absence of convolutions results from a migration defect: the majority of neurons fail to reach their final destinations. In classical lissencephaly (type I), cerebral cortex is thick and usually four-layered, whereas in cobblestone lissencephaly (type II), the cortex is chaotically organized with a partly smooth and partly pebbled surface and deficient lamination. The most severely affected parts of the brain are the cerebral cortex and hippocampus, with the cerebellum less affected. In fungus, the gene NudF was found to be essential for intracellular nuclear distribution, a translocation process also involved in mammalian cell migration. The human homologue of NudF is LIS-1 or PAFAH1B1, a mutation of which accounts for up to 60 percent of lissencephaly cases of type I pathology. The LIS-1 gene product interacts with microtubules and related motor components dynein and dynactin as well as doublecortin (DCX), which may regulate microtubule stability. Mutations in DCX gene result in X-linked lissencephaly in males and bands of heterotopic neurons in white matter in females, appearing as a “double cortex” on imaging studies, producing severe mental retardation and epilepsy. Other migratory defects occur when proteins associated with the actin cytoskeleton are affected, such as mutation in filamin 1 gene responsible for periventricular nodular heterotopias in humans and mutations of a regulatory phosphokinase enzyme, the CDK5/p35 complex. Cell migration also depends on molecules mediating cellular interactions, which provide cell adhesion to establish neuron–neuron and neuron–glia relationships or induce attraction or repulsion. Astrotactin is a major glial protein involved in neuronal migration on radial glial processes, whereas neuregulins and their receptors, ErbB2-4, play roles in neuronal–glial migratory interactions. Recent genetic studies associate neuregulin polymorphisms with schizophrenia, suggesting that this developmental disease may depend on altered oligodendrocyte numbers and activities and synaptic functions. Furthermore, some work suggests that early appearing neurotransmitters themselves, GABA and glutamate, and platelet-derived growth factor (PDGF) regulate migration speed. In contrast to radial migration from cortical VZ, GABA interneurons generated in ganglionic eminences employ different mechanisms to leave the ventral forebrain and migrate dorsally into the cerebral cortex. Several signaling systems have been identified, including the Slit protein and Robo receptor, the semaphorins and their neuropilin receptors, and hepatocyte growth factor and its c-Met receptor, all of which appear to repel GABA interneurons from basal forebrain, promoting tangential migration into cortex. Significantly, the c-Met receptor has recently been associated with autism spectrum disorders, suggesting that altered GABA interneuron migration into cortex and deficits in inhibitory signaling may contribute to the phenotype, including seizures and abnormal cognitive processing. Finally, several human forms of congenital

muscular dystrophy with severe brain and eye migration defects result from gene mutations in enzymes that transfer mannose sugars to serine/threonine –OH groups in glycoproteins, thereby interrupting interactions with several extracellular matrix molecules and producing type II cobblestone lissencephalies. DIFFERENTIATION AND NEURONAL PROCESS OUTGROWTH After newly produced neurons and glial cells reach their final destinations, they differentiate into mature cells. For neurons, this involves outgrowth of dendrites and extension of axonal processes, formation of synapses, and production of neurotransmitter systems, including receptors and selective reuptake sites. Most axons will become insulated by myelin sheaths produced by oligodendroglial cells. Many

of these events occur with a peak period from 5 months of gestation onward. During the first several years of life, many neuronal systems exhibit exuberant process growth and branching, which is later decreased by selective “pruning” of axons and synapses, dependent on experience, whereas myelination continues for several years after birth and into adulthood. Although there is tremendous synapse plasticity in adult brain, a fundamental feature of the nervous system is the point-to-point or topographic mapping of one neuron population to another. During development, neurons extend axons to innervate diverse distant targets, such as cortex and spinal cord. The structure that recognizes and responds to cues in the environment is the growth cone, located at the axon tip. The axonal process is structurally supported by microtubules that are regulated by numerous microtubule-associated proteins (MAPs), whereas the terminal growth cone exhibits a transition to actin-containing microfilaments. The growth cone has rod-like extensions called filopodia that bear receptors for specific guidance cues present on cell surfaces and in extracellular matrix. Interactions between filopodial receptors and environmental cues cause growth cones to move forward, turn, or retract. Recent studies have identified the actin-modulating proteins and kinases involved in rapid growth cone movements, such as LIMK kinase that causes the language phenotype associated with Williams’ syndrome. Perhaps surprising is that activation of growth cone receptors leads to local mRNA translation to produce synaptic proteins, whereas traditional concepts assumed that all proteins were transported to axon terminals from distant neuronal cell somas. The region-specific expression of extracellular guidance molecules, such as cadherins, regulated by patterning genes Pax6 and Emx2, results in highly directed outgrowth of axons, termed axonal pathfinding. These molecules affect the direction, speed, and fasciculation of axons, acting through either positive or negative regulation. Guidance molecules may be soluble extracellular factors or, alternatively, may be bound to extracellular matrix or cell membranes. In the latter class of signal is the newly discovered family of transmembrane proteins, the ephrins. Playing major roles in topographic mapping between neuron populations and their targets, ephrins act via the largest known family of tyrosine kinase receptors in brain, Eph receptors. Ephrins frequently serve as chemorepellent cues, negatively regulating growth by preventing

developing axons from entering incorrect target fields. For example, the optic tectum expresses ephrins A2 and A5 in a gradient that decreases along the posterior to anterior axis, whereas innervating retinal ganglion cells express a gradient of Eph receptors. Ganglion cell axons from posterior retina, which possess high Eph A3 receptor levels, will preferentially innervate the anterior tectum because the low level ephrin expression does not activate the Eph kinase that causes growth cone retraction. In the category of soluble molecules, netrins serve primarily as chemoattractant proteins secreted, for instance, by the spinal cord floor plate to stimulate spinothalamic sensory interneurons to grow into the anterior commissure, whereas Slit is a secreted chemorepulsive factor that through its roundabout (Robo) receptor regulates midline crossing and axonal fasciculation and pathfinding. THE NEURODEVELOPMENTAL BASIS OF PSYCHIATRIC DISEASE An increasing number of neuropsychiatric conditions are considered to originate during brain development, including schizophrenia, depression, autism, and attentiondeficit/hyperactivity disorder. Defining when a condition begins helps direct attention to underlying pathogenic mechanisms. The term neurodevelopmental suggests that the brain is abnormally formed from the very beginning due to disruption of fundamental processes, in contrast to a normally formed brain that is injured secondarily or that undergoes degenerative changes. However, the value of the term neurodevelopmental needs to be reconsidered, because of different use by clinicians and pathologists. In addition, given that the same molecular signals function in

both development and maturity, altering an early ontogenetic process by changes in growth factor signaling, for instance, probably means that other adult functions exhibit ongoing dysregulation as well. For example, clinical researchers of schizophrenia consider the disorder neurodevelopmental because at the time of onset and diagnosis, the prefrontal cortex and hippocampus are smaller and ventricles enlarged already at adolescent presentation. In contrast, the neuropathologist uses the term neurodevelopmental for certain morphological changes in neurons. If a brain region exhibits a normal cytoarchitecture but with neurons of smaller than normal diameter, reminiscent of "immature" stages, then this may be considered an arrest of development. However, if the same cellular changes are accompanied by inflammatory signs, such as gliosis and white blood cell infiltrate, then this is termed neurodegeneration. These morphological and cellular changes may no longer be adequate to distinguish disorders that originate from development versus adulthood, especially given the roles of glial cells, including astrocytes, oligodendrocytes, and microglia, as sources of neurotrophic support during both periods of life. Thus abnormalities in glial cells may occur in both epochs to promote disease or act as mechanisms of repair. Many neurodegenerative processes such as in Alzheimer's and Parkinson's diseases are associated with microglial cells. On the other hand, neuronal dysfunction in adulthood such as cell shrinkage may occur without inflammatory changes. In animal models, interruption of BDNF neurotrophic signaling in adult brain results in neuron and dendrite atrophy in cerebral cortex without eliciting

glial cell proliferation. Thus finding small neurons without gliosis in the brains of patients with schizophrenia or autism does not necessarily mean that the condition is only or primarily developmental in origin. In turn, several etiological assumptions about clinical brain conditions may require reexamination. Because the same processes that mediate development, including neurogenesis, gliogenesis, axonal growth and retraction, synaptogenesis, and cell death, also function during adulthood, a new synthesis has been proposed. All of these processes, although perhaps in more subtle forms, contribute to adaptive and pathological processes. Successful aging of the nervous system may require precise regulation of these processes, allowing the brain to adapt properly and counteract the numerous intrinsic and extrinsic events that could potentially lead to neuropathology. For example, adult neurogenesis and synaptic plasticity are necessary to maintain neuronal circuitry and ensure proper cognitive functions. Programmed cell death is crucial to prevent tumorigenesis that can occur as cells accumulate mutations throughout life. Thus dysregulation of these ontogenetic processes in adulthood will lead to disruption of brain homeostasis, expressing itself as various neuropsychiatric diseases. Schizophrenia The neurodevelopmental hypothesis of schizophrenia postulates that etiologic and pathogenetic factors occurring before the formal onset of the illness, that is, during gestation, disrupt the course of normal development. These subtle early alterations in specific neurons, glia, and circuits confer vulnerability to other later developmental factors, ultimately leading to malfunctions. Schizophrenia is clearly a multifactorial disorder, including both genetic and environmental factors. Clinical studies using risk assessment have identified some relevant factors, including prenatal and birth complications (hypoxia, infection, or substance and toxicant exposure), family history, body dysmorphia, especially structures of neural crest origin, and presence of mild premorbid deficits in social, motor, and cognitive functions. These risk factors may affect ongoing developmental processes such as experience-dependent axonal and dendritic production, programmed cell death, myelination, and synaptic pruning. An intriguing animal model using human influenza–induced pneumonia of pregnant mice shows that the inflammatory cytokine response produced by the mother may directly affect the offspring's brain development, with no evidence of the virus in the

fetus or placenta. Neuroimaging and pathology studies identify structural abnormalities at disease presentation, including smaller prefrontal cortex and hippocampus and enlarged ventricles, suggesting abnormal development. More severely affected patients exhibit a greater number of affected regions with larger changes. In some cases, ventricular enlargement and cortical gray matter atrophy increase with time. These ongoing progressive changes should lead us to reconsider the potential role of active degeneration in schizophrenia, whether due to the disease or its consequences, such as stress or drug treatment. However, classic signs of neurodegeneration with inflammatory cells are not present. Structural neuroimaging strongly supports the conclusion that the hippocampus in schizophrenia is significantly

smaller, perhaps by 5 percent. In turn, brain morphology has been used to assess etiological contributions of genetic and environmental factors. Comparisons of concordance for schizophrenia in monozygotic and dizygotic twins support roles for both factors. Among monozygotic twins, only 40 to 50 percent of both twins have the illness, indicating that genetic constitution alone does not ensure disease and suggesting that the embryonic environment also contributes. Neuroimaging, pharmacological, and pathological studies suggest that some genetic factors allow for susceptibility and that secondary insults, such as birth trauma or perinatal viral infection, provide the other. This model is consistent with imaging studies showing small hippocampus in both affected and unaffected monozygotic twins. Moreover, healthy, genetically at risk individuals show hippocampal volumes (smaller) more similar to affected probands than normal controls. Thus hippocampal volume reduction is not pathognomonic of schizophrenia but rather may represent a biological marker of genetic susceptibility. It is not difficult to envision roles for altered developmental regulators in producing a smaller hippocampus, which in turn limits functional capacity. A smaller hippocampus may result from subtle differences in the levels of transcription factors, such as NeuroD, Math1, or Lhx, signaling by Wnt3a and downstream mediator Lef1, or proliferative control mediated by bFGF, the family members of which exhibit altered expression levels in schizophrenia brain samples. Such genetic limitations may only become manifest following another developmental challenge, such as gestational infection, stressors, or toxicant exposure. A regional locus of schizophrenia pathology remains uncertain but may include hippocampus, entorhinal cortex, multimodal association cortex, limbic system, amygdala, cingulate cortex, thalamus, and medial temporal lobe. Despite size reductions in specific regions, attempts to define changes in cell numbers have been unrewarding, since most studies do not quantify the entire cell population but assess only regional cell density. Without assessing a region’s total volume, cell density measures alone are limited in revealing population size. Most studies have found no changes in cell density in diverse regions. A single study successfully examining total cell number in hippocampus found normal neuron density and a 5 percent volume reduction on the left and 2 percent on the right, yielding no significant change in total cell number. In contrast to total neuron numbers, using neuronal cell-type–specific markers, many studies have found a decreased density of nonpyramidal GABA interneurons in cortex and hippocampus. In particular, parvalbumin-expressing interneurons are reduced, whereas calretinin-containing cells are normal, suggesting a deficiency of an interneuron subtype. These morphometric data are supported by molecular evidence for decreased GABA neurons, including reduced mRNA and protein levels of the GABA-synthesizing enzyme, GAD67, in cortex and hippocampus. Another product of the adult GABA-secreting neurons, reelin, which initially appears in Cajal–Retzius cells in embryonic brain, is reduced 30 to 50 percent in schizophrenia and bipolar disorder with psychotic symptoms. Such a deficiency, leading to diminished GABA signaling, may underlie a potential compensatory increase in GABAA receptor

binding detected in hippocampal CA 2 to 4 fields by both pyramidal and nonpyramidal neurons, apparently selective since benzodiazepine binding is unchanged. More generally, deficiency in a subpopulation of GABA interneurons raises intriguing new possibilities for schizophrenia etiology. As indicated in the preceding gene patterning section, different subpopulations of forebrain GABA interneurons originate from distinct precursors located in the embryonic basal forebrain. Thus cortical and hippocampal GABA interneurons may derive primarily from the MGE under control of the patterning gene Nkx2.1, whereas SVZ and olfactory neurons derive from Gsh2-expressing LGE precursors. Furthermore, the timing and sequence of GABA interneuron generation may depend on a regulatory network including Mash1, Dlx1/2, and Dlx5/6, all gene candidates for schizophrenia risk. Indeed, DLX1 gene expression is reduced in the thalamus of patients with psychosis. Thus abnormal regulation of these factors may diminish selectively GABA interneuron formation, which in turn may represent a genetically determined vulnerability, and may contribute to diminished regional brain size and/or function. The most compelling neuropathological evidence for a developmental basis is the finding of aberrantly localized or clustered neurons especially in lamina II of the

entorhinal cortex and in the white matter underlying prefrontal cortex and temporal and parahippocampal regions. These abnormalities represent alterations of developmental neuronal migration, survival, and connectivity. In addition, in hippocampus and neocortex, pyramidal neurons appear smaller in many studies, exhibiting fewer dendritic arborizations and spines with reduced neuropil, findings that are associated with reductions in neuronal molecules, including MAP2, spinophilin, synaptophysin, and SNAP25. Although the genes associated with schizophrenia are reviewed extensively in other chapters, worth mentioning here is a particularly intriguing candidate gene DISC1, whose protein has roles during development including regulating cell migration, neurite outgrowth, and neuronal maturation as well as in adult brain, where it modulates cytoskeletal function, neurotransmission, and synaptic plasticity. DISC1 protein interacts with many other proteins intimately involved in neuronal cell migration and forms a protein complex with Lis1 and NudEL that is downstream of reelin signaling. Autism Spectrum Disorders Another condition that is clearly neurodevelopmental in origin is autism spectrum disorders (ASDs), a complex and heterogeneous group of disorders characterized by abnormalities in social interaction and communication and the presence of restricted or repetitive interests and activities. In the last edition of DSM (DSM-IV) the ASDs included classic autistic disorder, Asperger’s syndrome, and pervasive developmental disorder not otherwise specified. These three disorders were grouped together due to their common occurrence in families, indicating related genetic factors and shared signs and symptoms. Recent conceptualizations of ASDs propose that there are multiple “autisms” differing in underlying pathogenetic mechanisms and manifestations. It is likely that the different core symptom domains (or other endophenotypes) will be more heritable than the syndromic diagnosis, which was constructed to be inclusive. The large diversity of ASD signs and symptoms reflects the multiplicity of abnormalities observed in pathological and functional studies and include both forebrain and hindbrain regions. Forebrain neurons in the cerebral cortex and limbic system play critical roles in social interaction, communication, and learning and memory. For example, the amygdala, which connects to prefrontal and temporal cortices and fusiform gyrus, plays a prominent role in social and emotional cognition. In ASDs, the amygdala and fusiform gyrus demonstrate abnormal activation during facial recognition and emotional attribution tasks. Some investigators hypothesize that ASDs reflect dysfunctions in specific neural networks, such as the social network. On the other hand, neurophysiological tests of evoked cortical potentials and

oculomotor responses indicate normal perception of primary sensory information but disturbed higher cognitive processing. The functional impairments in higher-order cognitive processing and neocortical circuitry suggest a developmental disorder involving synaptic organization, a mechanism that may be uniformly present throughout the brain, a model in distinct contrast to abnormalities of specific neural networks. Earlier reference to the expression

of Wnt3a in cells that migrated widely during development and appear in auditory systems is one example of how developmental changes may affect single functional networks, whereas changes in common and widely expressed synaptic molecules, such as the neuroligins, would represent the other mechanism. The most important recent discovery in ASD pathogenesis has been the widely reported and replicated brain growth phenotype: Starting with probably normal size at birth, the brain exhibits an accelerated increase in volume by the end of the first year compared to the typically developing child, and this process continues from ages 2 to 4 years. These data derive from both neuroimaging studies as well as measures of head circumference performed by multiple labs. It is not known whether this reflects an acceleration of normal developmental processes or, alternatively, a disease-specific aberration in postnatal development, including changes in cell numbers, neuronal processes, synapse formation and modifications, or glial cell dysfunction, to name a few. The most prominent differences are observed in frontal and parietal cortex, cerebellar hemispheres, as well as the amygdala. These findings are also consistent with recent reports of macrocephaly in up to ~20 percent of ASD cases in brain and DNA banks. These findings raise many questions to be addressed by developmental neuroscientists. Functional neuroimaging studies indicate broad forebrain but also cerebellar dysfunctions in ASD, and classical pathological studies have suggested abnormalities restricted to limbic and cerebellar structures. However, classical studies were hampered by small sample sizes, poor control for comorbidities such as epilepsy and mental retardation that affect neuroanatomy, and the use of tissue cell density measures as opposed to unbiased stereological methods to estimate regional neuron numbers. Although previous studies described increased densities of small neurons in interconnecting limbic nuclei, including CA fields, septum, mammillary bodies, and amygdala, these results have not been replicated by other laboratories. In contrast, the most consistent neuropathology has been observed in the cerebellum (21 of 29 brains), showing reductions in the number of Purkinje neurons without signs of acquired postnatal lesions, such as gliosis, empty baskets, and retrograde loss of afferent inferior olive neurons, suggesting prenatal origins. A more recent study identifies widespread and nonuniform abnormalities, suggesting dysregulation of many processes, including neuron proliferation, migration, survival, organization, and programmed cell death. Four of six brains were macrocephalic, consistent with increased size defined by numerous pathology and neuroimaging studies. In cerebral cortex, there was thickened or diminished gray matter, disorganized laminar patterns, misoriented pyramidal neurons, ectopic neurons in both superficial and deep white matter, and increased or decreased neuron densities. This evidence of abnormal cortical neurogenesis and migration accords well with the deficits in cognitive functions. In brainstem, neuronal disorganization appeared as discontinuous and malpositioned neurons in olivary and dentate nuclei, ectopic neurons in medulla and cerebellar peduncles, and aberrant fiber tracts. There were widespread patchy or diffuse decreases of Purkinje neurons, sometimes associated with increased Bergmann glia, or ectopic Purkinje neurons in the molecular layer. Hippocampal neuronal atrophy was not observed, and quantitative stereology found no consistent change in neuron density or number. Moreover, a single recent neuropathological study using multiple immunological indices has reported increased levels of immune cytokines in the

cerebrospinal fluid of patients and in brain tissues as well as astrocytes expressing high levels of glial fibrillary acidic protein in frontal and cingulated cortex, white matter, and cerebellum, all suggesting potential immune activation without evidence of an inflammatory process. We await confirmation of these important findings.

Although seemingly incompatible, these various data support a model of developmental abnormalities occurring at different times, altering regions according to specific schedules of neurogenesis and differentiation. It is notable that a similar range of abnormalities was found in classical studies but was excluded, since these abnormalities did not occur in every brain examined. Moreover, in 15 children exposed to the teratogen thalidomide during days 20 to 24 of gestation, when cranial and Purkinje neurogenesis occurs in brainstem, four cases exhibited autism. On the basis of these data, autism is associated with insults at 3 weeks for thalidomide, 12 weeks when inferior olivary neurons are migrating, and ~30 weeks when olivary axons make synapses with Purkinje cells. These diverse abnormalities in cell production, survival, migration, organization, and differentiation in both hindbrain and forebrain indicate disturbed brain development over a range of stages. Recent genetic studies have defined two genetic polymorphisms associated reproducibly with ASD in several datasets, both of which influence brain developmental processes. The first is ENGRAILED-2, the cerebellar patterning gene whose dysregulation causes deficits in Purkinje and granule neurons in animal models and acts to control proliferation and differentiation. The second is the hepatocyte growth factor receptor cMET, whose function affects tangential migration of GABA interneurons from the ventral forebrain ganglionic eminences, potentially leading to imbalances of excitatory and inhibitory neurotransmission. Furthermore, although the cellular derangements may be directly responsible for the core symptoms of autism, there is an alternative hypothesis: Disturbed regulation of developmental processes produces an as-yet unidentified biochemical cellular lesion that may be associated with autism. This proposal is supported by the currently known genetic causes of autism that account for 10 percent of cases, including tuberous sclerosis, neurofibromatosis, Smith-Lemli-Opitz syndrome, Rett syndrome, and fragile X mental retardation. These genetic etiologies interfere with cell proliferation control, cholesterol biosynthesis and Shh function, and synaptic and dendrite protein translation and function, fundamental processes in the sequence of development. An intriguing potential link in these monogenetic causes of autism symptoms is their participation in protein synthesis in the synapse, especially as regulated via the PI3K/Akt signaling pathway and the mammalian target of rapamycin (mTOR) complex, an area of active research.

THE REMARKABLE DISCOVERY OF ADULT NEUROGENESIS In the last decade, there has been a fundamental shift in paradigm regarding the limits of neurogenesis in the brain, with important implications for neural plasticity, mechanisms of disease etiology and therapy, and possibilities of repair. Until recently, it has generally been maintained that we do not produce new neurons in the brain after birth (or soon thereafter, considering cerebellar EGL); thus brain plasticity and repair depend on modifications of a numerically static neural network. We now have strong evidence to the contrary: new neurons are generated throughout life in certain regions, well documented across the phylogenetic tree, including birds, rodents, primates, and humans. As an area of intense interest and investigation, we may expect rapid progress over the next two decades, likely altering models described herein. The term neurogenesis has been used inconsistently in different contexts, indicating sequential production of neural elements during development, first neurons then glial cells, but frequently connoting only neuron generation in adult brain, in contrast to

gliogenesis. For this discussion, we use the first, more general meaning, and distinguish cell types as needed. The first evidence of mammalian neurogenesis, or birth of new neurons, in adult hippocampus was reported in the 1960s in which 3H-thymidine-labeled neurons were documented. As a common marker for cell production, these studies used nuclear incorporation of 3H-thymidine into newly synthesized DNA during chromosome replication, which occurs before cells undergo division. After a delay, cells divide, producing two 3H-thymidine-labeled progeny. Cell proliferation is defined as an absolute increase in cell number, which occurs only if cell production is not balanced by cell death. Because there is currently little evidence for a progressive increase in brain size with age, except perhaps for rodent hippocampus, most neurogenesis in adult brain is apparently compensated for by cell loss. More recent studies of neurogenesis employ the more convenient thymidine analog BrdU, which can be injected into living animals and then detected by immunohistochemistry. During embryonic development, neurons are produced from almost all regions of the ventricular neuroepithelium. Neurogenesis in the adult, however, is largely restricted to two regions: the SVZ lining the lateral ventricles and a narrow proliferative zone underlying the dentate gyrus granule layer (subgranular zone) in hippocampus. In mice, rodents, and monkeys, newly produced neurons migrate from the SVZ in an anterior direction into the olfactory bulb to become GABA interneurons. The process has been elegantly characterized at both ultrastructural and molecular levels. In the SVZ, the neuroblasts (A cells) on their way to olfactory bulb create chains of cells and migrate through a scaffold of glial cells supplied by slowly dividing astrocytes (B cells). Within this network of cell chains, there are groups of rapidly dividing neural precursors (C cells). Evidence suggests that the B cells give rise to the C cells, which in turn develop into the A cells, the future olfactory bulb interneurons. The existence of a sequence of precursors with progressively restricted abilities to generate diverse neural cell types makes defining mechanisms that regulate adult neurogenesis in vivo a great challenge.

As in developing brain, adult neurogenesis is also subject to regulation by extracellular signals that control precursor proliferation and survival and in many cases the very same factors. After initial discovery of adult neural stem cells generated under EGF stimulation, other regulatory factors were defined including bFGF, IGF-I, BDNF, and LIF/CNTF. Although the hallmark of neural stem cells includes the capacity to generate neurons, astrocytes, and oligodendroglia, termed multipotentiality, specific signals appear to produce relatively different profiles of cells that may migrate to distinct sites. Intraventricular infusion of EGF promotes primarily gliogenesis in the SVZ, with cells migrating to olfactory bulb, striatum, and corpus callosum, whereas bFGF favors the generation of neurons destined for the olfactory bulb. Both factors appear to stimulate mitosis directly, with differential effects on the cell lineage produced. In contrast, BDNF may increase neuron formation in SVZ as well as striatum and hypothalamus, though effects may be primarily through promoting survival of newly generated neurons that otherwise undergo cell death. Finally, CNTF and related LIF may promote gliogenesis or, alternatively, support self-renewal of adult stem cells rather than enhancing a specific cell category. Remarkably, in addition to direct intraventricular infusions, adult neurogenesis is also affected by peripheral levels of growth factors, hormones, and neuropeptides. Peripheral administration of both bFGF and IGF-I stimulate neurogenesis, increasing selectively mitotic labeling in the SVZ and hippocampal subgranular zone, respectively, suggesting that there are specific mechanisms for factor transport across the BBB. Of interest, elevated prolactin levels, induced by peripheral injection or natural pregnancy, stimulate proliferation of progenitors in the mouse SVZ, leading to increased olfactory bulb interneurons, potentially playing roles in learning new infant scents. This may be relevant to changes in prolactin

seen in psychiatric disease. Conversely, in behavioral paradigms of social stress, such as territorial challenge by male intruders, activation of the hypothalamic-pituitary-adrenal axis with increased glucocorticoids leads to reduced neurogenesis in the hippocampus, apparently through local glutamate signaling. Inhibition is also observed after peripheral opiate administration, a model for substance abuse. Thus neurogenesis may be one target process affected by changes of hormones and neuropeptides associated with several psychiatric conditions. The discovery of adult neurogenesis naturally leads to questions about whether new neurons can integrate into the complex cytoarchitecture of the mature brain and to speculation about its functional significance, if any. In rodents, primates, and humans, new neurons are generated in the dentate gyrus of the hippocampus, an area important for learning and memory. Some adult-generated neurons in humans have been shown to survive for at least 2 years. Furthermore, newly generated cells in adult mouse hippocampus indeed elaborate extensive dendritic and axonal arborizations appropriate to the neural circuit and display functional synaptic inputs and action potentials. From a functional perspective, the generation and/or survival of new neurons correlates strongly with multiple instances of behavioral learning and experience. For example, survival of newly generated neurons is markedly enhanced by hippocampal-dependent learning tasks and by an enriched, behaviorally complex environment. Of perhaps greater importance, a reduction in dentate gyrus neurogenesis impairs the formation of trace memories, that is, when an animal must associate stimuli that are separated in time, a hippocampal-dependent task. Finally, in songbirds, neurogenesis is activity dependent and is increased by foraging for food and learning new song, whether it occurs seasonally or is induced by steroid hormone administration. From clinical and therapeutic perspectives, fundamental questions are whether changes in neurogenesis contribute to disease and whether newly formed neurons undergo migration to and integration into regions of injury, replacing dead cells and

leading to functional recovery. A neurogenetic response has now been shown for multiple conditions in the adult, including brain trauma, stroke, and epilepsy. For instance, ischemic stroke in the striatum stimulates adjacent SVZ neurogenesis with neurons migrating to the injury site. Furthermore, in a highly selective paradigm not involving local tissue damage, degeneration of layer 3 cortical neurons elicited SVZ neurogenesis and cell replacement. These studies raise the possibility that newly produced neurons normally participate in recovery and may be stimulated as a novel therapeutic strategy. However, in contrast to potential reconstructive functions, neurogenesis may also play roles in pathogenesis: In a kindling model of epilepsy, newly generated neurons were found to migrate to incorrect positions and participate in aberrant neuronal circuits, thereby reinforcing the epileptic state. Conversely, reductions in neurogenesis may contribute to several conditions that implicate dysfunction or degeneration of the hippocampal formation. Dentate gyrus neurogenesis is inhibited by increased glucocorticoid levels observed in aged rats and can be reversed by steroid antagonists and adrenalectomy, observations potentially relevant to the correlation of elevated human cortisol levels with reduced hippocampal volumes and the presence of memory deficits. Similarly, stress-induced increases in human glucocorticoids may contribute to decreased hippocampal volumes seen in schizophrenia, depression, and posttraumatic stress disorder. A potential role for altered neurogenesis in disease has gained the most support in recent studies of depression. A number of studies in animals and humans suggest a correlation of decreased hippocampal size with depressive symptoms, whereas clinically effective antidepressant therapy elicits increased hippocampal volume and enhanced neurogenesis, with causal relationships still being defined. For example, postmortem and brain imaging studies

indicate cell loss in corticolimbic regions in bipolar disorder and major depression. Significantly, mood stabilizers, such as lithium ion and valproic acid, as well as antidepressants and electroconvulsive therapy activate intracellular pathways that promote neurogenesis and synaptic plasticity. Furthermore, in a useful primate model, the adult tree shrew, the chronic psychosocial stress model of depression elicited ~15 percent reductions in brain metabolites and a 33 percent decrease in neurogenesis (BrdU mitotic labeling), effects that were prevented by coadministration of antidepressant, tianeptine. More importantly, although stress exposure elicited small reductions in hippocampal volumes, stressed animals treated with antidepressant exhibited increased hippocampal volumes. Similar effects have been found in rodent models of depression. In addition to the foregoing structural relationships, recent evidence has begun defining the roles of relevant neurotransmitter systems to antidepressant effects on behavior and neurogenesis. In a most exciting finding, a causal link between antidepressant-induced neurogenesis and a positive behavioral response has been demonstrated. In the serotonin 1A receptor null mouse, fluoxetine, a selective serotonin reuptake inhibitor [SSRI], produced neither enhanced neurogenesis nor behavioral improvement. Furthermore, when hippocampal neuronal precursors were selectively reduced (85 percent) by X-irradiation, neither fluoxetine nor imipramine induced neurogenesis or behavioral recovery. Finally, one study using hippocampal cultures from normal and mutant rodents strongly supports a neurogenetic role for endogenous NPY, which is contained in dentate gyrus hilar interneurons. NPY stimulates precursor proliferation selectively via the Y1 (not Y2 or Y5) receptor, a finding consistent with the receptor-mediating antidepressive effects of NPY in animal models and the impact of NPY levels on both hippocampal-dependent learning and responses to stress. In aggregate, these observations suggest that volume changes observed with human depression and therapy may directly relate to alterations in ongoing neurogenesis. More generally, the discovery of adult neurogenesis has led to major changes in our perspectives

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including monoamines. This complexity poses a major challenge to understanding the precise molecular, cellular, and systems level pathways through which various monoamine neurotransmitters affect neuropsychiatric disorders. However, recent advances in

human genetics and genomics, as well as in experimental neuroscience, have shed light on this question. Molecular cloning has identified a large number of genes that regulate monoaminergic neurotransmission, such as the enzymes, receptors, and transporters that mediate the synthesis, cellular actions, and cellular reuptake of these neurotransmitters, respectively. Human genetics studies have provided evidence of tantalizing links between allelic variants in specific monoamine-related genes and psychiatric disorders and trait abnormalities, whereas the ability to modify gene function and cellular activity in experimental animals has clarified the roles of specific genes and neural pathways in mediating behavioral processes. Monoamines act on target cells by binding to specific cell-surface receptors. There are multiple receptor subtypes for each monoamine, which are expressed in diverse regions and subcellular locales and which engage a variety of intracellular signaling pathways. This panoply of receptors thus allows each monoamine neurotransmitter to modulate target cells in many ways; the same molecule may activate some cells while inhibiting others, depending on which receptor subtype is expressed by each cell. The various monoamines are discussed below. Serotonin Although only one in a million CNS neurons produces serotonin, these cells influence virtually all aspects of CNS function. The cell bodies of these serotonergic neurons are clustered in the midline raphe nuclei of the brainstem; the rostral raphe nuclei send ascending axonal projections throughout the brain, whereas the descending caudal raphe nuclei send projections into the medulla, cerebellum, and spinal cord (Fig. 1.4-1). The descending serotonergic fibers that innervate the dorsal horn of the spinal cord have been implicated in the suppression of nociceptive pathways, a finding that may relate to the pain-relieving effects of some antidepressants. The tonic firing of CNS serotonin neurons varies across the sleep–wake cycle, with an absence of activity during rapid eye movement (REM) sleep. Increased serotonergic firing is observed during rhythmic motor behaviors and suggests that serotonin modulates some forms of motor output. FIGURE 1.4-1 Brain serotonergic pathways (in rats). Serotonergic neurons are located in brainstem midline raphe nuclei and project throughout the neuraxis. (There is an approximate similarity between monoamine pathways in rats and in humans.) AMG, amygdala; CBM, cerebellum; cc, corpus callosum; CP, caudate putamen; CRN, caudal raphe nuclei; CTX, neocortex; DR, dorsal raphe nucleus; HI, hippocampus; HY, hypothalamus; LC, locus ceruleus; MR, median raphe nucleus; NAc, nucleus accumbens; OB, olfactory bulb;

SN, substantia nigra; TE, tectum; TH, thalamus; TM, tuberomammillary nucleus of hypothalamus. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:65.) Most serotonergic innervation of the cortex and limbic system arises from the dorsal and median raphe nuclei in the midbrain; the serotonergic neurons in these areas send projections through the medial forebrain bundle into target forebrain regions. The median raphe provides most of the serotonergic fibers that innervate the limbic system, whereas the dorsal raphe nucleus provides most of the serotonergic fibers that innervate the striatum and thalamus. In addition to the different target fields of these serotonergic nuclei, there are also cellular differences between their constituent neurons. Dorsal raphe serotonergic fibers are fine, with small vesicle-coated swellings called varicosities, whereas median raphe fibers have large spherical or beaded varicosities. It is unclear to what extent serotonin acts as a true synaptic or “private” neurotransmitter versus action as a local endocrine hormone or

“social transmitter,” or whether its roles differ depending on the fiber type from which it is released. These fibers show differential sensitivity to the neurotoxic effects of the amphetamine analog 3,4-methylenedioxy-methamphetamine (MDMA, “ecstasy”), which lesions the fine axons of the dorsal raphe while sparing the thick beaded axons of the median raphe. The significance of these morphological differences is unclear, although recent work has identified functional differences between the serotonergic neurons of the dorsal and median raphe nuclei. Dopamine Dopamine neurons are more widely distributed than those of other monoamines, residing in the midbrain substantia nigra and ventral tegmental area and in the periaqueductal gray, hypothalamus, olfactory bulb, and retina. In the periphery, dopamine is found in the kidney where it functions to produce renal vasodilation, diuresis, and natriuresis. Three dopamine systems are highly relevant to psychiatry: The nigrostriatal, mesocorticolimbic, and tuberohypophyseal system (Fig. 1.4-2). Degeneration of the nigrostriatal system causes Parkinson’s disease and has led to an intense research focus on the development and function of dopamine neurons in the midbrain substantia nigra nuclei. Dopamine cell bodies in the pars compacta division of this region send ascending projections to the dorsal striatum (especially to the caudate and putamen) and thereby modulate motor control. The extrapyramidal effects of antipsychotic drugs are thought to result from the blockade of these striatal dopamine receptors.

FIGURE 1.4-2 Brain dopaminergic pathways (in rats).The three principal dopaminergic pathways: (1) nigrostriatal pathway, (2) mesocorticolimbic pathway, and (3) tuberohypophyseal pathway. AMG, amygdala; CBM, cerebellum; cc, corpus callosum; CP, caudate putamen; CTX, neocortex; HI, hippocampus; HY, hypothalamus; LC, locus ceruleus; NAc, nucleus accumbens; OB, olfactory bulb; PFC, prefrontal cortex; PI, pituitary; SNC, substantia nigra pars compacta; TE, tectum; TH, thalamus; VTA, ventral tegmental area. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:66.) The midbrain ventral tegmental area (VTA) lies medial to the substantia nigra and contains dopaminergic neurons that give rise to the mesocorticolimbic dopamine system. These neurons send ascending projections that innervate limbic structures, such as the nucleus accumbens and amygdala; the mesoaccumbens pathway is a central element in the neural representation of reward, and intense research has been devoted to this area in recent years. All known drugs of abuse activate the mesoaccumbens dopamine pathway, and plastic changes in this pathway are thought to underlie drug addiction. The mesolimbic projection is believed to be a major target for the antipsychotic properties of dopamine receptor antagonist drugs in controlling the positive symptoms of schizophrenia, such as hallucinations and delusions. VTA dopamine neurons also project to cortical structures, such as the prefrontal cortex, and modulate working memory and attention; decreased activity in this pathway is proposed to underlie negative symptoms of schizophrenia. Thus antipsychotic drugs that decrease positive symptoms by blocking dopamine receptors in the mesolimbic pathway may simultaneously worsen these negative symptoms by blocking similar dopamine receptors in the mesocortical pathway. The decreased risk of extrapyramidal side effects seen with clozapine (Clozaril; versus other typical antipsychotic medications) is thought to be due to its relatively selective effects on this mesocortical projection. The tuberohypophyseal system consists of dopamine neurons in the hypothalamic arcuate and paraventricular nuclei that project to the pituitary gland and thereby inhibit prolactin release. Antipsychotic drugs that block dopamine receptors in the pituitary may thus disinhibit prolactin release and cause galactorrhea. Norepinephrine and Epinephrine The postganglionic sympathetic neurons of the autonomic nervous system release norepinephrine, resulting in widespread

peripheral effects including tachycardia and elevated blood pressure. The adrenal medulla releases epinephrine, which produces similar effects; epinephrine-secreting pheochromocytoma tumors produce bursts of sympathetic activation, central arousal, and anxiety. Norepinephrine-producing neurons are found within the brain in the pons and medulla in two major clusterings: The locus ceruleus (LC) and the lateral tegmental noradrenergic nuclei (Fig. 1.4-3). Noradrenergic projections from both of these regions ramify extensively as they project throughout the neuraxis. In humans, the LC is found in the dorsal portion of the caudal pons and contains approximately 12,000 tightly packed neurons on each side of the brain. These cells provide the major noradrenergic projections to the neocortex, hippocampus, thalamus, and midbrain tectum. The activity of LC

neurons varies with the animal's level of wakefulness. Firing rates are responsive to novel and/or stressful stimuli, with largest responses to stimuli that disrupt ongoing behavior and reorient attention. Altogether, physiological studies indicate a role for this structure in the regulation of arousal state, vigilance, and stress response. The projections from lateral tegmental nucleus neurons, which are loosely scattered throughout the ventral pons and medulla, partially overlap those of the LC. Fibers from both cell groups innervate the amygdala, septum, and spinal cord. Other regions, such as the hypothalamus and lower brainstem, receive adrenergic inputs predominantly from the lateral tegmental nucleus. The relatively few neurons that utilize epinephrine as a neurotransmitter are located in the caudal pons and medulla, intermingled with noradrenergic neurons. Projections from these groups ascend to innervate the hypothalamus, LC, and visceral efferent and afferent nuclei of the midbrain. FIGURE 1.4-3 Brain noradrenergic pathways (in rats). Projections of noradrenergic neurons located in the locus ceruleus (LC) and lateral tegmental noradrenergic nuclei (LTN). AMG, amygdala; CBM, cerebellum; cc, corpus callosum; CP, caudate putamen; CTX, neocortex; HI, hippocampus; HY, hypothalamus; OB, olfactory bulb; TE, tectum; TH, thalamus. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:66.) Histamine Histamine is perhaps best known for its role in allergies. It is an inflammatory mediator stored in mast cells and released upon cellular interaction with allergens. Once released, histamine causes vascular leakage and edema and other facial and topical allergy symptoms. In contrast, central histaminergic neural pathways have only more recently been characterized by immunocytochemistry using antibodies to the synthetic enzyme histidine decarboxylase and to histamine. Histaminergic cell bodies are located within a region of the posterior hypothalamus termed the tuberomammillary nucleus. The activity of tuberomammillary neurons is characterized by firing that varies across the sleep–wake cycle, with the highest activity during the waking state, slowed firing during slow-wave sleep, and absence of firing during REM sleep. Histaminergic fibers project diffusely throughout the brain and spinal cord (Fig. 1.4-4). Ventral ascending projections course through the medial forebrain bundle and then innervate the hypothalamus, diagonal band, septum, and olfactory bulb. Dorsal ascending projections

innervate the thalamus, hippocampus, amygdala, and rostral forebrain. Descending projections travel through the midbrain central gray to the dorsal hindbrain and spinal cord. The fibers have varicosities that are seldom associated with classical synapses, and histamine has been proposed to act at a distance from its sites of release, like a local hormone. The hypothalamus receives the densest histaminergic innervation, consistent with a role for this transmitter in the regulation of autonomic and neuroendocrine processes. In addition, strong histaminergic innervation is seen in monoaminergic and cholinergic nuclei. FIGURE 1.4-4 Brain histaminergic pathways (in rats).

Histaminergic neurons are located in the tuberomammillary nucleus of the caudal hypothalamus (TM) and project to the hypothalamus (HY) and more distant brain regions. CBM, cerebellum; cc, corpus callosum; CP, caudate putamen; CTX, neocortex; HI, hippocampus; NAc, nucleus accumbens; OB, olfactory bulb; TE, tectum; TH, thalamus. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:67.) Acetylcholine Within the brain, the axonal processes of cholinergic neurons may either project to distant brain regions (projection neurons) or contact local cells within the same structure (interneurons). Two large clusters of cholinergic projection neurons are found within the brain: The basal forebrain complex and the mesopontine complex (Fig. 1.45). The basal forebrain complex provides most of the cholinergic innervation to the nonstriatal telencephalon. It consists of cholinergic neurons within the nucleus basalis of Meynert, the horizontal and vertical diagonal bands of Broca, and the medial septal nucleus. These neurons project to widespread areas of the cortex and amygdala, to the anterior cingulate gyrus and olfactory bulb, and to the hippocampus, respectively. In Alzheimer's disease there is significant degeneration of neurons in the nucleus basalis, leading to substantial reduction in cortical cholinergic innervation. The extent of neuronal loss correlates with the degree of dementia, and the cholinergic deficit may contribute to the cognitive decline in this disease, consistent with the beneficial effects

of drugs that promote acetylcholine signaling in this disorder. FIGURE 1.4-5 Brain cholinergic projection pathways (in rats). The majority of cholinergic projection neurons are located in the basal forebrain complex (BFC) and the mesopontine complex (MPC). AMG, amygdala; CBM, cerebellum; cc, corpus callosum; CP, caudate putamen; CTX, neocortex; HI, hippocampus; HY, hypothalamus; LC, locus ceruleus; NAc, nucleus accumbens; OB, olfactory bulb; SN, substantia nigra; TE, tectum; TH, thalamus. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:67.) The mesopontine complex consists of cholinergic neurons within the pedunculopontine and laterodorsal tegmental nuclei of the midbrain and pons and provides cholinergic innervation to the thalamus and midbrain areas (including the dopaminergic neurons of the ventral tegmental area and substantia nigra) and descending innervation to other brainstem regions such as the LC, dorsal raphe, and cranial nerve nuclei. In contrast to central serotonergic, noradrenergic, and histaminergic neurons, cholinergic neurons may continue to fire during REM sleep and have been proposed to play a role in REM sleep induction. Acetylcholine is also found within interneurons of several brain regions, including the striatum. The modulation of striatal cholinergic transmission has been implicated in the antiparkinsonian actions of anticholinergic agents. Within the periphery, acetylcholine is a prominent neurotransmitter, located in motoneurons innervating skeletal muscle, preganglionic autonomic neurons, and postganglionic parasympathetic neurons. Peripheral acetylcholine mediates the characteristic postsynaptic effects of the parasympathetic system, including bradycardia and reduced blood pressure, and enhanced digestive function. MONOAMINE SYNTHESIS, STORAGE, AND DEGRADATION In addition to neuroanatomic similarities, monoamines are also synthesized, stored, and degraded in similar ways (Fig. 1.4-6). Monoamines are synthesized within neurons from common amino acid precursors (Fig. 1.4-6, step 1) and taken up into synaptic vesicles by way of a vesicular monoamine transporter (Fig. 1.4-6, step 2). On stimulation, vesicles within nerve terminals fuse with the presynaptic terminal and release the neurotransmitter into the synaptic cleft (Fig. 1.4-6, step 3). Once released, the monoamines interact with postsynaptic receptors to alter the function of postsynaptic cells (Fig. 1.4-6, step 4), and they may also act on presynaptic autoreceptors on the

nerve terminal to suppress further release (Fig. 1.4-6, step 5). In addition, released monoamines may be taken back up from the synaptic cleft into the nerve terminal by plasma membrane transporter proteins (Fig. 1.4-6, step 6), a process known as reuptake. Reuptake plays an important role in limiting the total magnitude and temporal duration of monoamine signaling. Once monoamines are taken up, they may be subject to enzymatic degradation (Fig. 1.4-6, step 7), or they may be protected from degradation by uptake into vesicles. The processing of acetylcholine differs from this scheme and is described later in this section. FIGURE 1.4-6 Schematic diagram of a monoaminergic synapse. Steps involved in synaptic transmission are described in the text. MAO, monoamine oxidase. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:68.) SEROTONIN The CNS contains less than 2 percent of the serotonin in the body; peripheral serotonin is located in platelets, mast cells, and enterochromaffin cells. More than 80 percent of all the serotonin in the body is found in the gastrointestinal system, where it modulates motility and digestive functions. Platelet serotonin promotes aggregation and clotting through a most unusual mechanism: The covalent linkage of serotonin molecules to small GTP-binding proteins, which can then activate these proteins, is a process termed "serotonylation." Peripheral serotonin cannot cross the blood–brain barrier, so serotonin is synthesized within the brain as well. Serotonin is synthesized from the amino acid tryptophan, which is derived from the diet. The rate-limiting step in serotonin synthesis is the hydroxylation of tryptophan by the enzyme tryptophan hydroxylase to form 5hydroxytryptophan (4-HT) (Fig. 1.4-7). Two isoforms of tryptophan hydroxylase exist— one isoform is found mainly in the periphery, whereas the second isoform is restricted to

the CNS. FIGURE 1.4-7 Synthesis and catabolism of serotonin. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:68.)

Under normal circumstances, tryptophan concentration is rate limiting in serotonin synthesis. Therefore, much attention has focused on the factors that determine tryptophan availability. Unlike serotonin, tryptophan is taken up into the brain by way of a saturable active carrier mechanism. Because tryptophan competes with other large neutral amino acids for transport, brain uptake of this amino acid is determined both by the amount of circulating tryptophan and by the ratio of tryptophan to other large neutral amino acids. This ratio may be elevated by carbohydrate intake, which induces insulin release and the uptake of many large neutral amino acids into peripheral tissues. Conversely, high-protein foods tend to be relatively low in tryptophan, thus lowering this ratio. Moreover, the administration of specialized low tryptophan diets produces significant declines in brain serotonin levels. After tryptophan hydroxylation, 5-hydroxytryptophan is rapidly decarboxylated by aromatic amino acid decarboxylase (an enzyme also involved in dopamine synthesis) to form serotonin. The first step in the degradation of serotonin is mediated by monoamine oxidase type A (MAOA), which oxidizes the amino group to form an aldehyde. MAOA is located in mitochondrial membranes and is nonspecific in its substrate specificity; in addition to serotonin, it oxidizes norepinephrine. The elevation of serotonin levels by MAO inhibitors (MAOIs) is believed to underlie the antidepressant efficacy of these drugs. After oxidation by MAOA, the resulting aldehyde is further oxidized to 5-hydroxyindoleacetic acid (5-HIAA). Levels of 5-HIAA are often measured as a correlate of serotonergic system activity, although the relationship of these levels to serotonergic neuronal activity remains unclear. Catecholamines The catecholamines are synthesized from the amino acid tyrosine, which is taken up into the brain via an active transport

mechanism (Fig. 1.4-8). Within catecholaminergic neurons, tyrosine hydroxylase catalyzes the addition of a hydroxyl group to the meta position of tyrosine, yielding L-dopa. This rate-limiting step in catecholamine synthesis is subject to inhibition by high levels of catecholamines (end-product inhibition). Because tyrosine hydroxylase is normally saturated with substrate, manipulation of tyrosine levels does not readily affect the rate of catecholamine synthesis. Once formed, L-dopa is rapidly converted to dopamine by dopa decarboxylase, which is located in the cytoplasm. It is now recognized that this enzyme acts not only on L-dopa but also on all naturally occurring aromatic L-amino acids, including tryptophan, and thus it is more properly termed aromatic amino acid decarboxylase. In noradrenergic and adrenergic neurons, dopamine is actively transported into storage vesicles, where it is oxidized by dopamine β-hydroxylase to form norepinephrine. In adrenergic neurons and the adrenal medulla, norepinephrine is converted to epinephrine by phenylethanolamine Nmethyltransferase (PNMT), which is located within the cytoplasmic compartment.

FIGURE 1.4-8 Synthesis of catecholamines. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:69.) Two enzymes that play major roles in the degradation of catecholamines are monoamine oxidase and catechol-Omethyltransferase (COMT). MAO is located on the outer membrane of mitochondria, including those within the terminals of adrenergic fibers, and oxidatively deaminates catecholamines to their corresponding aldehydes. Two MAO isozymes with differing substrate specificities have been identified: MAOA, which preferentially deaminates serotonin and

norepinephrine, and MAO type B (MAOB), which deaminates dopamine, histamine, and a broad spectrum of phenylethylamines. Neurons contain both MAO isoforms. The blockade of monoamine catabolism by MAO inhibitors produces elevations in brain monoamine levels. MAO is also found in peripheral tissues such as the gastrointestinal tract and liver, where it prevents the accumulation of toxic amines. For example, peripheral MAO degrades dietary tyramine, an amine that can displace norepinephrine from sympathetic postganglionic nerve endings, producing hypertension if tyramine is present in sufficient quantities. Thus patients treated with MAO inhibitors are cautioned to avoid pickled and fermented foods that typically have high levels of tyramine. Catechol-O-methyltransferase (COMT) is located in the cytoplasm and is widely distributed throughout the brain and peripheral tissues, although little to none is found in adrenergic neurons. It has a wide substrate specificity, catalyzing the transfer of methyl groups from S-adenosyl methionine to the m-hydroxyl group of most catechol compounds. The catecholamine metabolites produced by these and other enzymes are frequently measured as indicators of the activity of catecholaminergic systems. In humans, the predominant metabolites of dopamine and norepinephrine are homovanillic acid (HVA) and 3-methoxy-4hydroxyphenylglycol (MHPG), respectively. Histamine As is the case for serotonin, the brain contains only a small portion of the histamine found in the body. Histamine is distributed throughout most tissues of the body, predominantly in mast cells. Because it does not readily cross the blood–brain barrier, it is believed that histamine is synthesized within the brain. In the brain, histamine is formed by the decarboxylation of the amino acid histidine by a specific L-histidine decarboxylase. This enzyme is not normally saturated with substrate, so synthesis is sensitive to histidine levels. This is consistent with the observation that the peripheral administration of histidine elevates brain histamine levels. Histamine is metabolized in the brain by histamine N-methyltransferase, producing methylhistamine. In turn, methylhistamine undergoes oxidative deamination by MAOB. Acetylcholine Acetylcholine is synthesized by the transfer of an

acetyl group from acetyl coenzyme A (ACoA) to choline in a reaction mediated by the enzyme choline acetyltransferase (ChAT). The majority of choline within the brain is transported from the blood rather than being synthesized de novo. Choline is taken up into cholinergic neurons by a highaffinity active transport mechanism, and this uptake is the rate-limiting step in acetylcholine synthesis. The rate of choline transport is regulated such that increased cholinergic neural activity is associated with enhanced choline uptake. After synthesis, acetylcholine is stored in synaptic vesicles through the action of a vesicular acetylcholine transporter. After vesicular release, acetylcholine is rapidly broken down by hydrolysis by acetylcholinesterase, located in the synaptic cleft. Much of the choline produced by this hydrolysis is then taken back into the presynaptic terminal via the choline transporter. Of note, although acetylcholinesterase is localized primarily to cholinergic neurons and synapses, a second class of cholinesterase termed butyrylcholinesterase is found primarily in the liver and plasma as well as in glia. In the

treatment of Alzheimer’s disease, strategies aimed at enhancing cholinergic function, primarily through the use of cholinesterase inhibitors to prevent normal degradation of acetylcholine, have shown moderate efficacy in ameliorating cognitive dysfunction as well as behavioral disturbances. Cholinesterase inhibitors are also used in the treatment of myasthenia gravis, a disease characterized by weakness due to blockade of neuromuscular transmission by autoantibodies to acetylcholine receptors. Transporters A great deal of progress has been made in the molecular characterization of the monoamine plasma membrane transporter proteins. These membrane proteins mediate the reuptake of synaptically released monoamines into the presynaptic terminal. This process also involves cotransport of Na^+ and Cl^- ions and is driven by the ion concentration gradient generated by the plasma membrane Na^+/K^+ ATPase. Monoamine reuptake is an important mechanism for limiting the extent and duration of activation of monoaminergic receptors. Reuptake is also a primary mechanism for replenishing terminal monoamine neurotransmitter stores. Moreover, transporters serve as molecular targets for a number of antidepressant drugs, psychostimulants, and monoaminergic neurotoxins. Whereas transporter molecules for serotonin (SERT), dopamine (DAT), and norepinephrine (NET) have been well characterized, transporters selective for histamine and epinephrine have not been demonstrated. Among drugs of abuse, cocaine binds with high affinity to all three known monoamine transporters, although the stimulant properties of the drug have been attributed primarily to its blockade of DAT. This view has been recently supported by the absence of cocaine-induced locomotor stimulation in a strain of mutant mice engineered to lack this molecule. In fact, psychostimulants produce a paradoxical locomotor suppression in these animals that has been attributed to their blockade of the serotonin transporter. The rewarding properties of cocaine have also been attributed primarily to dopamine transporter inhibition, although other targets mediate these effects as well, since cocaine still has rewarding effects in mice lacking the dopamine transporter. It appears that serotonergic as well as dopaminergic mechanisms may be involved. Transporters may also provide routes that allow neurotoxins to enter and damage monoaminergic neurons; examples include the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the serotonergic neurotoxin MDMA. Vesicular Monoamine Transporter In addition to the reuptake of monoamines into the presynaptic nerve terminal, a second transport process serves to concentrate and store monoamines within synaptic vesicles. The transport and storage of monoamines in vesicles may serve several purposes: (1) to enable the regulated release of transmitter under appropriate physiological stimulation, (2) to protect monoamines from degradation by MAO, and (3) to protect neurons from the toxic effects of free radicals produced by the oxidation of cytoplasmic

monoamines. In contrast with the plasma membrane transporters, a single type of vesicular monoamine transporter is believed to mediate the uptake of

monoamines into synaptic vesicles within the brain. Consistent with this, blockade of this vesicular monoamine transporter by the antihypertensive drug reserpine (Serpasil) has been found to deplete brain levels of serotonin, norepinephrine, and dopamine and to increase the risk of suicide and affective dysfunction. RECEPTORS Ultimately, the effects of monoamines on CNS function and behavior depend on their interactions with receptor molecules. The binding of monoamines to these plasma membrane proteins initiates a series of intracellular events that modulate neuronal excitability. Unlike the transporters, multiple receptor subtypes exist for each monoamine neurotransmitter (Table 1.4-1). Table 1.4-1 Monoamine Receptors: Overview Serotonin Receptors The 5-hydroxytryptophan type 1 (5-HT1) receptors comprise the largest serotonin receptor subfamily, with human subtypes designated 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, and 5-HT1F. All five 5-HT1 receptor subtypes display intronless gene structures, high affinities for serotonin, and adenylate cyclase inhibition. The most intensively studied of

these has been the 5-HT1A receptor. This subtype is found on postsynaptic membranes of forebrain neurons, primarily in the hippocampus, cortex, and septum and on serotonergic neurons, where it functions as an inhibitory somatodendritic autoreceptor. There is significant interest in the 5-HT1A receptor as a modulator of both anxiety and depression. The downregulation of 5-HT1A autoreceptors by the chronic administration of serotonin reuptake inhibitors has been implicated in their antidepressant effects, and SSRIs may produce some behavioral effects via increases in hippocampal neurogenesis mediated by postsynaptic 5-HT1A receptor activation. In addition, partial 5-HT1A receptor agonists such as buspirone (BuSpar) display both anxiolytic and antidepressant properties. Much recent attention has focused on the contributions of 5-HT2A/C receptors to the actions of atypical antipsychotic drugs such as clozapine (Clozaril), risperidone (Risperdal), and olanzapine (Zyprexa). Analysis of the receptor-binding properties of these drugs has led to the hypothesis that 5-HT2A receptor blockade correlates with the therapeutic effectiveness of atypical antipsychotics. Of interest, the 5-HT2A receptor has also been implicated in the cognitive process of working memory, a function believed to be impaired in schizophrenia. The 5-HT2C receptor is expressed at high levels in many CNS regions, including the hippocampal formation, prefrontal cortex, amygdala, striatum, hypothalamus, and choroid plexus. Stimulation of 5-HT2C receptors has been proposed to produce anxiogenic effects as well as anorectic effects, which may result from interactions with the hypothalamic melanocortin and leptin pathways. 5-HT2C receptors may also play a role in the weight gain and development of type 2 diabetes mellitus associated with atypical antipsychotic treatment. Indeed, a line of mice lacking this receptor subtype exhibits an obesity syndrome associated with overeating and enhanced seizure susceptibility, suggesting that this receptor regulates neuronal network excitability. A variety of antidepressant and antipsychotic drugs antagonize 5-HT2C receptors with high affinity. Conversely, hallucinogens such as lysergic acid diethylamide (LSD) display agonist activity at 5-HT2 (and other) serotonin receptor subtypes. 5-HT2C receptor transcripts also undergo RNA editing, producing isoforms of the receptor with significantly altered basal versus serotonin-induced activity. Alterations in 5-HT2C receptor messenger ribonucleic acid (mRNA) editing have been found in the brains of suicide victims with a history of major depression, and SSRIs have been shown to alter these editing patterns. Dopamine Receptors In 1979, it was clearly recognized that the actions of dopamine are mediated by more than one receptor subtype. Two dopamine receptors, termed D1

and D2, were

distinguished on the basis of differential binding affinities of a series of agonists and antagonists, distinct effector mechanisms, and distinct distribution patterns within the CNS. It was subsequently found that the therapeutic efficacy of antipsychotic drugs correlated strongly with their affinities for the D2 receptor, implicating this subtype as an important site of antipsychotic drug action. Recent molecular cloning studies have identified three additional dopamine receptor genes encoding the D3, D4, and D5 dopamine receptors. On the basis of their structure, pharmacology, and primary effector mechanisms, the D3 and D4 receptors are considered to be “D2-like,” and the D5 receptor “D1-like.” The functional roles of the recently discovered subtypes remain to be definitively elucidated. The D1 receptor was initially distinguished from the D2 subtype by its high affinity for the antagonist SCH 23390 and relatively low affinity for butyrophenones such as haloperidol (Haldol). Whereas D1 receptor activation stimulates cyclic adenosine monophosphate (cAMP) formation, D2 receptor stimulation produces the opposite effect. Adrenergic Receptors As for the α1 receptors, the functions of α2 receptor subtypes (designated α2A, α2B, and α2C) have been difficult to determine due to a lack of selective agonists and antagonists; α2 receptors display both presynaptic autoreceptor and postsynaptic actions, and all appear to inhibit cAMP formation and to activate potassium channels with resultant membrane hyperpolarization. These receptors regulate neurotransmitter release from peripheral sympathetic nerve endings. Within the brain the stimulation of α2 autoreceptors (likely the α2A subtype) inhibits firing of the noradrenergic neurons of the LC, which have been implicated in arousal states. This mechanism has been proposed to underlie the sedative effects of the α2 receptor agonist clonidine (Catapres). In addition, the stimulation of brainstem α2 receptors has been proposed to reduce sympathetic and to augment parasympathetic nervous system activity. This action may relate to the utility of clonidine in lowering blood pressure and in suppressing the sympathetic hyperactivity associated with opiate withdrawal. Activation of α2 receptors inhibits the activity of serotonin neurons of the dorsal raphe nucleus, whereas activation of local α1 receptors stimulates the activity of these neurons, and this is thought to be a major activating input to the serotonergic system. Histamine Receptors Histaminergic systems have been proposed to modulate arousal, wakefulness, feeding behavior, and neuroendocrine responsiveness. Four histaminergic receptor subtypes have been identified and termed H1, H2, H3, and H4. The H4 receptor was identified

recently and is detected predominantly in the periphery, in regions such as the spleen, bone marrow, and leukocytes. The other three histamine receptors have prominent expression in the CNS. H1 receptors are expressed throughout the body, particularly in smooth muscle of the gastrointestinal tract and bronchial walls as well as on vascular endothelial cells. H1 receptors are widely distributed within the CNS, with particularly high levels in the thalamus, cortex, and cerebellum. H1 receptor activation is associated with Gq activation and stimulation of phosphoinositide turnover and tends to increase excitatory neuronal responses. These receptors are the targets of classical antihistaminergic agents used in the treatment of allergic rhinitis and conjunctivitis. The well-known sedative effects of these compounds have been attributed to their actions in the CNS and have implicated histamine in the regulation of arousal and the sleep–wake cycle. Accordingly, a line of mutant mice lacking histamine displays deficits in waking and attention. In addition, the sedation and weight gain produced by a number of antipsychotic and antidepressant drugs have been attributed to H1 receptor antagonism. Conversely, H1 receptor agonists stimulate arousal and suppress food intake in animal models. Cholinergic Receptors M1

receptors are the most abundantly expressed muscarinic receptors in the forebrain, including the cortex, hippocampus, and striatum. Pharmacological evidence has suggested their involvement in memory and synaptic plasticity, and recent evaluation of mice lacking the M1 receptor gene revealed deficits in memory tasks believed to require interactions between the cortex and the hippocampus. Nicotinic receptors have been implicated in cognitive function, especially working memory, attention, and processing speed. Cortical and hippocampal nicotinic acetylcholine receptors appear to be significantly decreased in Alzheimer's disease, and nicotine administration improves attention deficits in some patients. The acetylcholinesterase inhibitor galantamine used in the treatment of Alzheimer's disease also acts to positively modulate nicotinic receptor function. The α7 nicotinic acetylcholine receptor subtype has been implicated as one of many possible susceptibility genes for schizophrenia, with lower levels of this receptor being associated with impaired sensory gating. Some rare forms of the familial epilepsy syndrome autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) are associated with mutations in the α4 or β2 subunits of the nicotinic acetylcholine receptor. Finally, the reinforcing properties of tobacco use are proposed to involve the stimulation of nicotinic acetylcholine receptors located in mesolimbic dopaminergic reward pathways. AMINO ACID NEUROTRANSMITTERS For more than 50 years, biogenic amines have dominated thinking about the role of neurotransmitters in the pathophysiology of psychiatric disorders. However, over the last decade, evidence has accumulated from postmortem, brain imaging, and genetic

studies that the amino acid neurotransmitters, in particular glutamic acid and γaminobutyric acid (GABA), play an important, if not central, role in the pathophysiology of a broad range of psychiatric disorders including schizophrenia, bipolar disorder, major depression, Alzheimer's disease, and anxiety disorders. Glutamic Acid Glutamate mediates fast excitatory neurotransmission in the brain and is the transmitter for approximately 80 percent of brain synapses, particularly those associated with dendritic spines. The repolarization of neuronal membranes that have been depolarized by glutamatergic neurotransmission may account for as much as 80 percent of the energy expenditure in the brain. The concentration of glutamate in brain is 10 mM, the highest of all amino acids, of which approximately 20 percent represents the neurotransmitter pool of glutamate. The postsynaptic effects of glutamate are mediated by two families of receptors. The first are the glutamate-gated cation channels that are responsible for fast neurotransmission. The second type of glutamate receptor are the metabotropic glutamate receptors (mGluR), which are G-protein-coupled receptors like α-adrenergic receptors and dopamine receptors. The mGluRs primarily modulate glutamatergic neurotransmission. Major Glutamatergic Pathways in the Brain. All primary sensory afferent systems appear to use glutamate as their neurotransmitter including retinal ganglion cells, cochlear cells, trigeminal nerve, and spinal afferents. The thalamocortical projections that distribute afferent information broadly to the cortex are glutamatergic. The pyramidal neurons of the corticolimbic regions, the major source of intrinsic, associational, and efferent excitatory projections from the cortex, are glutamatergic. A temporal lobe circuit that figures importantly in the development of new memories is a series of four glutamatergic synapses: The perforant path innervates the hippocampal granule cells that innervate CA3 pyramidal cells that innervate CA1 pyramidal cells. The climbing fibers innervating the cerebellar cortex are glutamatergic as well as the corticospinal tracks. Ionotropic Glutamate Receptors. Three families of ionotropic glutamate receptors have been identified on the basis of selective activation by conformationally restricted or synthetic analogs of glutamate. These include α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainic acid (KA), and N-methyl-D-

aspartic acid (NMDA) receptors. Subsequent cloning revealed 16 mammalian genes that encode structurally related proteins, which represent subunits that assemble into functional receptors. Glutamate-gated ion channel receptors appear to be tetramers, and subunit composition affects both the pharmacologic and the biophysical features of the receptor. Metabotropic Glutamate Receptors. These receptors are so designated because their effects are mediated by G proteins. All mGluRs are activated by glutamate, although their sensitivities vary remarkably. To date, eight mGluRs have been cloned. These genes encode for seven membrane-spanning proteins that are members of the superfamily of G-protein-coupled receptors.

The Role of Astrocytes. Specialized end-feet of the astrocyte surround glutamatergic synapses. The astrocyte expresses the two Na+-dependent glutamate transporters that play the primary role in removing glutamate from the synapse, thereby terminating its action: EAAT1 and EAAT2 (excitatory amino acid transporter). The neuronal glutamate transporter, EAAT3, is expressed in upper motor neurons, whereas EAAT4 is expressed primarily in cerebellar Purkinje cells and EAAT5 in retina. Mice homozygous for null mutations of either EAAT1 or EAAT2 exhibit elevated extracellular glutamate and excitotoxic neurodegeneration. Notably, several studies have described the loss of EAAT2 protein and transport activity in the ventral horn in amyotrophic lateral sclerosis. The astrocytes express AMPA receptors so that they can monitor synaptic glutamate release. GlyT1, which maintains subsaturating concentrations of glycine in the synapse, is expressed on the astrocyte plasma membrane. GlyT1 transports three Na+ out for each molecule of glycine transported into the astrocyte. This stoichiometry results in a robust reversal of the direction of transport when glutamate released in the synapse activates the AMPA receptors on the astrocyte, thus depolarizing the astrocyte. Thus glycine release in the synapse by GlyT1 is coordinated with glutamatergic neurotransmission. Similarly, activation of the astrocyte AMPA receptors causes GRIP to dissociate from the AMPA receptor and bind to serine racemase, activating it to synthesize D-serine. D-Serine levels are also determined by D-amino acid oxidase (DAAO) with low D-serine levels in the cerebellum and brainstem where DAAO expression is high, and high D-serine levels are found in corticolimbic brain regions where DAAO expression is quite low. In contrast, the expression of GlyT1 is highest in the cerebellum and brainstem. This distribution suggests that D-serine is the primary modulator of the NMDA receptor in the forebrain, whereas glycine is more prominent in the brainstem and cerebellum. Plasticity in Glutamatergic Neurotransmission. The extinction of conditioned fear has been shown to be an active process mediated by the activation of NMDA receptors in the amygdala. Treatment of rats with NMDA receptor antagonists prevents the extinction of conditioned fear, whereas treatment with the glycine modulatory site partial agonist D-cycloserine facilitates the extinction of conditioned fear. (DCycloserine is an antibiotic used to treat tuberculosis that has 50 percent of the efficacy of glycine at the NMDA receptor.) To determine whether the phenomenon generalizes to humans, patients with acrophobia were administered either placebo or a single dose of D-cycloserine along with cognitive behavioral therapy (CBT). D-Cycloserine plus CBT resulted in a highly significant reduction in acrophobic symptoms that persisted for at least 3 months as compared to placebo plus CBT. Other placebo-controlled clinical trials support the notion that D-cycloserine is a robust enhancer of CBT, suggesting that pharmacologically augmenting neural plasticity may be used to bolster psychological interventions. Fragile X mental retardation protein (FMRP), which is deficient in individuals with fragile X syndrome, appears to be synthesized locally within the spine during times of NMDA receptor activation and also plays a role in transporting specific mRNAs to the spine for translation. Notably, mice in which the FMRP gene has been inactivated through a null mutation

as well as patients with fragile X syndrome have fewer dendritic spines, the preponderance of which have an immature morphology. Loss of FMRP exaggerates responses of mGluR5, which stimulates dendritic protein synthesis, and treatment with an mGluR5 antagonist reverses the fragile-X-like phenotype in mice with the FMRP gene inactivated. Excitotoxicity. In the early 1970s, it was shown that the systemic administration of large amounts of monosodium glutamate to immature animals resulted in the degeneration of neurons in brain regions where the blood–brain barrier was deficient. Excitotoxicity has also been implicated in the proximate cause of neuronal degeneration in Alzheimer's disease. Most evidence points to the toxic consequences of

aggregates of β-amyloid, especially β-amyloid1–42. The β-amyloid fibrils depolarize neurons, resulting in loss of the Mg2+ block and enhanced NMDA receptor sensitivity to glutamate. The fibrils also impair glutamate transport into astrocytes, thereby increasing the extracellular concentration of glutamate. β-Amyloid directly promotes oxidative stress through inflammation that further contributes to neuronal vulnerability to glutamate. Thus, several mechanisms contribute to neuronal vulnerability to NMDAreceptor-mediated excitotoxicity in Alzheimer's disease. Memantine, a recently approved treatment for mild to moderate Alzheimer's disease, is a weak noncompetitive inhibitor of NMDA receptors. It reduces tonic sensitivity of NMDA receptors to excitotoxicity but does not interfere with "phasic" neurotransmission, thereby attenuating neuronal degeneration in Alzheimer's disease. Inhibitory Amino Acids: GABA GABA is the major inhibitory neurotransmitter in the brain, where it is broadly distributed and occurs in millimolar concentrations. In view of its physiological effects and distributions, it is not surprising that the dysfunction of GABAergic neurotransmission has been implicated in a broad range of neuropsychiatric disorders including anxiety disorders, schizophrenia, alcohol dependence, and seizure disorders. Chemically, GABA differs from glutamic acid, the major excitatory neurotransmitter, simply by the removal of a single carboxyl group from the latter. GABA is synthesized from glutamic acid by glutamic acid decarboxylase (GAD), which catalyzes the removal of the α-carboxyl group. In the CNS, the expression of GAD appears to be restricted to GABAergic neurons, although in the periphery it is expressed in pancreatic islet cells. Two distinct but related genes encode GAD. GAD65 is localized to nerve terminals, where it is responsible for synthesizing GABA that is concentrated in the synaptic vesicles. Consistent with its role in fast inhibitory neurotransmission, mice homozygous for a null mutation of GAD65 have an elevated risk for seizures. GAD67 appears to be the primary source for neuronal GABA because mice homozygous for a null mutation of GAD67 die at birth, have a cleft pallet, and exhibit major reductions in brain GABA. GABA is catabolized by GABA transaminase (GABA-T) to yield succinic semialdehyde. Transamination generally occurs when the parent compound, α-ketoglutarate, is present to receive the amino group, thereby regenerating glutamic acid. Succinic semialdehyde is oxidized by succinic semialdehyde dehydrogenase (SSADH) into succinic acid, which re-enters the Krebs cycle. GABA-T is a cell surface, membrane-bound enzyme expressed by neurons and glia, which is oriented toward the extracellular compartment. As would be anticipated, drugs that inhibit the catabolism of GABA have anticonvulsant properties. One of the mechanisms of action of valproic acid is the competitive inhibition of GABA-T. γ-Vinyl-GABA is a suicide substrate inhibitor of GABA-T that is used as an anticonvulsant in Europe (vigabatrin [Sabril]). The synaptic action of GABA is also terminated by high-affinity transport back into the presynaptic terminal as well as into astrocytes. Four genetically distinct GABA high-

affinity transporters have been identified with differing kinetic and pharmacological characteristics. They all share homology with other neurotransmitter transporters with the characteristic of 12 membrane-spanning domains. The active transport is driven by the sodium gradient so that upon depolarization, transportation of GABA out of the neuron is favored. GABA transported into astrocytes is catabolyzed by GABA-T and ultimately converted to glutamic acid and then to glutamine, which is transported back into the presynaptic terminal for GABA synthesis. Tiagabine (Gabitril) is a potent GABA transport inhibitor that is used to treat epilepsy. Preliminary results suggest that it also may be effective in panic disorder. GABAA Receptors. GABAA receptors are distributed throughout the brain. The GABAA complex, when activated, mediates an increase in membrane conductance with an equilibrium potential near the resting membrane potential of –70 mV (Fig. 1.4-9). In the mature neuron, this typically results with an influx of Cl−, causing membrane hyperpolarization. Hyperpolarization is inhibitory because it increases the threshold for generating an action potential. In immature neurons, which have unusually high levels of intracellular Cl−, activating the GABAA receptor can counterintuitively cause depolarization. For this reason, anticonvulsants that act by enhancing GABAA receptor activity may actually exacerbate seizures in the neonatal period. FIGURE 1.4-9 Schematic representation of the GABAA receptor. The receptor-channel complex is a heteropentamer. The GABA binding site is at the interface of the α and β subunits. The benzodiazepine binding site is at the interface between the γ and α subunits. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:81.)

Barbiturates such as phenobarbital and pentobarbital are noted for their sedative and anticonvulsant activities. Barbiturates allosterically increase the affinities of the binding sites for GABA and benzodiazepines at concentrations that are pharmacologically relevant. Barbiturates also affect channel dynamics by markedly increasing the long open state and reducing the short open state, thereby increasing Cl− inhibition. Chemically modified analogs of progesterone and corticosterone have been shown in behavioral studies to have sedative and anxiolytic effects through their interaction with the GABAA receptor complex. They share features with barbiturates, although they act at a distinctly different site. Thus, they allosterically enhance agonist ligand binding to the receptor and increase the duration of chloride channel opening. A variety of behavioral effects associated with steroid administration or fluctuation of endogenous steroids and sex-specific effects of GABAergic drugs have been linked to the action of endogenous neurosteroids. With regard to GABAA receptor antagonists, picrotoxin, like the barbiturates, alters channel dynamics but in the opposite direction by reducing long open states and favoring the briefest open state. The proconvulsant pentylenetetrazol also acts by reducing chloride channel permeability. Penicillin, which at high concentrations is proconvulsant, binds to the positively charged residues in the channel, thereby occluding it. As a general class, anesthetics including barbiturates, steroids, and volatile anesthetics increase chloride conductance, thereby inhibiting neurotransmissions. Amino acids in the membrane-spanning domain of the GABA receptor subunits confer sensitivity to anesthetics. The precise mechanism whereby ethanol enhances GABAA receptor function remains unclear due to inconsistent results, suggesting that subunit composition may be important. However, recent studies suggest that ethanol increases the response of the tonic GABA-activated currents, which contain the δ subunit and exhibit remarkably high affinity to GABA. Recently, recombinant DNA strategies exploiting site-directed mutagenesis have permitted the identification of sites on the specific subunits that mediate the pharmacological action of drugs such as the benzodiazepines. Removal of the binding ability for benzodiazepines has established

that the α1 subunit plays a major role in the sedative and amnestic effects of benzodiazepines, whereas inactivating the benzodiazepine site on the α2 subunit eliminates the anxiolytic effect of benzodiazepines GABAB Receptors. The GABAB receptors are distinguished pharmacologically from GABAA receptors by the fact that they are insensitive to the canonical GABAA receptor antagonist bicuculline and that they are potently activated by baclofen [β-(4chlorophenyl)-γ-aminobutyric acid], which is inactive at GABAA receptors. They are members of the G-protein-coupled superfamily of receptors but are highly unusual, as they are made of a dimer of two seven-transmembrane-spanning subunits. GABAB receptors are widely distributed throughout the nervous system and are localized both presynaptically and postsynaptically. The postsynaptic GABAB receptors cause a longlasting hyperpolarization by activating potassium channels. Presynaptically, they act as autoreceptors and heteroreceptors to inhibit neurotransmitter release.

Glycine as a Neurotransmitter. Glycine is an inhibitory neurotransmitter primarily in the brainstem and spinal cord, although the expression of glycine receptor subunits in the thalamus, cortex, and hippocampus suggest a broader role. Glycine is a nonessential amino acid that is synthesized in the brain from L-serine by serine hydroxymethyltransferase. Glycine is concentrated within synaptic vesicles by H+- dependent vesicular inhibitory amino acid transporter (VIAAT or VGAT), which also transports GABA. Termination of the synaptic action of glycine is through reuptake into the presynaptic terminal by the glycine transporter II (GlyT2), which is quite distinct from GlyT1 that is expressed in astrocytes and modulates NMDA receptor function. The inhibitory effects of glycine are mediated by a ligand-gated chloride channel, which can also respond to β-alanine, taurine, L-alanine, L-serine, and proline, but not to GABA. The canonical antagonist for the glycine receptor is the plant alkaloid strychnine. The receptor was first identified through the specific binding of [3H]strychnine. [3H]Glycine binds to two sites: One that is displaceable by strychnine and represents the glycine A receptor and a second that is insensitive to strychnine and is designated the glycine B receptor, representing the glycine modulatory site on the NMDA receptor. Neuropsychiatric Implications of Amino Acid Transmitters Schizophrenia. Evidence accumulating from postmortem, pharmacological, and genetic studies is shifting the focus of the pathophysiology of schizophrenia from dopamine to glutamate and GABA. Indeed, after the use of D2 receptor antagonists as the sole treatment of schizophrenia for the last 50 years, more than two thirds of the treated patients remain substantially disabled. Early postmortem studies indicated a reduction in the activity of GAD in the cortex in patients with schizophrenia as compared to suitable controls. With the advent of immunocytochemistry and gene expression techniques, it has been possible to more precisely define the GABAergic deficit in schizophrenia. It appears that the parvalbumin-positive GABAergic interneurons in the intermediate layers of the cortex bear the brunt of the pathology, which includes reduced expression of GAD67, parvalbumin, and the GABA transporter (GAT). The finding that GABAA receptors are upregulated, as measured by autoradiography or with antibodies, supports the theory that these changes reflect hypofunction of the presynaptic GABAergic neurons. These particular GABAergic interneurons, which include the chandelier cells, play an important role in negative feedback inhibition to the pyramidal cells in the cortex. Despite this highly reproducible neuropathology, genes related to GABAergic function have not figured prominently in genomewide searches, suggesting that GABAergic deficits may be a downstream consequence of some more proximal genetic defects. The theory that hypofunction of NMDA receptors is an etiologic factor in schizophrenia initially arose from the observation that phencyclidine (PCP) and related dissociative anesthetics that block NMDA receptors produce a syndrome that can be indistinguishable from schizophrenia (Fig. 1.4-10). Dissociative anesthetics

are so named because they prevent

the acquisition of new memories while the patient is apparently conscious. In fact under laboratory conditions, low-dose infusion of ketamine can produce the positive symptoms, negative symptoms, and specific cognitive deficits associated with schizophrenia in clear consciousness. Subsequent studies indicated that low-dose ketamine can also cause enhanced amphetamine-induced subcortical dopamine release as is observed in schizophrenia as well as abnormal cortical eventrelated potentials (ERPs) and disruption of prepulse inhibition in experimental animals. FIGURE 1.4-10 Pathological circuit in schizophrenia. The NMDA receptors on the rapidly firing parvalbumin (PV) expressing GABAergic interneurons in the intermediate levels of the cortex are disproportionately sensitive to antagonists or loss of the coagonist, D-serine. NMDA receptor hypofunction causes reduced expression of PV, GAD67, and the GABA transporter and upregulation of GABAA receptors on pyramidal neurons. Disinhibition of the pyramidal neurons causes cognitive dysfunction and negative symptoms and drives excessive subcortical dopamine release resulting in psychosis. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:83.) A number of putative risk genes for schizophrenia are closely associated with NMDA receptor function. DAAO, which encodes a protein that activates D-amino acid oxidase, has been repeatedly linked to the risk of schizophrenia. D-Amino acid oxidase itself has been associated with increased risk. Recently an allelic variant of serine racemase in the promoter region has also been associated with the risk for schizophrenia. Each of these gene variants could reduce the availability of D-serine in the cortex, thereby impairing NMDA receptor function. Notably, cerebrospinal fluid (CSF) and blood levels of D-serine are significantly reduced in patients with schizophrenia. Neuregulin 1 appears to be a convincing risk gene and interacts directly with NMDA receptors. Dysbindin, another risk gene, is expressed in glutamatergic terminals. mGluR3, which downregulates glutamate release, has also been associated with schizophrenia. Recent findings have provided a link between the GABAergic neuropathology and NMDA receptor hypofunction. Chronic treatment of rats with NMDA receptor antagonists causes a downregulation of GAD67, parvalbumin, and GAT. The sensitive subpopulation of GABAergic neurons is the rapidly firing interneurons that provide the perisomatic innervation of the pyramidal cells. Their NMDA receptors appear to be much more sensitive to antagonists than those less active

GABAergic neurons and pyramidal cells. The subtly reduced GABAergic inhibition results in a disinhibition of the glutamatergic pyramidal output. This degradation of the inhibitory feedback could account for the cognitive deficits and negative symptoms in schizophrenia, and the disinhibited output also results in elevated subcortical dopamine release and psychosis. Thus psychosis would be considered a downstream event resulting from a disruption in critical glutamatergic– GABAergic synaptic function in the cerebral cortex. Anxiety and Depression. GABAergic dysfunction has been associated with anxiety disorders, especially panic disorder, as well as with major depressive disorder. Clinically, there is considerable comorbidity between anxiety and affective disorders. Decreased levels of the GABAA receptor modulators, the three α-reduced neuroactive steroids, have been found both in plasma and in CSF in major depressive disorder. Effective treatment with selective serotonin reuptake inhibitors (SSRIs) increases the neurosteroid levels. In contrast, in patients with panic disorder, the plasma neurosteroid levels were significantly elevated, perhaps as a compensatory mechanism. Magnetic resonance spectroscopy (MRS) has disclosed significant reductions in GABA levels in the anterior cingulate

and in the basal ganglia of medicated patients with panic disorder. Positron emission tomography (PET) scanning reveals a highly selective reduction in benzodiazepine receptor sites bilaterally in the insular cortex in panic disorder. A genomewide screen has shown significant linkage at 15q in a region containing GABAA receptor subunit genes and panic disorder. MRS reveals significant reductions in both GABA and glutamate/glutamine (Glx) in the prefrontal cortex in major depressive disorder. Postmortem studies indicate upregulation of the GABAA receptor α1 and β3 subunits in the cerebral cortices of depressed patients who committed suicide, consistent with a reduction in GABAergic neurotransmission. The reduced levels of GABA in the occipital cortex in episodes of major depressive disorder normalized with effective treatment with SSRI or with electroconvulsive therapy. Glutamatergic dysfunction has also been implicated in depression. NMDA receptor antagonists have antidepressant effects in several animal models of depression including forced swim, tail suspension, and learned helplessness. A single injection of ketamine provides protection from the induction of behavioral despair in rats for up to 10 days. Chronic treatment with antidepressants alters the expression of NMDA receptor subunits and decreases glycine receptor B binding. Two placebo-controlled clinical trials have shown that a single dose of ketamine can produce a rapid, substantial, and persistent reduction in symptoms in patients with major depressive disorder. Alcoholism. Ethanol at concentrations associated with intoxication has a dual action of enhancing GABAergic receptor function and attenuating NMDA receptor function. The GABA receptor effects may be associated with the anxiolytic effects of ethanol. Persistent abuse and dependency on ethanol result in a downregulation of GABAA receptors and an upregulation of NMDA receptors such that acute discontinuation of ethanol results in a hyperexcitable state characterized by delirium tremens. Furthermore, supersensitive NMDA receptors in the context of thiamine

deficiency may contribute to the excitotoxic neuron degeneration of Wernicke– Korsakoff's syndrome. Acamprosate is a derivative of homotaurine that was developed as an agent to reduce alcohol consumption, craving, and relapse in alcoholic patients, for which it exhibits moderate efficacy in clinical trials. Because of taurine's resemblance to GABA, it was thought that acamprosate acted via GABAA receptors, but electrophysiological studies found little evidence to support this hypothesis. Subsequent studies demonstrated that it inhibited NMDA receptor responses in cortical slices and recombinant NMDA receptors. The precise mechanism whereby acamprosate alters NMDA receptor function, however, remains unclear. Fetal alcohol syndrome is the most common preventable cause of mental retardation. Convincing evidence has been developed that the microencephaly associated with fetal alcohol exposure results from inhibition of NMDA receptor function, resulting in widespread neuronal apoptosis in the immature cortex. NMDA receptor activation is essential for immature neuronal survival and differentiation. NEUROPEPTIDES Neuropeptides represent the most diverse class of signaling molecules in the CNS. Initially discovered for their role in the hypothalamic regulation of pituitary hormone secretion, the complex role of peptides in brain function has emerged over the last 30 years. Many neuropeptides and their receptors are widely distributed within the CNS where they have an extraordinary array of direct or neuromodulatory effects, ranging from modulating neurotransmitter release and neuronal firing patterns to the regulation of emotionality and complex behaviors. More than 100 unique biologically active neuropeptides have been identified in the brain, a subset of which is presented in Table 1.4-2. Adding to the complexity of neuropeptide systems in the CNS, the actions of many peptides are mediated via multiple receptor subtypes localized in different brain regions. In fact, the discovery of new peptides and receptor subtypes has outpaced our understanding of the roles

of these peptides in normal or aberrant CNS function. Pharmacological, molecular, and genetic approaches are now leading the way in our understanding of the contribution of neuropeptide systems in psychiatric disorders. Table 1.4-2 Selected Neuropeptide Transmitters

Neuropeptides have been implicated in the regulation of a variety of behavioral and physiological processes, including thermoregulation, food and water consumption, sex, sleep, locomotion, learning and memory, responses to stress and pain, emotion, and social cognition. Involvement in such behavioral processes suggests that neuropeptidergic systems may contribute to the symptoms and behaviors exhibited in major psychiatric illnesses such as psychoses, mood disorders, dementias, and autism spectrum disorders. Investigating Neuropeptide Function The roles of neuropeptides in CNS function and behavior have been examined using a multitude of experimental techniques. The levels of analysis include the following:

Molecular structure and biosynthesis of the peptide and its receptor(s), the neuroanatomical localization of the peptide and its receptor(s), the regulation of the expression and release of the peptide, and the behavioral effects of the peptide. Most information on neuropeptide biology is derived from laboratory animal studies; however, there is a growing database on the localization, activity, and potential psychiatric relevance of several neuropeptide systems in humans. Most neuropeptide structures have been identified based on the chemical analysis of purified biologically active peptides, leading ultimately to the cloning and characterization of the genes encoding them. Characterization of the gene structure of peptides and their receptors has provided insight into the molecular regulation of these systems, and their chromosomal localization is useful in genetic studies examining the potential roles of these genes in psychiatric disorders. Structural characterization permits the production of immunological and molecular probes that are useful in determining peptide distribution and regulation in the brain. Quantitative radioimmunoassays on microdissected brain regions or immunocytochemistry on brain sections are typically used to localize the distribution of peptide within the brain. Both techniques use specific antibodies generated against the neuropeptide to detect the presence of the peptide. Immunocytochemistry allows researchers to visualize the precise cellular localization of peptide-synthesizing cells as well as their projections throughout the brain, although the technique is generally not quantitative. With molecular probes homologous to the mRNA encoding the peptides or receptor, in situ hybridization can be used to localize and quantify gene expression in brain sections. This is a powerful technique for examining the molecular regulation of neuropeptide synthesis with precise neuroanatomical resolution, which is impossible for other classes of nonpeptide neurotransmitters that are not derived directly from the translation of mRNAs, such as dopamine, serotonin, and norepinephrine. Generally, the behavioral effects of neuropeptides are initially investigated by infusions of the peptide directly into the brain. Unlike many nonpeptide neurotransmitters, most neuropeptides do not penetrate the blood–brain barrier in amounts sufficient enough to produce CNS effects. Furthermore, serum and tissue enzymes tend to degrade the peptides before they reach their target sites. The degradation is usually the result of the cleavage of specific amino acid sequences targeted by a specific peptidase designed for that purpose. Thus, intracerebroventricular (ICV) or site-specific infusions of peptide in animal models are generally required to probe for behavioral effects of peptides. However, there are some examples of delivery of neuropeptides via intranasal infusions in human subjects, which in some cases has been shown to permit access of the peptide to the brain. One of the greatest impediments for exploring the roles and potential therapeutic values of neuropeptides is the inability of the peptides or their agonists/antagonists to penetrate

the blood–brain barrier. Thus the behavioral effects of most peptides in humans are largely uninvestigated, with the exception of a few studies utilizing intranasal delivery. However, in some instances small-molecule, nonpeptide agonists/antagonists have been developed that can be administered peripherally and permeate the blood–brain barrier in sufficient quantities to affect receptor activation. The use of pretreatment and posttreatment CSF samples or of samples obtained during the active disease state versus when the patient is in remission addresses some of

the serious limitations in study design. For such progressive diseases as schizophrenia or Alzheimer's disease, serial CSF samples may be a valuable indicator of disease progression or response to treatment. Even with these constraints, significant progress has been made in describing the effects of various psychiatric disease states on neuropeptide systems in the CNS. Biosynthesis Unlike other neurotransmitters, the biosynthesis of a neuropeptide involves the transcription of an mRNA from a specific gene, translation of a polypeptide preprohormone encoded by that mRNA, and then posttranslational processing involving proteolytic cleavage of the preprohormone to yield the active neuropeptide. Over the last 25 years the gene structures and biosynthetic pathways of many neuropeptides have been elucidated. The gene structure of selected neuropeptides is illustrated in Figure 1.411. Neuropeptide genes are generally composed of multiple exons that encode a protein preprohormone. The N-terminus of the preprohormone contains a signal peptide sequence, which guides the growing polypeptide to the rough endoplasmic reticulum (RER) membrane. The single preprohormone molecule often contains the sequences of multiple peptides that are subsequently separated by proteolytic cleavage by specific enzymes. For example, translation of the gene encoding NT yields a preprohormone, which upon enzymatic cleavage produces both NT and neuromedin N.

FIGURE 1.4-11 Schematics illustrating the gene structure, preprohormone messenger RNA (mRNA), and processed neuropeptides of thyrotropin-releasing hormone (TRH), corticotropinreleasing factor (CRF), oxytocin (OT), arginine vasopressin (AVP), and neurotensin (NT). Boxed regions indicate the locations of the exons in the respective genes. Shaded or hatched regions indicate coding regions. Each preprohormone begins with a signal

peptide (SP) sequence. Black boxes indicate the locations of the sequences encoding the neuropeptide. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:87.) Distribution and Regulation Although many neuropeptides were originally isolated from pituitary and peripheral tissues, the majority of neuropeptides were subsequently found to be widely distributed throughout the brain. Those peptides involved in regulating pituitary secretion are concentrated in the hypothalamus. Hypothalamic releasing and inhibiting factors are produced in neurosecretory neurons adjacent to the third ventricle that send projections to the median eminence where they contact and release peptide into the hypothalamohypophysial portal circulatory system. Peptides produced in these neurons are often subject to regulation by the peripheral hormones that they regulate. For example, thyrotropin-releasing hormone (TRH) regulates the secretion of thyroid hormones, and thyroid hormones negatively feedback on TRH gene expression. However, neuropeptide-expressing neurons and their projections are found in many other brain regions, including limbic structures, midbrain, hindbrain, and spinal cord. Neuropeptide Signaling Neuropeptides may act as neurotransmitters, neuromodulators, or neurohormones. Neurotransmitters are typically released from axonal terminals into a synapse where they change

the postsynaptic membrane potential, either depolarizing or hyperpolarizing the cell. For classical neurotransmitters, this often involves direct modulation of voltage-gated ion channels. In contrast, neuromodulators and neurohormones do not directly affect the firing of the target cell itself but may alter the response of the cell to other neurotransmitters through the modulation of second messenger pathways. Neuropeptide release is not restricted to synapses or axon terminals but may occur throughout the axon or even from dendrites. The cellular signaling of neuropeptides is mediated by specific neuropeptide receptors. Thus understanding neuropeptide receptor function is essential for understanding neuropeptide biology. Neuropeptide receptors have undergone the same process of discovery and characterization that receptors for other neurotransmitters have enjoyed. Most neuropeptide receptors are G-protein-coupled, seventransmembrane domain receptors belonging to the same family of proteins as the monoamine receptors. Molecular technology has made it possible to clone and characterize neuropeptide receptor genes and complementary DNAs (cDNAs). This is most often accomplished in one of three ways. First, the neuropeptide receptor protein is biochemically purified and partially sequenced, which allows the development of oligonucleotide probes that can be used to isolate the cDNA encoding the protein from a cDNA library. A second approach involves producing expression libraries in which cells containing the receptor cDNA can be isolated based on their ability to bind to a radiolabeled peptide ligand. Finally, many neuropeptide receptors are now isolated based on their sequence homology with other known peptide

receptors. Once the cDNA of the receptor has been isolated, it can be used to produce purified receptor protein for structural and functional studies. By mutation of specific amino acids in the receptor structure and determination of relative binding affinities of peptides with various amino acid substitutions, it is possible to elucidate the nature of the ligand–receptor interaction. This information facilitates the development of drugs that specifically modulate receptor function, including nonpeptide drugs, leading to the ability to manipulate peptide systems in ways that are currently enjoyed by the more classic neurotransmitters. The availability of cDNAs encoding the receptor also permits the neuroanatomical mapping of the receptor-producing cells in the brain, which is critical for understanding the neural circuits modulated by the peptide. Finally, with the cloned receptor in hand, it is possible to use transgenic techniques, such as targeted gene overexpression or gene knockouts, to further elucidate the functions of these receptors. siRNA techniques now allow the targeted synthesis disruption of specific receptor populations, allowing researchers to examine the roles of these receptor populations on physiology and behavior. The following three factors determine the biological roles of a neuropeptide hormone: (1) the temporal–anatomical release of the peptide, (2) functional coupling of the neuropeptide receptor to intracellular signaling pathways, and (3) the cell type and circuits in which the receptor is expressed. Genetic studies have demonstrated that regulatory sequences flanking the receptor coding region determine the expression pattern of the receptor and thus the physiological and behavioral response to the neuropeptide. Peptidases Unlike monoamine neurotransmitters, peptides are not actively taken up by presynaptic nerve terminals. Rather, released peptides are degraded into smaller fragments, and eventually into single amino acids by specific enzymes termed peptidases. The enzymes may be found bound to presynaptic or postsynaptic neural membranes or in solution in the cytoplasm and extracellular fluid, and they are distributed widely in peripheral organs and serum as well as in the CNS. As a result, neuropeptides generally have halflives on the order of minutes once released. Specific Neuropeptides as Prototypes of Neuropeptide Biology Thyrotropin-Releasing Hormone. In 1969, TRH, a

pyroglutamylhistidylprolinamide tripeptide (Table 1.4-3), became the first of the hypothalamic releasing hormones to be isolated and characterized. The discovery of the structure of this hormone led to the conclusive demonstration that peptide hormones secreted from the hypothalamus regulate the secretion of hormones from the anterior pituitary. The gene for TRH in humans resides on chromosome 3q13.3-q21. In the rat it consists of three exons (coding regions) separated by two introns (noncoding sequences) (see Fig. 1.4-11). The first exon contains the 5′ untranslated region of the mRNA encoding the TRH preprohormone, the second exon contains the signal peptide (SP) sequence and much of the remaining N-terminal end of the precursor peptide, and the third contains the remainder of the sequence, including five copies of the TRH precursor sequence, the C-terminal region, and the 3′ untranslated region. The 5′ flanking of the

gene, or promoter, contains sequences homologous to the glucocorticoid receptor and the thyroid hormone receptor DNA binding sites, providing a mechanism for the regulation of this gene by cortisol and negative feedback by thyroid hormone. Enzymatic processing of TRH begins with excision of the progenitor peptides by carboxypeptidases, amidation of the C-terminal proline, and cyclization of the Nterminal glutamine to yield five TRH molecules per prohormone molecule. TRH is widely distributed in the CNS with TRH immunoreactive neurons being located in the olfactory bulbs, entorhinal cortices, hippocampus, extended amygdala, hypothalamus, and midbrain structures. As is the case for most neuropeptides, the TRH receptor is also a member of the seven-transmembrane domain, G-protein-coupled receptor family. Table 1.4-3 Selected Neuropeptide Structures Hypothalamic TRH neurons project nerve terminals to the median eminence; there they release TRH into the hypothalamohypophyseal portal system where it is transported to the adenohypophysis, causing the release of thyroid-stimulating hormone (TSH) into systemic circulation. TSH subsequently stimulates the release of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) from the thyroid gland. TRH neurons in the paraventricular nucleus (PVN) contain thyroid hormone receptors and respond to increases in thyroid hormone secretion with a decrease in TRH gene expression and synthesis. This negative feedback of thyroid hormones on the TRH-synthesizing neurons was first demonstrated by a decrease in TRH content in the median eminence, but not in the PVN of the hypothalamus, after thyroidectomy. This effect can be reversed with exogenous thyroid hormone treatment. The treatment of normal rats with exogenous thyroid hormone decreases TRH concentration in the PVN and the posterior nucleus of the hypothalamus. With a probe against the TRH preprohormone mRNA, in situ hybridization studies have demonstrated that TRH mRNA is increased in the PVN 14 days after thyroidectomy. The ability of thyroid hormones to regulate TRH mRNA can be

superseded by other stimuli that activate the hypothalamic–pituitary–thyroid (HPT) axis. In that regard, repeated exposure to cold (which releases TRH from the median eminence) induces increases in the levels of TRH mRNA in the PVN despite concomitantly elevated concentrations of thyroid hormones. Further evidence of the different levels of communication of the HPT axis are seen in the ability of TRH to regulate the production of mRNA for the pituitary TRH receptor and for TRH concentrations to regulate the mRNA coding for both the α and β subunits of the thyrotropin (TSH) molecule. In addition, TRH-containing synaptic boutons have been observed in contact with TRH-containing cell bodies in the medial and periventricular subdivisions of the paraventricular nucleus, thus providing anatomical evidence for ultrashort feedback regulation of TRH release. Negative feedback by thyroid hormones may be limited to the hypothalamic TRH neurons because negative feedback on TRH synthesis by thyroid hormones has not been found in extrahypothalamic

TRH neurons. The early availability of adequate tools to assess HPT axis function (i.e., radioimmunoassays and synthetic peptides), coupled with observations that primary hypothyroidism is associated with depressive symptomatology, ensured extensive investigation of the involvement of this axis in affective disorders. Early studies established the hypothalamic and extrahypothalamic distribution of TRH. This extrahypothalamic presence of TRH quickly led to speculation that TRH might function as a neurotransmitter or neuromodulator. Indeed, a large body of evidence supports such a role for TRH. Within the CNS, TRH is known to modulate several different neurotransmitters, including dopamine, serotonin, acetylcholine, and the opioids. TRH has been shown to arouse hibernating animals and counteracts the behavioral response and hypothermia produced by a variety of CNS depressants including barbiturates and ethanol. The use of TRH as a provocative agent for the assessment of HPT axis function evolved rapidly after its isolation and synthesis. Clinical use of a standardized TRH stimulation test, which measures negative feedback responses, revealed blunting of the TSH response in approximately 25 percent of euthyroid patients with major depression. These data have been widely confirmed. The observed TSH blunting in depressed patients does not appear to be the result of excessive negative feedback due to hyperthyroidism because thyroid measures such as basal plasma concentrations of TSH and thyroid hormones are generally in the normal range in these patients. It is possible that TSH blunting is a reflection of pituitary TRH receptor downregulation as a result of median eminence hypersecretion of endogenous TRH. Indeed, the observation that CSF TRH concentrations are elevated in depressed patients as compared to those of controls supports the hypothesis of TRH hypersecretion but does not elucidate the regional CNS origin of this tripeptide. In fact, TRH mRNA expression in the PVN of the hypothalamus is decreased in patients with major depression. However, it is not clear whether the altered HPT axis represents a causal mechanism underlying the symptoms of depression or simply a secondary effect of depression-associated alterations in other neural systems. Corticotropin-Releasing Factor (CRF) and Urocortins. There is convincing evidence to support the hypothesis that CRF and the urocortins play a complex role in integrating the endocrine, autonomic, immunological, and behavioral responses of an organism to stress. Although it was originally isolated because of its functions in regulating the hypothalamic–pituitary–adrenal (HPA) axis, CRF is widely distributed throughout the brain. The PVN of the hypothalamus is the major site of CRF-containing cell bodies that influence anterior pituitary hormone secretion. These neurons originate in the

parvocellular region of the PVN and send axon terminals to the median eminence, where CRF is released into the portal system in response to stressful stimuli. A small group of PVN neurons also projects to the brainstem and spinal cord where they regulate autonomic aspects of the stress response. CRF-containing neurons are also found in other hypothalamic nuclei, the neocortex, the extended amygdala, brainstem, and spinal cord. Central CRF infusion into laboratory animals produces physiological changes and behavioral effects similar to those observed following stress, including increased locomotor activity, increased responsiveness to an acoustic startle, and decreased exploratory behavior in an open field. The physiological and behavioral roles of the urocortins are less understood, but several studies suggest that urocortins 2 and 3 are anxiolytic and may dampen the stress response. This has led to the hypothesis that CRF and the urocortins act in opposition, but this is likely an oversimplification. Urocortin 1 is primarily synthesized in the Edinger–Westphal nucleus, lateral olivary nucleus, and supraoptic hypothalamic nucleus. Urocortin 2 is synthesized primarily in the hypothalamus, while urocortin 3 cell bodies are found more broadly in the extended amygdala, perifornical area, and preoptic area. Hyperactivity of the HPA

axis in major depression remains one of the most consistent findings in biological psychiatry. The reported HPA axis alterations in major depression include hypercortisolemia, resistance to dexamethasone suppression of cortisol secretion (a measure of negative feedback), blunted adrenocorticotropic hormone (ACTH) responses to intravenous CRF challenge, increased cortisol responses in the combined dexamethasone/CRF test, and elevated CSF CRF concentrations. The exact pathological mechanism(s) underlying HPA axis dysregulation in major depression and other affective disorders remains to be elucidated. Mechanistically, two hypotheses have been advanced to account for the ACTH blunting following exogenous CRF administration. The first hypothesis suggests that pituitary CRF receptor downregulation occurs as a result of hypothalamic CRF hypersecretion. The second hypothesis postulates altered sensitivity of the pituitary to glucocorticoid negative feedback. Substantial support has accumulated favoring the first hypothesis. However, neuroendocrine studies represent a secondary measure of CNS activity; the pituitary ACTH responses principally reflect the activity of hypothalamic CRF rather than that of the corticolimbic CRF circuits. The latter of the two are more likely to be involved in the pathophysiology of depression. Of particular interest is the demonstration that the elevated CSF CRF concentrations in drug-free depressed patients are significantly decreased after successful treatment with electroconvulsive therapy (ECT), indicating that CSF CRF concentrations, like hypercortisolemia, represent a state rather than a trait marker. Other recent studies have confirmed this normalization of CSF CRF concentrations following successful treatment with fluoxetine. One group demonstrated a significant reduction of elevated CSF CRF concentrations in 15 female patients with major depression who remained depression free for at least 6 months following antidepressant treatment, as compared to little significant treatment effect on CSF CRF concentrations in 9 patients who relapsed in this 6-month period. This suggests that elevated or increasing CSF CRF

concentrations during antidepressant treatment may be the harbinger of a poor response in major depression despite early symptomatic improvement. Of interest, treatment of normal subjects with desipramine or, as noted above, of individuals with depression with fluoxetine is associated with a reduction in CSF CRF concentrations. If CRF hypersecretion is a factor in the pathophysiology of depression, then reducing or interfering with CRF neurotransmission might be an effective strategy to alleviate depressive symptoms. Over the last several years, a number of pharmaceutical companies have committed considerable effort to the development of small-molecule CRF1 receptor antagonists that can effectively penetrate the blood–brain barrier. Several compounds have been produced with reportedly promising characteristics. Oxytocin (OT) and Vasopressin (AVP). The vasopressor effects of posterior pituitary extracts were first described in 1895, and the potent extracts were named AVP. OT and AVP mRNAs are among the most abundant messages in the hypothalamus, being heavily concentrated in the magnocellular neurons of the PVN and the supraoptic nucleus of the hypothalamus, which send axonal projections to the neurohypophysis. These neurons produce all of the OT and AVP that is released into the bloodstream where these peptides act as hormones on peripheral targets. OT and AVP are generally synthesized in separate neurons within the hypothalamus. OT released from the pituitary is most often associated with functions associated with female reproduction, such as regulating uterine contractions during parturition and the milk ejection reflex during lactation. AVP, also known as antidiuretic hormone, regulates water retention in the kidney and vasoconstriction through interactions with vasopressin V2 and V1a receptor subtypes, respectively. AVP is released into the bloodstream from the neurohypophysis following a variety of stimuli including plasma osmolality, hypovolemia,

hypertension, and hypoglycemia. The actions of OT are mediated via a single receptor subtype (oxytocin receptor, OTR), which is distributed in the periphery and within the limbic CNS. In contrast to the OTR there are three vasopressin receptor subtypes, V1a, V1b, and V2 receptors, each of which are G-protein-coupled, seventransmembrane domain receptors. The V2 receptor is localized in the kidney and is not found in the brain. The V1a receptor is distributed widely in the CNS and is thought to mediate most of the behavioral effects of AVP. The V1b receptor is concentrated in the anterior pituitary, and some reports describe V1b receptor mRNA in the brain, although its function is unknown. Neurotensin (NT) Although NT is found in a number of brain regions, it has been most thoroughly investigated in terms of its association with other neurotransmitter systems, particularly the mesolimbic dopamine system, and has gained interest in research on the pathophysiology of schizophrenia. There are several lines of evidence suggesting that NT and its receptors should be considered as potential targets for pharmacological intervention in this disorder. First, the NT system is positioned anatomically to modulate

the neural circuits implicated in schizophrenia. Second, peripheral administration of antipsychotic drugs has been shown to consistently modulate NT systems. Third, there is evidence that central NT systems are altered in patients with schizophrenia. NT was first shown to interact with dopamine systems while undergoing characterization of its potent hypothermicpotentiating and sedative-potentiating activities. Subsequent work indicated that NT possessed many properties that were also shared by antipsychotic drugs, including the ability to inhibit avoidance, but not escape responding in a conditioned active avoidance task; the ability to block the effects of indirect dopamine agonists or endogenous dopamine in the production of locomotor behavior; and the ability to elicit increases in dopamine release and turnover. Perhaps most importantly, both antipsychotic drugs and NT neurotransmission enhance sensorimotor gating. Sensorimotor gating is the ability to screen or filter relevant sensory input, deficits in which may lead to an involuntary flooding of indifferent sensory data. Increasing evidence suggests that deficits in sensorimotor gating are a cardinal feature of schizophrenia. Both dopamine agonists and NT antagonists disrupt performance on tasks designed to gauge sensorimotor gating. Unlike antipsychotic drugs, NT is not able to displace dopamine from its receptor. As noted earlier, NT is colocalized in certain subsets of dopamine neurons and is co-released with dopamine in the mesolimbic and medial prefrontal cortex dopamine terminal regions that are implicated as the sites of dopamine dysregulation in schizophrenia. Antipsychotic drugs that act at D2 and D4 receptors increase the synthesis, concentration, and release of NT in those dopamine terminal regions but not in others. That effect of antipsychotic drugs in increasing NT concentrations persists after months of treatment and is accompanied by the expected increase in NT mRNA concentrations as well as expression of the “immediate early gene” cfos within hours of initial drug treatment. The altered regulation of NT expression by antipsychotic drugs apparently extends to the peptidases that degrade the peptide, because recent reports have revealed decreased NT metabolism in rat brain slices 24 hours after the acute administration of haloperidol. When administered directly into the brain, NT preferentially opposes dopamine transmission in the nucleus accumbens but not the caudate putamen. In the nucleus accumbens, NT receptors are located predominantly on GABAergic neurons, which release GABA on dopamine terminals, thereby inhibiting release. Decreased CSF NT concentrations have been reported in several populations of patients with schizophrenia when compared to those of controls or other psychiatric disorders. Although treatment with antipsychotic drugs has been observed to increase NT concentrations in the CSF, it is not known whether this increase is causal or merely accompanies the decrease in psychotic symptoms seen with successful treatment.

Postmortem studies have shown an increase in NT concentrations in the dopamine-rich Brodmann's area 32 of the frontal cortex, but that result may have been confounded by premortem antipsychotic treatment. Other researchers have found no postmortem alterations in NT concentrations of a wide sampling of subcortical regions. Decreases in NT receptor densities in the entorhinal cortex have been reported in entorhinal cortices of schizophrenic postmortem samples. A critical test of the hypothesis that NT may act as an endogenous antipsychotic-like substance awaits the development of an NT receptor agonist that can penetrate the blood–brain barrier. Other Neuropeptides A number of other neuropeptides have been implicated in the pathophysiology of psychiatric disorders. These include,

but are not limited to, cholecystokinin (CCK), substance P, and neuropeptide Y. CCK, originally discovered in the gastrointestinal tract, and its receptor are found in areas of the brain associated with emotion, motivation, and sensory processing (e.g., cortex, striatum, hypothalamus, hippocampus, and amygdala). CCK is often colocalized with dopamine in the VTA neurons that comprise the mesolimbic and mesocortical dopamine circuits. Like NT, CCK decreases dopamine release. Infusions of a CCK fragment have been reported to induce panic in healthy individuals, and patients with panic disorder exhibit increased sensitivity to the CCK fragment compared to that of normal controls. Pentagastrin, a synthetic CCK agonist, dose-dependently produced increased blood pressure, pulse, HPA activation, and physical symptoms of panic. Recently, a CCK receptor gene polymorphism has been associated with panic disorder. The undecapeptide substance P is localized in the amygdala, hypothalamus, periaqueductal gray, LC, and parabrachial nucleus and is colocalized with norepinephrine and serotonin. Substance P serves as a pain neurotransmitter, and administration to animals elicits behavioral and cardiovascular effects resembling the stress response. More recent data suggest a role for substance P in major depression and PTSD. Both depressed and PTSD patients had elevated CSF substance P concentrations. Furthermore, in PTSD patients, marked increases in CSF substance P concentrations were detected following precipitation of PTSD symptoms. One study has indicated that a substance P receptor (termed the neurokinin 1 [NK1] receptor) antagonist capable of passing the BBB is more effective than placebo and as effective as paroxetine in patients with major depression with moderate to severe symptom severity, although subsequent studies have been unable to confirm these findings. Neuropeptide Y (NPY) is a 36 amino acid peptide found in the hypothalamus, brainstem, spinal cord, and several limbic structures and is involved in the regulation of appetite, reward, anxiety, and energy balance. NPY is colocalized with serotonergic and noradrenergic neurons and is thought to facilitate the containment of negative effects following exposure to stress. Suicide victims with a diagnosis of major depression are reported to have a pronounced reduction in NPY levels in the frontal cortex and caudate nucleus. Furthermore, CSF NPY levels are decreased in depressed patients. Chronic administration of antidepressant drugs increases neuropeptide Y concentrations in the neocortex and hippocampus in rats. Plasma NPY levels were found to be elevated in soldiers subjected to the "uncontrollable stress" of interrogation, and NPY levels were correlated with the feelings of dominance and confidence during the stress. In addition, low NPY response to stress has been associated with increased vulnerability to depression and PTSD. NOVEL NEUROTRANSMITTERS Nitric Oxide The discovery that gases could function as neurotransmitters revealed that highly atypical modes of signaling existed between neurons. In the early 1990s, nitric oxide was the first gas to be ascribed a neurotransmitter function and proved to be an atypical neurotransmitter for several reasons. First, it was not stored in or released from synaptic vesicles, as it was a small gas it could freely diffuse into the target neuron. Second, its target was not a specific receptor on the

surface of a target neuron, but intracellular proteins whose activity could directly be modulated by nitric oxide, leading to neurotransmission. Nitric oxide also lacks a reuptake mechanism to remove it from the synapse. Although enzymatic inactivation of it is postulated to exist, nitric oxide appears to have a very short half-life of a few seconds. Nitric oxide was initially discovered as a bactericidal compound released from macrophages, and as an endothelial cell it derived relaxation factor allowing for the

dilation of blood vessels. A role for nitric oxide in the brain followed, revealing a role for the gas in neurotransmission, learning and memory processes, neurogenesis, and neurodegenerative disease. Nitric Oxide and Behavior Nitric oxide neurotransmission can play a role in behavior, as neuronal nitric oxide synthase (nNOS)–deficient male mice display exaggerated aggressive tendencies and increased sexual activity. In female mice the contrary is true, as they have reduced aggression. As manic bipolar patients may show both hypersexuality and aggression, the nitric oxide pathway may participate in the psychopathology of affective states. In the periphery, nNOS localizes to neurons that innervate blood vessels of the penis, including the corpus cavernosa. Stimulation of these nerves releases nitric oxide, leading to cyclic guanosine monophosphate (cGMP) formation, blood vessel wall relaxation and vasodilation, penile engorgement, and initial erection. The sustained phase of erection also depends on nitric oxide; turbulent blood flow leads to phosphorylation of eNOS and sustained nitric oxide production. Drugs used in treatment of erectile dysfunction—sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra)—act to inhibit phosphodiesterase type 5 (PDE5), an enzyme that degrades cGMP in the penis (Fig. 1.4-12), thereby potentiating nitric oxide neurotransmission and penile erection.

FIGURE 1.4-12 Neurotransmitter and signaling functions of nitric oxide (NO) via production of cyclic guanosine monophosphate (cGMP). Gaseous nitric oxide is enzymatically generated and freely diffuses into an adjacent neuron (upper right). In comparison to traditional neurotransmitters (upper left), nitric oxide (NO) does not act via a specific neurotransmitter receptor on the surface membrane of a neuron. In contrast, NO freely diffuses across the neuronal membrane and activates the enzyme, guanylyl cyclase, which converts guanosine 5’-triphosphate (GTP) into the second messenger, cGMP. Nitric oxide effects are mediated, in part, by cGMP activation of neuronal protein kinases, new gene expression, and effects on neuronal long-term potentiation (LTP) and long-term depression (LTD). ATP, adenosine triphosphate. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:104.) Numerous lines of evidence have suggested a role for nitric oxide in the regulation of sleep–wake cycles. nNOS expressing neurons occur in several areas that initiate REM sleep, including the pons, dorsal raphe nucleus, laterodorsal tegmentum, and pedunculopontine tegmentum. In animal models, microinjection of compounds that release nitric oxide

result in decreased wakefulness and increased slow wave sleep. Consistent with this, NOS inhibitors show a trend toward decreasing slow wave and REM sleep. Studies of NOS-deficient mice suggest that nitric oxide may serve a more complex role than merely promoting sleep. nNOS-deficient animals also show reduced REM sleep; however, inducible nitric oxide synthase (iNOS)–deficient mice demonstrate the reverse, suggesting a complex interplay between NOS enzymatic isoforms. Nitric Oxide and Mood Disorders. NOS-expressing neurons are well represented in areas implicated in depression, including the dorsal raphe nucleus and prefrontal cortex. A role for nitric oxide has been suggested in antidepressant response, as SSRI

antidepressants can directly inhibit NOS activity. Moreover, in animal studies such as the forced swim test, NOS and soluble guanylyl cyclase inhibitors can achieve antidepressant-like effects. Plasma nitric oxide levels were elevated in patients with bipolar disorder compared to healthy controls. However, in depressed subjects, studies have found decreased nitric oxide levels and increased plasma nitrite, a byproduct of nitric oxide. Reduced NOS has also been described in the paraventricular nucleus of patients with schizophrenia and depression compared to controls. Nitric oxide has been questioned as to its ability to regulate neurotransmission at serotonin, norepinephrine, and dopamine nerve termini. However, there has been no clear consensus, and nitric oxide appears to be able to increase or decrease activity at these neurons depending on the timing of its activation and the region of the brain studied. Nitric Oxide and Schizophrenia. Nitric oxide has been investigated as a candidate molecule contributing to symptoms of schizophrenia. Two genetic studies have identified schizophrenia-associated single nucleotide polymorphisms (SNPs) in CAPON, a protein that associates with nNOS. SNPs in nNOS itself have been associated with schizophrenia, although others have not been able to reproduce such findings. Changes in NOS levels have been reported in postmortem brain samples of individuals with schizophrenia. Abnormalities have been noted in the cortex, cerebellum, hypothalamus, and brainstem, although no specific trend can be discerned. Elevated NOS activity has been noted in platelets from drug-naive and drug-treated individuals with schizophrenia. Some investigators find increased nitric oxide activity and others the reverse. In autopsy samples, schizophrenic patients were found to have abnormally localized NOS expressing neurons in the prefrontal cortex, hippocampus, and lateral temporal lobe, consistent with abnormal migration of these neuronal types during development. In a rat model, prenatal stress led to reduced NOS expressing neurons in the fascia dentate and hippocampus. Neuropathological Roles of Nitric Oxide. Abundant evidence exists that nitric oxide is a direct participant in a variety of neuropathic events. Superoxide, a byproduct of cellular metabolism, can react with nitric oxide to form peroxynitrite (chemical formula $ONOO^-$). This labile and toxic compound forms chemical adducts with protein tyrosine residues, a process termed protein nitration, and deoxyribonucleic acid (DNA),

leading to cellular dysfunction. Cell loss resulting from ischemic stroke is mediated in part by overstimulation of the glutamate NMDA receptor, a process termed excitotoxicity. Nitric oxide produced by NMDA activation appears to mediate a significant portion of this excitotoxic neuronal death, and stroke damage is reduced in mice with a genetic deletion of nNOS. S-Nitrosylation has also been implicated in pathologic processes in the brain. Mutations in the Parkin protein are associated with early onset Parkinson’s disease. Parkin is an E3 ubiquitin ligase, adding ubiquitin molecules to proteins and targeting them for destruction in the cell proteasome. In sporadic Parkinson’s disease (i.e., without the early onset mutation), nitric oxide can nitrosylate the Parkin protein and inhibit its protective E3 ubiquitin ligase function. An overabundance of nitric oxide signaling may thus predispose to the dysfunction and cell death of dopaminergic neurons in Parkinson’s disease by interfering with proteins essential for cell functioning. In Alzheimer’s disease excess oxidation of brain protein, lipids, and carbohydrates has long been appreciated, but nitrosative stress from excess nitric oxide also appears to participate in the disease. Protein disulfide isomerase (PDI) is a cellular protective protein that may help combat the accumulation of misfolded proteins such as the amyloid fibrils occurring in the disease. In both Alzheimer’s and Parkinson’s disease brains, PDI appears to be S-nitrosylated in a harmful way that impedes its cellular protective function. The discovery that nitric oxide participates in neurodegenerative processes raises the possibility for improved diagnostic processes, such as detecting damage to

cellular components produced by nitric oxide prior to the onset of fullblown symptoms. In addition, drugs may be designed to attenuate the damage to crucial neuronal proteins that protect against disease onset. However, completely and nonspecifically inhibiting or stimulating NOS is likely to produce significant side effects because of its wide-ranging activities throughout the body. Carbon Monoxide Although carbon monoxide (CO) is most well known as an air pollutant derived from combustion reactions, it is produced physiologically in a great variety of organisms ranging from human to bacterium. Once thought to be a toxic byproduct of metabolic reactions, carbon monoxide is increasingly recognized to play an important role in regulating a variety of physiological processes in the brain and other organs. These varied effects include regulation of olfactory neurotransmission, blood vessel relaxation, smooth muscle cell proliferation, and platelet aggregation. Carbon monoxide is far better known for its toxic effects than its activities at physiologic concentrations. It binds tightly to heme molecules within hemoglobin, forming carboxyhemoglobin, which can no longer transport oxygen to tissues. One- to two-pack per day smokers typically have 3 to 8 percent of their hemoglobin as carboxyhemoglobin, with nonsmokers having less than 2 percent. Following acute carbon monoxide poisoning, 5 to 10 percent carboxyhemoglobin is associated with impaired alertness and cognition, and 30 to 50 percent carboxyhemoglobin leads to significant drops in oxygen transport to tissues. Carbon Monoxide and Neurotransmission. Carbon monoxide appears to participate in the neurotransmission of odorant perception. Odorants lead to carbon

monoxide production and subsequent cGMP synthesis that promotes long-term adaptation to odor stimuli. Carbon monoxide has the potential to regulate a variety of perceptual and cognitive processes that are yet untested. Similarly, in the rat retina, long periods of light exposure led to increased HO1 expression, carbon monoxide production, and cGMP signaling. Carbon monoxide may also participate in adaptation to chronic pain. HO2-deficient animals manifest reduced hyperalgesia and allodynia after exposure to chronic pain stimuli. Carbon monoxide may thus set the threshold for pain perception, although it is unclear whether the effect occurs in the central or peripheral nervous system. Aside from its role in promoting cGMP production, carbon monoxide may also directly bind to and open the calcium-activated big potassium (BKCa) channel, leading to as yet uncharacterized effects on neurotransmission. In the gastrointestinal (GI) nervous system, carbon monoxide serves as a neurotransmitter to relax the internal anal sphincter in response to nonadrenergic noncholinergic (NANC) nerve stimulation and vasoactive intestinal peptide (VIP). Carbon monoxide has been implicated in the development of hippocampal LTP, although lines of evidence are contradictory. Carbon monoxide and tetanic stimulation of nerves leads to increased excitatory postsynaptic potentials (EPSPs). HO inhibitors that block carbon monoxide production lead to impaired induction of LTP and reduced calcium-dependent release of glutamate neurotransmitter. However, HO2-deficient animals fail to manifest any differences in LTP. These disparate findings may be explained by a role for HO1 in LTP, or an ability of HO inhibitors to nonspecifically block some other processes important to LTP induction. At toxic levels, carbon monoxide is well known to impair oxygen transport by binding to hemoglobin with a higher affinity than oxygen. Amazingly, carbon monoxide itself plays a physiological role in the mechanism by which the carotid body senses oxygen. HO, expressed in glomus cells of the carotid body, uses oxygen as a substrate in the production of carbon monoxide (Fig. 1.4-13). When oxygen levels drop, so does carbon monoxide production, leading to a resetting of the threshold in which the carotid body senses oxygen. The molecular mechanism may occur via carbon monoxide regulation of the carotid body BK ion channel.

FIGURE 1.4-13 Synthesis of carbon monoxide (CO), an unexpected neurotransmitter. Gaseous carbon monoxide is enzymatically synthesized in neurons by way of the enzyme heme oxygenase, also converting heme into the molecule biliverdin and liberating free iron (Fe). Similar to nitric oxide, CO is not stored in neuronal vesicles and can freely diffuse across neuronal membranes. CO also similarly activates soluble guanylyl cyclase, and leads to activation of multiple intracellular signaling molecules such as p38 MAP kinase. CO exerts its neurotransmitter and signaling functions at concentrations far below that at which classical CO toxicity occurs. The significance of this pathway in neurons is underlined by the existence of two distinct heme oxygenase enzymes, one of which is predominantly expressed in the brain. Biliverdin is converted to bilirubin via the enzyme biliverdin reductase. Similar to CO, bilirubin is no longer relegated to the status of toxic byproduct and may be an important antioxidant. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:107.) Endocannabinoids: From Marijuana to Neurotransmission Whether known as cannabis, hemp, hashish, ma-fen, or a variety of slang terms, marijuana has been cultivated and utilized by human populations for thousands of years. Despite long debate as to whether its risks and benefits are evenly matched, it has only been in recent decades that some of the mystery has been revealed by which marijuana exerts its effects on the brain. The "high" users experience, euphoria and tranquility, relates to cannabis acting on a neural pathway involving cannabinoids endogenous to the human brain, or endocannabinoids.

The first described medicinal use of cannabis dates to approximately 2700 BC in the pharmacopeia of Chinese Emperor Shen Nung, who recommended its use for a variety of ailments. At this time, adverse properties were also apparent, and large amounts of the fruits of hemp could lead to "seeing devils," or a user might "communicate with spirits and lightens one's body." For centuries, cannabis was employed in India as an appetite stimulant; habitual marijuana users remain well acquainted with "the munchies." For many years the mechanisms by which the active components of marijuana, cannabinoids, exerted their psychoactive effects remained a mystery. Chemists sought to isolate the psychoactive components of cannabis from the many components of the plant oil (Table 1.4-4). Table 1.4-4 Selected Discoveries in Cannabinoid Research Discovery of the Brain Endocannabinoid System. Estimates suggest that 20 to 80 µg of tetrahydrocannabinol (THC) reaches the brain after one smokes a marijuana cigarette (i.e., "joint"). This is comparable to the 100 to 200 µg of norepinephrine

neurotransmitter present in the entire human brain. Thus the effects of THC might be explained by the effects on neurotransmitter systems. In the 1960s, there were at least two schools of thought on how THC exerted its psychoactive effects. One held that THC worked in a manner similar to that of the inhaled volatile anesthetics (i.e., no specific receptor existed), and it might have a generalized effect on neuronal membranes or widespread actions on neurotransmitter receptors. A competing school of thought speculated that specific receptors for cannabinoids existed in the brain, but they were difficult to identify due to the lipophilic nature of these chemicals. Novel cannabinoids were synthesized that were more water soluble, and in the late 1980s, this allowed for the discovery of a specific cannabinoid receptor, CB1. Several additional endocannabinoids were soon discovered, 2-arachidonylglycerol (2-AG), N-arachidonyldopamine (NADA), 2-arachidonoylglycerol ether (noladin ether), and virodhamine (Fig. 1.4-14). The reason for having several different endocannabinoids may lie with their differing affinities for the cannabinoid receptors, CB1 and CB2. Anandamide appears to have the greatest selectivity for the CB1 receptor,

followed by NADA and noladin ether. In contrast, virodhamine prefers CB2 receptors and has only partial agonist activity at CB1. 2-AG appears not to discriminate between CB1 and CB2.

FIGURE 1.4-14 Endogenous cannabinoids. At least five endocannabinoids exist in the mammalian brain, each differing in affinity for CB1 and CB2 cannabinoid receptors. All are derived from the essential omega-6 fatty acid, arachidonic acid, which is also a substrate in the

formation of prostaglandins and leukotrienes. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:111.) Biosynthesis of Endocannabinoids. Arachidonic acid is utilized as a building block for biosynthesis of endocannabinoids, prostaglandins, and leukotrienes and is found within cellular phospholipids of the plasma membrane and other intracellular membranes. Synthesis of anandamide requires the sequential action of two enzymes (Fig. 1.4-15). In the first reaction the enzyme N-acetyltransferase (NAT) transfers an arachidonic acid side chain from a phospholipid to phosphatidylethanolamine (PE), generating NAPE (N-arachidonyl-phosphatidylethanolamine). In the second reaction the enzyme N-arachidonyl-phosphatidylethanolamine phospholipase (NAPD-PLD) converts NAPE to anandamide. Because NAPE is already a natural component of mammalian membranes, it is the second step that generates anandamide, which is most crucial to neurotransmission.

FIGURE 1.4-15 Retrograde neurotransmission of the endocannabinoids, anandamide, and 2arachidonylglycerol (2-AG). Anandamide is synthesized on demand for

neurotransmission via a two-step process. The enzyme NAT transfers the arachidonic acid chain from a phospholipid (APL) to phosphatidylethanolamine (PE), thereby producing NAPE. A second enzyme, NAPE-PLD, generates anandamide. 2-AG is similarly synthesized in two steps by the enzymes PLC and DAGL. The endocannabinoids made in a postsynaptic neuron cross the synapse and activate presynaptic CB1 receptors, and suppress neurotransmission of the presynaptic neuron (although activation of the presynaptic neuron occurs in some cases). Enzymes involved in endocannabinoid synthesis are yellow, those that break them down in red. 2-AG is predominantly inactivated in the presynaptic neuron by MAGL, whereas anandamide is destroyed in the postsynaptic neuron by FAAH. PE, phosphatidylethanolamine; APL, arachidonyl phospholipids; NAT, N-acyltransferase; NAPE, N-arachidonyl-phosphatidylethanolamine; NAPE-PLD, N-arachidonyl-phosphatidylethanolamine phospholipase D; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; PLC, phospholipase C; DAG, diacylglycerol; DAGL, diacylglycerol lipase; R1-R3, various acyl or akyl side chains of phospholipids; R', side chain of phospholipid head group. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:112.) Endocannabinoids are not stored in synaptic vesicles for later use, but are synthesized on demand as is done for the gaseous neurotransmitters. An important criterion for a signaling molecule to be considered a neurotransmitter is that neuronal depolarization should lead to its release. Depolarization leads to increases in cellular calcium, which in turn promotes synthesis of the endocannabinoids and their release. The mechanism is explained in part by calcium activation of NAPE-PLD and DAGL, leading to augmented biosynthesis of anandamide and 2-AG, respectively. Endocannabinoids generated in a neuron must cross the synaptic cleft to act on cannabinoid receptors. Similar to THC, endocannabinoids are highly lipophilic and thus poorly soluble in CSF. It is hypothesized that a

specific endocannabinoid transporter exists to allow endocannabinoids to cross the synaptic cleft and allow entry into the target neuron. Inactivation of Endocannabinoids. Neurotransmitters are typically inactivated either by reuptake from the neurons that release them or by degradation by highly specific enzymes, such as the example of acetylcholine being hydrolyzed by acetylcholinesterase. At least two enzymes exist to target the destruction of endocannabinoids and attenuate their neurotransmission. Fatty acid amide hydrolase (FAAH) converts anandamide to arachidonic acid and ethanolamine (Fig. 1.4-15). FAAH is found in regions of the brain where CB1 receptors are predominant and localizes to postsynaptic neurons where anandamide is made. Rapid degradation of anandamide in part explains its relatively low potency compared to THC. Confirming a role of FAAH in anandamide inactivation, knockout mice without FAAH exhibit a 15-fold increase of anandamide, but not 2-AG. These mice have greater behavioral responses to exogenous anandamide, owing to its decreased degradation. The endocannabinoid 2-AG is inactivated by FAAH, but also by a monoacylglycerol lipase (MAGL) located in presynaptic neurons. Pharmacologic inhibitors of FAAH have analgesic effects and reduce anxiety in animal

models, but do not have the undesirable effects of THC such as immobility, lowered body temperature, or greater appetite. Such a pharmacological strategy would be analogous to MAOIs and COMT inhibitors (COMTIs). MAOIs, used to treat depression, slow the breakdown of serotonin and other monoamines, thereby increasing serotonin, whereas COMTIs serve an analogous role in blocking destruction of dopamine and other catecholamines. Cannabinoid Receptors. Underscoring their importance in neural functions, CB1 receptors are possibly the most abundant G-protein-coupled receptors in the brain. They occur at highest density in the basal ganglia, cerebellum, hippocampus, hypothalamus, anterior cingulate cortex, and cerebral cortex, particularly the frontal cortex. Humans or animals that receive large doses of THC develop catalepsy, a reduction of spontaneous movement, and freeze in bizarre and unnatural postures. The action of cannabinoids in the basal ganglia and cerebellum may be associated with these behaviors, which may prove relevant in understanding catatonic symptoms in schizophrenia. CB1 receptors are predominantly found on axons and nerve termini, with little present on neuronal dendrites and the cell body. CB1 receptors tend to be localized to the presynaptic rather than postsynaptic side of the neuronal cleft, suggesting a role in regulation of neurotransmission. A second cannabinoid receptor, CB2, is predominantly expressed on the surface of white blood cells of the immune system, but small amounts appear to be present in the brainstem. EFFECTS ON NEUROTRANSMISSION. The cannabinoid CB1 receptor is associated with G proteins that mediate its intracellular signaling, in part, through inhibition of adenylyl cyclase. This leads to a decrease in levels of the important second messenger, cyclic adenosine monophosphate. Activation of the CB1 receptor also leads to activation of potassium channels and inhibition of N-type calcium channels. Because calcium is integral to neurotransmitter release, cannabinoids can block neurotransmission through this mechanism. Cannabinoid receptors also activate mitogen-activated protein kinases. With the use of cell culture models and slices of brain, cannabinoids have been shown to block the release of a variety of neurotransmitters, including GABA, norepinephrine, and acetylcholine. Norepinephrine and acetylcholine tend to be excitatory neurotransmitters, and cannabinoid inhibition of their release would be expected to have an overall inhibitory effect. However, GABA is an inhibitory neurotransmitter, and cannabinoid inhibition of it would lead to overall excitatory effects, demonstrating that cannabinoids can have complex effects on neurotransmission depending on the specific context. Cannabinoids also appear to increase the release of brain endorphin neurotransmitters and increase dopamine release in the nucleus accumbens, a “reward center”

relevant to addiction and learning. The endocannabinoids have been implicated in a variety of forms of synaptic plasticity, including LTP and long-term depression (LTD). Endocannabinoids in Anxiety and Mood. Endocannabinoid

neurotransmission may be an important regulator of anxiety, and cannabis users regularly describe a tranquilizing effect of THC. Loss of signaling by the endocannabinoid system appears to promote anxiety-like states in animal studies. CB1 receptor–deficient animals exhibit more pronounced anxiety behavior when exposed to stress or new environs. The endocannabinoid pathway may represent an attractive target in understanding posttraumatic stress responses and phobias. Although one cannot yet safely measure endocannabinoid levels in human subjects, this model is supported by clinical trials of the cannabinoid receptor blocker, rimonabant (Acomplia), which may offer promise as a strategy for weight loss (see below). A frequent adverse reaction to the drug is increased anxiety and depression. ADDICTION. The endocannabinoid system may be an attractive target for understanding addiction. Mice deficient in CB1 receptors are unsurprisingly resistant to the behavioral effects of cannabinoids; they also appear to have reduced addiction to and withdrawal from opiates. Further interaction has also been found between the opioid and cannabinoid systems, as cannabinoids appear to increase the release of dopamine in the nucleus accumbens, a key reward area of the brain implicated in addiction. This dopamine release appears to require μ-opioid receptors, as pharmacological inhibition of these receptors blocks the ability of cannabinoids to increase dopamine release. Rats with a preference for alcohol have decreased FAAH activity, suggestive of greater cannabinoid signaling. CB1 receptor antagonists dampen their alcohol consumption, whereas inhibiting FAAH increases their alcohol consumption. Furthermore, CB1deficient animals also appear to have reduced alcohol intake. A single amino acid mutation in human FAAH has been found to be associated with drug abuse, and this abnormal enzyme appears to be less stable than its wild-type counterpart. Endocannabinoids in Psychosis. Heavy use of cannabis can produce psychotic symptoms in individuals with no prior history of psychiatric disorder, although it is unclear whether this is solely due to the drug or to an underlying vulnerability to psychosis in such persons. Cannabis use often worsens psychosis in schizophrenia, and heavy use has been associated with developing schizophrenia, although some suggest that this association is an accelerated development of symptoms in those who would eventually manifest schizophrenia. Nonetheless, the endocannabinoid system has implications for the pathophysiology of schizophrenia, as cannabinoid signaling appears to increase the release of dopamine. Medications that act as antagonists of D2 receptors will likely remain a component of schizophrenia treatment for some time. FEEDING. Following drug ingestion, THC users develop an increased appetite (“the munchies”), and cannabis has been utilized as an appetite stimulant for centuries. This effect may depend on CB1 receptors present in the hypothalamus. Endocannabinoid levels increase in the hypothalamus and limbic system when animals are deprived of food. Mice genetically deficient in CB1 receptors become resistant to developing obesity

after being given a high-fat diet. Similarly, the CB1 receptor antagonist, rimonabant, appears to facilitate weight loss by blocking cannabinoid signaling. In a clinical trial of more than 3,000 obese patients, those treated with 20 mg per day of rimonabant lost 6.3 kg at 1 year, compared to 1.6 kg in the placebo group. Nausea was the most common side effect reported. A 2007 meta-analysis of clinical trials reported an overall 4.7 kg weight loss with rimonabant treatment, besting the weight-loss drugs orlistat (Xenical; 2.9 kg) and sibutramine (Meridia; 4.2 kg). Effects on Brain Injury and Pain. In mouse models of traumatic brain injury, 2AG appears neuroprotective, reducing brain

edema, infarct size, and cell death, while improving functional outcomes. Anandamide also protected against brain injury in a model of multiple sclerosis (MS), and human patients with the disease have increased production of anandamide. A study of cannabinoid agonist, HU-211, led to more rapid clinical improvement following head trauma. FAAH inhibitors improved motor symptoms in a mouse model of Parkinson's disease, likely via cannabinoids increasing dopamine neurotransmission. There is increasing evidence that neurotransmission via the endocannabinoid pathway regulates pain perception. THC and cannabinoid agonists have proven effective in animal models of acute and chronic pain, ranging from burn injury to nerve damage and inflammation. The CB1 receptor plays an important role in these effects, as the analgesic effects of cannabinoid drugs are lost when CB1 antagonist rimonabant is given. Similarly, the analgesic effect of THC is lost in mice that are genetically deficient in the CB1 receptor. Stress has long been associated with diminished pain perception, such as in cases of injured military personnel who demonstrate remarkable pain tolerance, a phenomenon known as stress-induced analgesia. The endocannabinoid system may mediate these effects. Animal models reveal anandamide and 2-AG production after stress, and stressinduced analgesia is blocked by the CB1 blocker, rimonabant, in these animals. Endocannabinoid regulation of pain perception appears to be distinct from that of the endogenous opiate system, but the two pathways may share overlapping neural pathways. Evidence for this has been provided using CB1 blocker, rimonabant, and naloxone (Narcan), which blocks opiate receptors. Rimonabant attenuates analgesia provided by THC and cannabinoids, but only partly blocks the response to morphine. However, the opposite is true for opiates: Naloxone blocks morphine-induced analgesia but also partially blocks the analgesia of THC and cannabinoid drugs. Combinations of cannabinoid and opiate drugs evince synergistic analgesic effects in animal models. Although it was initially assumed that cannabinoids exert their analgesic effects via the CNS, in animal models it has been shown that localized administration of cannabinoids may also be effective, including drugs selective for the CB2 receptor, whose expression is minimal in the CNS. Endocannabinoids may also influence pain sensitivity by mechanisms that do not involve the CB1 and CB2 receptors. Both anandamide and NADA can also activate a calcium channel known as the vanilloid receptor (also known as transient receptor potential vanilloid type 1 [TRPV-1]) that is found on sensory nerves. This same receptor is also famous for being

activated by capsaicin, which causes the hot sensation after eating chili peppers. Thus endocannabinoids can exert opposing functions: Promoting analgesia through the CB1 and CB2 receptors, but potentially increasing pain via TRP channels. Although CB2 receptors are largely expressed in the periphery, postmortem analyses reveal an upregulation in brain from those with Alzheimer's disease. The rapid development of novel cannabinoid drugs may allow for targeting of specific symptoms, rather than elicit all of the typical effects of THC. For instance, ajulemic acid demonstrates analgesic and anti-inflammatory properties, but may offer a benefit of limited psychoactive side effects. In a randomized clinical trial of this compound, Mathias Karst and colleagues found efficacy in reducing chronic neuropathic pain. Effects in the Periphery. Cannabinoids lead to direct relaxation of vascular smooth muscle by local CB1 receptors. This vasodilation extends to the conjunctiva, leading to a "bloodshot" appearance in some cannabis users. Relaxation of ocular arteries by cannabinoids may offer utility as a treatment for glaucoma, a condition of high intraocular pressure, and activation of CB1 receptors in the kidney can improve renal blood flow. A role in generalized blood pressure regulation is unproven, and blood pressure is unaltered in persons treated with rimonabant or animals deficient in CB1 receptors. Cannabinoid signaling may also be relevant to ectopic pregnancy, as CB1deficient mice retain many embryos in

the oviduct. Nonpsychoactive Cannabinoids Although THC is the principal psychoactive component of cannabis, the many nonpsychoactive cannabinoids also have intriguing properties and may regulate neurotransmission. Cannabidiol may offer potential therapeutic effects and appears to stimulate TRPV-1 receptors and influence endocannabinoid degradation. In addition, cannabidiol demonstrated a protective effect in a mouse model of inflammatory arthritis. Although results have been mixed, purified cannabidiol may also exert antipsychotic activity, although the net effect of plant cannabis use typically exacerbates schizophrenia symptoms owing to THC. Tetrahydrocannabivarin is a plant cannabinoid that antagonizes CB1 receptors. It is a candidate marker to distinguish whether a patient has been using plant-derived cannabis or prescription THC, which contains no tetrahydrocannabivarin. Eicosanoids Overview. Clinical findings suggest that the dietary supplements omega-3 fatty acids, eicosapentaenoic acid (EPA), its ester ethyl-eicosapentaenoic (E-EPA), and docosahexaenoic acid (DHA), help relieve symptoms of depression, bipolar illness, schizophrenia, and cognitive impairment. DHA and EPA may help reduce behavioral outbursts and improve attention in children. Chemistry. Essential fatty acids are a group of polyunsaturated fats that contain a carbon–carbon double bond in the third position from the methyl end group in the fatty

acid chain. They are essential because unlike monosaturated and saturated fatty acids, polyunsaturated fatty acids cannot be synthesized de novo and can be acquired only through diet from natural fats and oils. Linoleic acid (LA) is the parent compound of omega-6 fatty acids, and α-linolenic acid (ALA) is the parent compound of omega-3 fatty acids. Both omega-3 and omega-6 groups use the same enzymes for desaturation and chain elongation. Omega-3 fatty acids are synthesized by algae and plankton. Fish such as herring, salmon, mackerel, and anchovy feed on these aquatic species and become a rich dietary source of omega-3. EPA and DHA are highly unsaturated omega-3 fatty acids that contain 6 and 5 double bonds on their long structural chain, respectively. They are positioned in the cell membrane by phospholipids and play a crucial role in cell membrane signaling. Effects on Specific Organs and Systems. The strongest scientific evidence for treatment with fatty acid supplements comes from the cardiovascular literature. Several human trials have demonstrated that omega-3 fatty acids lower blood pressure, reduce the rate of recurrent myocardial infarction, and lower triglyceride levels. In the nervous system, fatty acids are essential components of neurons, immune cells, and glial phospholipid membrane structures. They increase cerebral blood flow, decrease platelet aggregation, and delay progression of atherosclerosis in the cardiovascular system. Omega-6 fatty acids appear to reduce inflammation and neuronal apoptosis and decrease phosphatidylinositol second messenger activity. Omega-3 fatty acids have been suggested to alter gene expression. In the CNS, fatty acids are selectively concentrated in neuronal membranes and involved in cell membrane structure. Omega-6 arachidonic acid has been shown to enhance glutamate neurotransmission, stimulate stress hormone secretion, and trigger glial cell activation in the setting of oxidative toxicity and neurodegeneration. The omega-3 fatty acids DHA and EPA appear to protect neurons from inflammatory and oxidative toxicities. Increases in serotonin, enhancement of dopamine, and regulation of CRF have been demonstrated in cell culture models. In rodent models of depression, chronic EPA treatment normalized behavior in open field tests. Serotonin and norepinephrine were also increased in the limbic regions. Mice fed omega-3 poor diets had reduced memory, altered learning patterns, and more behavioral problems. Therapeutic Indications. Clinical research with the use of fish oil for mood disorders was based on epidemiology studies where there appears to be negative correlation between fish consumption and depressive symptoms. Countries with lower per

capita fish consumption had up to 60 times increased rates of major depression, bipolar disorder, and postpartum depression. Observational studies concluded that the lower incidence of seasonal affective disorder in Iceland and Japan, rather than latitude predicted, is related to the amount of fatty acid these populations consume in their diet. A study in Norway showed that use of cod liver oil decreased depressive symptoms. Depression after a myocardial infarction shows higher arachidonic acid to EPA ratio.

Postmortem studies in brains of patients diagnosed with major depressive disorder show reduced DHA in the orbitofrontal cortex. The first randomized, controlled pilot study of omega-3 fatty acids focused on adjunctive treatment in both bipolar and unipolar patients with depression in addition to their standard lithium (Eskalith) or valproic acid (Depakene) treatment. The omega-3 fatty acid group had significant improvement on the Hamilton Depression scale and a longer period of remission than the placebo group. A subsequent larger study supported a benefit from treatment with E-EPA for bipolar illness. However, a study of a group of patients with either bipolar disorder or rapid cycling treated with E-EPA showed no significant difference on any outcome measure between the EPA and placebo groups. Bleeding time was also increased in the treatment group. There are no current data on monotherapy in bipolar illness or depression. The most convincing evidence comes from early brain development and learning studies. Pregnant mothers who consumed foods rich in DHA gave birth to infants who had improved problem-solving skills, but not necessarily improved memory. Visual acuity and eye development are also associated with DHA supplementation during pregnancy. Reports of behavioral studies of prisoners in England who consumed higher amounts of seafood containing omega-3 fatty acids showed a decrease in assault rates. A Finnish study of violent criminals identified lower levels of omega-3 fatty acids in their system compared to the nonviolent offenders. The negative and psychotic symptoms of schizophrenia may be improved with supplementation with omega-3 fatty acids. Antipsychotic medications like haloperidol (Haldol) appear to have fewer extrapyramidal side effects when combined with antioxidants and omega-3 fatty acids. EPA and DHA have been associated with decreased dementia incidence. After reviewing the Rotterdam study of a longitudinal cohort of more than 5,300 patients, fish consumption appeared to be inversely related to development of new cases of dementia. A later analysis of the study after 6 years demonstrated that low intake of omega-3 fatty acids was not associated with increased risk of dementia. In contrast, the Zutphen study, also in the Netherlands, concluded that high fish consumption was inversely related with cognitive decline at 3-year follow-up and after 5 years. Well-designed clinical trials are needed before omega-3 fatty acids can be recommended for prevention of cognitive impairment. Precautions and Adverse Reactions. The most adverse complication of eicosanoid use is increased risk for bleeding. Dietary sources can contain heavy metals, and there is no standard preparation for capsule formulations. Treatment studies have yielded a variety of different doses, but evidence for the therapeutic dose and clinical guidelines are almost nonexistent. The length of treatment still needs to be determined. Neurosteroids

Background. Although steroids are critical for the maintenance of body homeostasis, neurosteroids are synthesized from cholesterol in the brain and independent of peripheral formation in the adrenals and gonads. Neurosteroids are produced by a sequence of enzymatic processes governed by cytochrome P450 (CYP) and non-CYP enzymes, either within or outside the mitochondria of several types of CNS and peripheral nervous system (PNS) cells. Recent work has shown that neurosteroids can operate through a nongenomic pathway to regulate neuronal excitability through

their effects on neurotransmittergated ion channels. Receptors are generally located in the nucleus, membrane, or microtubules of the CNS and PNS. Although steroids and neurosteroids can act on the same nuclear receptors, neurosteroids differ from steroids in their topological distribution and regional synthesis. The most well-known effect of neurosteroids is on the GABA receptor, particularly the GABAA receptor. Neurosteroids acting primarily at this site include allopregnanolone (3α,5α-tetrahydroprogesterone), pregnanolone (PREG), and tetrahydrodeoxycorticosterone (THDOC). Dehydroepiandrosterone sulfate (DHEA-S), the most prevalent neurosteroid, acts as a noncompetitive modulator of GABA, and its precursor dehydroepiandrosterone (DHEA) has also been shown to exert inhibitory effects at the GABA receptor. Some neurosteroids may also act at the NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propanoic acid (AMPA), kainate, glycine, serotonin, sigma type-1, and nicotinic acetylcholine receptors. Progesterone is also considered a neurosteroid and has the ability to regulate gene expression at progesterone receptors. Neurosteroids in Neurodevelopment and Neuroprotection. In general, neurosteroids stimulate axonal growth and promote synaptic transmission. Specific neuroprotective effects are unique to each neurosteroid. DHEA acts to regulate brain serotonin and dopamine levels, suppress cortisol, increase hippocampal primed burst potentiation and cholinergic function, decrease amyloid-β protein, inhibit the production of proinflammatory cytokines, and prevent free radical scavenging. DHEA and DHEA-S have both been shown to have a role in glial development and neuronal growth and to promote their survival in animals; the injection of these substances into the brains of mice promoted long-term memory while reversing amnesia. Progesterone is linked to myelinating processes like aiding in the repair of damaged neural myelination (Color Plate 1.4-16). Allopregnanolone contributes to the reduction of contacts during axonal regression. Role of Neurosteroids in Mental Illness. Neurosteroids have distinct implications for the maintenance of normal neurologic function and also may contribute to neuropathology. Neurosteroids are differentially regulated in males and females and may affect the manifestation of psychological disorders in these two populations. Specifically, they play a distinct role in depression and anxiety disorders and may be targeted by psychiatric medications in the near future.

DEPRESSION. When compared with nondepressed controls, studies show that depressed patients have lower plasma and CSF concentrations of allopregnanolone. In addition, this research has elucidated an inverse relationship between allopregnanolone concentrations and severity of depressive illness. However, there are no allopregnanolone-based therapies available for humans, so its direct efficacy is unsubstantiated. Antidepressant drugs, specifically fluoxetine (Prozac), have been shown in multiple studies to increase the levels of certain neurosteroids. Nonetheless, there is debate over the therapeutic properties of neurosteroids, prompting the investigation of neurosteroid concentrations in patients undergoing nonpharmacological therapies. Preliminary results indicate that the lack of modifications in neurosteroid levels during nonpharmacological treatments supports the validity of the pharmacological properties of antidepressants, not their therapeutic action, in the elevation of neurosteroid levels in medicated populations. ANXIETY DISORDERS. In patients with anxiety disorders, the major mechanism of action is on the GABA receptor. Homeostasis characterized by normal GABAergic activity is restored after panic attacks as neurosteroids are released in response to stress. Allopregnanolone stimulates GABAergic activity with 20 times the strength of benzodiazepines and 200 times the potency of barbiturates. Both positive and negative regulation of the GABAA receptor are correlated with anxiolytic and anxiogenic action, respectively. PSYCHOTIC DISORDERS. In addition to their primary relevance to the pharmacological treatment of mood and anxiety disorders, neurosteroids contribute to

psychotic, childhood, substance abuse, eating, and postpartum disorders. The effect of neurosteroids on psychotic disorders such as schizophrenia is mediated by DHEA and DHEA-S. DHEA has been dispensed to decrease anxiety in patients with schizophrenia, as DHEA and DHEA-S suppress GABA inhibition and heighten the neuronal response at the NMDA and sigma receptors. DHEA and DHEA-S levels are typically elevated in the initial episode of a patient with schizophrenia, indicating neurosteroids are upregulated by the onset of psychosis. Because neurosteroid levels are studied across various illness stages, some questions still exist regarding the role of neurosteroids in psychosis. CHILDHOOD MENTAL ILLNESS. In children, the clinical symptomology of ADHD is inversely correlated with DHEA and pregnenolone levels. SUBSTANCE ABUSE. Alcohol is theorized to regulate the GABA receptor and induce de novo steroid synthesis in the brain; specifically, pregnenolone, allopregnanolone, and allotetrahydrodeoxycorticosterone levels are increased in the brain and periphery in response to increases in peripheral alcohol levels. It is hypothesized that sharp increases in ethanol concentration may mimic the acute stress response and elevate neurosteroid concentrations by the HPA axis. To prevent ethanol dependence, researchers are investigating fluctuations in neurosteroid levels and in vivo neurosteroid responsiveness. Neurosteroids (increased allopregnanolone levels in particular) are

associated with drug abuse. However, DHEA-S may actually check the acquisition of morphine tolerance. Past research has shown that DHEA-S levels were also increased in patients who abstained from cocaine use in a treatment program, and as patients relapsed DHEA-S concentrations decreased accordingly. EATING DISORDERS. With regard to eating disorders, DHEA has been shown to diminish food intake, temper obesity, moderate insulin resistance, and lower lipids in rats with a model of youth-onset, hyperphagic, and genetic obesity. By regulating the serotonergic system, DHEA is hypothesized to promote a reduced caloric load. Although hypothetical, low levels of DHEA and DHEA-S are recorded in young women with anorexia nervosa, and 3 months of oral DHEA supplementation increased bone density and tempered the emotional problems associated with the disorder. POSTPARTUM AND GYNECOLOGICAL DISORDERS. Because estrogen and progesterone levels fluctuate during the course of pregnancy and drop markedly after delivery, neurosteroids are thought to contribute to postpartum disorders. Low postpartum DHEA concentrations have been linked to mood instability. In addition, allopregnanolone levels correlated with mood disorders during pregnancy and in premenstrual syndrome (PMS). It has been noted that women with premenstrual dysphoric disorder have higher allopregnanolone/progesterone ratios than normal controls; women treated for this disorder reported improvement as allopregnanolone levels decreased. NEUROSTEROIDS, MEMORY DISORDERS, AND AGING. Neurosteroid levels may be irregular in neurodegenerative disorders and aging conditions such as Alzheimer’s disease and Parkinson’s disease. DHEA levels at age 70 are only about 20 percent of their maximum value recorded in the late 20s, and some researchers believe DHEA supplementation can prevent or slow the cognitive declines associated with the aging process. However, conflicting studies have indicated that DHEA administration does not improve cognitive measures in patients. In addition, in patients with Alzheimer’s disease, DHEA concentrations have been found to be markedly decreased. REFERENCES Abi-Dargham A. The neurochemistry of schizophrenia: A focus on dopamine and glutamate. In: Charney DS, Nestler E, eds. Neurobiology of Mental Illness. 3rd ed. New York: Oxford University Press; 2009:321. Berger M, Honig G, Wade JM, Tecott LH. Monoamine neurotransmitters. In: Sadock BJ, Sadock VA, Ruiz P, eds. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. Butler JS, Foxe JJ, Fiebelkorn IC, Mercier MR, Molholm S. Multisensory

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hormone (CRH), which stimulates adrenocorticotropin (adrenocorticotropic hormone [ACTH]); thyrotropin-releasing hormone (TRH), which stimulates release of thyroid-stimulating hormone (TSH); gonadotropin-releasing hormone (GnRH), which stimulates release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH); and somatostatin (somatotropin

release-inhibiting factor [SRIF]) and growth-hormone-releasing hormone (GHRH), which influence growth hormone (GH) release. Chemical signals cause the release of these neurohormones from the median eminence of the hypothalamus into the portal hypophyseal bloodstream and subsequent transport to the pituitary to regulate the release of target hormones. Pituitary hormones in turn act directly on target cells (e.g., ACTH on the adrenal gland) or stimulate the release of other hormones from peripheral endocrine organs. In addition, these hormones have feedback actions that regulate secretion and exert neuromodulatory effects in the CNS. Table 1.5-1 Examples of Regulating Hormones Hormones are divided into two general classes: (1) proteins, polypeptides, and glycoproteins, and (2) steroids and steroid-like compounds (Table 1.5-2); these are secreted by an endocrine gland into the bloodstream and are transported to their sites of action. Table 1.5-2 Classifications of Hormones

DEVELOPMENTAL PSYCHONEUROENDOCRINOLOGY Hormones can have both organizational and activational effects. Exposure to gonadal hormones during critical stages of neural development directs changes in brain morphology and function (e.g., sex-specific behavior in adulthood). Similarly, thyroid hormones are essential for the normal development of the CNS, and thyroid deficiency during critical stages of postnatal life will severely impair growth and development of the brain, resulting in behavioral disturbances that may be permanent if replacement therapy is not instituted. ENDOCRINE ASSESSMENT Neuroendocrine function can be studied by assessing baseline measures and by measuring the response of the axis to some neurochemical or hormonal challenge. The first method has two approaches. One approach is to measure a single time point—for example, morning levels of growth hormone; this approach is subject to significant error because of the pulsatile nature of the release of most hormones. The second approach is to collect blood samples at multiple points or to collect 24-hour urine samples; these measurements are less susceptible to major errors. The best approach, however, is to perform a neuroendocrine challenge test, in which the person is given a drug or a hormone that perturbs the endocrine axis in some standard way. Persons with no disease show much less variation in their responses to such challenge studies than in their baseline measurements.

HYPOTHALAMIC–PITUITARY–ADRENAL AXIS Since the earliest conceptions of the stress response, by Hans Selye and others, investigation of hypothalamic–pituitary–adrenal (HPA) function has occupied a central position in psychoendocrine research. CRH, ACTH, and cortisol levels all rise in response to a variety of physical and psychic stresses and serve as prime factors in maintaining homeostasis and developing adaptive responses to novel or challenging stimuli. The hormonal response depends both on the characteristics of the stressor itself and on how the individual assesses and is able to cope with it. Aside from generalized effects on arousal, distinct effects on sensory processing, stimulus habituation and sensitization, pain, sleep, and memory storage and retrieval have been documented. In primates, social status can influence adrenocortical profiles and, in turn, be affected by exogenously induced changes in hormone concentration. Pathological alterations in HPA function have been associated primarily with mood disorders, posttraumatic stress disorder (PTSD), and dementia of the Alzheimer’s type, although recent animal evidence

points toward a role of this system in substance use disorders as well. Disturbances of mood are found in more than 50 percent of patients with Cushing's syndrome (characterized by elevated cortisol concentrations), with psychosis or suicidal thought apparent in more than 10 percent of patients studied. Cognitive impairments similar to those seen in major depressive disorder (principally in visual memory and higher cortical functions) are common and relate to the severity of the hypercortisolemia and possible reduction in hippocampal size. In general, reduced cortisol levels normalize mood and mental status. Conversely, in Addison's disease (characterized by adrenal insufficiency), apathy, social withdrawal, impaired sleep, and decreased concentration frequently accompany prominent fatigue. Replacement of glucocorticoid (but not of electrolyte) resolves behavioral symptomatology. Similarly, HPA abnormalities are reversed in persons who are treated successfully with antidepressant medications. Failure to normalize HPA abnormalities is a poor prognostic sign. Alterations in HPA function associated with depression include elevated cortisol concentrations, failure to suppress cortisol in response to dexamethasone, increased adrenal size and sensitivity to ACTH, a blunted ACTH response to CRH, and, possibly, elevated CRH concentrations in the brain. HYPOTHALAMIC–PITUITARY–GONADAL AXIS The gonadal hormones (progesterone, androstenedione, testosterone, estradiol, and others) are steroids that are secreted principally by the ovary and testes, but significant amounts of androgens arise from the adrenal cortex as well. The prostate gland and adipose tissue, also involved in the synthesis and storage of dihydrotestosterone, contribute to individual variance in sexual function and behavior. The timing and presence of gonadal hormones play a critical role in the development of sexual dimorphisms in the brain. Developmentally, these hormones direct the organization of many sexually dimorphic CNS structures and functions, such as the size

of the hypothalamic nuclei and corpus callosum, neuronal density in the temporal cortex, the organization of language ability, and responsivity in Broca's motor speech area. Women with congenital adrenal hyperplasia—a deficiency of the enzyme 21hydroxylase, which leads to high exposure to adrenal androgens in prenatal and postnatal life, in some studies—have been found to be more aggressive and assertive and less interested in traditional female roles than control female subjects. Sexual dimorphisms may also reflect acute and reversible actions of relative steroid concentrations (e.g., higher estrogen levels transiently increase CNS sensitivity to serotonin). Testosterone Testosterone is the primary androgenic steroid, with both androgenic (i.e., facilitating linear body growth) and somatic growth functions. Testosterone is associated with increased violence and aggression in animals and in correlation studies in humans, but anecdotal reports of increased aggression with testosterone treatment have not been substantiated in investigations in humans. In hypogonadal men, testosterone improves mood and decreases irritability. Varying effects of anabolic–androgenic steroids on mood have been noted anecdotally. A prospective, placebo-controlled study of anabolic– androgenic steroid administration in normal subjects reported positive mood symptoms, including euphoria, increased energy, and sexual arousal, in addition to increases in the negative mood symptoms of irritability, mood swings, violent feelings, anger, and hostility. Testosterone is important for sexual desire in both men and women. In males, muscle mass and strength, sexual activity, desire, thoughts, and intensity of sexual feelings depend on normal testosterone levels, but these functions are not clearly augmented by supplemental testosterone in those with normal androgen levels. Adding small amounts of testosterone to normal hormonal replacement in postmenopausal women has proved, however, to be as beneficial as its use in hypogonadal men. Dehydroepiandrosterone DHEA and DHEA sulfate (DHEA-S) are adrenal androgens secreted in response to ACTH and represent the

most abundant circulating steroids. DHEA is also a neurosteroid that is synthesized in situ in the brain. DHEA has many physiological effects, including reduction in neuronal damage from glucocorticoid excess and oxidative stress. Behavioral interest has centered on its possible involvement in memory, mood, and a number of psychiatric disorders. Adrenarche is the prepubertal onset of adrenal production of DHEA-S and may play a role in human maturation through increasing the activity of the amygdala and hippocampus and promoting synaptogenesis in the cerebral cortex. DHEA has been shown to act as an excitatory neurosteroid and to enhance memory retention in mice, but studies of DHEA administration to humans have not consistently shown any improvement in cognition. Several trials of DHEA administration point to an improvement in well-being, mood, energy, libido, and

functional status in depressed individuals. Administration of DHEA to women with adrenal insufficiency (e.g., Addison's disease) has repeatedly been demonstrated to enhance mood, energy, and sexual function; effects in men remain to be assessed. Mood, fatigue, and libido improved in human immunodeficiency virus (HIV)–positive patients treated with DHEA in one study, and DHEA and DHEA-S have been found to be inversely correlated with severity in attention-deficit/hyperactivity disorder (ADHD). Women diagnosed with fibromyalgia have significantly decreased DHEA-S levels, but supplementation does not improve outcome. Several cases of possible DHEA-induced mania have been reported, and DHEA has been reported to be inversely related to extrapyramidal symptoms (EPS) in patients with schizophrenia who are treated with antipsychotics. DHEA administration in these cases improves EPS. Double-blind treatment studies have shown antidepressant effects of DHEA in patients with major depression, midlife-onset dysthymia, and schizophrenia, although beneficial effects on memory have not been reliably demonstrated. A small, double-blind trial of DHEA treatment of Alzheimer's disease failed to reveal significant benefit, although a near-significant improvement in cognitive function was seen after 3 months of treatment. Animal studies suggest that DHEA may be involved in eating behavior, aggressiveness, and anxiety as well, with its effects resulting from its transformation into estrogen, testosterone, or androsterone from its antiglucocorticoid activity, or from direct effects on GABAA, N-methyl-D-aspartate (NMDA), and σ receptors. Because of the putative antiglucocorticoid effects, the ratio of cortisol to DHEA levels may be particularly important in understanding adaptive responses to stress. Both cortisol and DHEA appear to be involved in fear conditioning, with the cortisol/DHEA ratio hypothesized to be an index of the degree to which an individual is buffered against the negative effects of stress. This ratio has been found to be related to some measures of psychopathology and response to treatment, predicting the persistence of the first episode major depression and being related to degree of depression, anxiety, and hostility in patients with schizophrenia and response to antipsychotic treatment. Patients with PTSD have higher DHEA levels and lower cortisol/DHEA ratios related to symptom severity, suggesting a role in PTSD recovery. Fear-potentiated startle is larger in individuals with high as compared to low cortisol/DHEA-S ratios and is positively associated with cortisol and negatively with DHEA-S. Greater DHEA response to ACTH is related to lower PTSD ratings, and the cortisol/DHEA ratio to negative mood symptoms. A genetic variation in an ACTH receptor promoter has been found to influence DHEA secretion in response to dexamethasone and may underlie some individual differences in stress response. Estrogen and Progesterone Estrogens can influence neural activity in the hypothalamus and limbic system directly through modulation of neuronal excitability, and they have complex multiphasic effects on nigrostriatal dopamine receptor sensitivity. Accordingly, evidence indicates that the antipsychotic effect of psychiatric drugs can change over the menstrual

cycle and that the risk of tardive dyskinesia depends partly on estrogen concentrations. Several studies have suggested that gonadal steroids modulate spatial cognition and verbal memory and are involved in impeding age-related neuronal degeneration. Increasing evidence also suggests that estrogen administration decreases the risk and severity of dementia of the

Alzheimer's type in postmenopausal women. Estrogen has mood-enhancing properties and can also increase sensitivity to serotonin, possibly by inhibiting monoamine oxidase. In animal studies, long-term estrogen treatment results in a decrease in serotonin 5-HT1 receptors and an increase in 5-HT2 receptors. In oophorectomized women, significant reductions in tritiated imipramine binding sites (which indirectly measures presynaptic serotonin uptake) were restored with estrogen treatment. The association of these hormones with serotonin is hypothetically relevant to mood change in premenstrual and postpartum mood disturbances. In premenstrual dysphoric disorder, a constellation of symptoms resembling major depressive disorder occurs in most menstrual cycles, appearing in the luteal phase and disappearing within a few days of the onset of menses. No definitive abnormalities in estrogen or progesterone levels have been demonstrated in women with premenstrual dysphoric disorder, but decreased serotonin uptake with premenstrual reductions in steroid levels has been correlated with the severity of some symptoms. Most psychological symptoms associated with the menopause are actually reported during perimenopause rather than after complete cessation of menses. Although studies suggest no increased incidence of major depressive disorder, reported symptoms include worry, fatigue, crying spells, mood swings, diminished ability to cope, and diminished libido or intensity of orgasm. Hormone replacement therapy (HRT) is effective in preventing osteoporosis and reinstating energy, a sense of well-being, and libido; however, its use is extremely controversial. Studies have shown that combined estrogen– progestin drugs (e.g., Premarin) cause small increases in breast cancer, heart attack, stroke, and blood clots among menopausal women. Studies of the effects of estrogen alone in women who have had hysterectomies (because estrogen alone increases the risk for uterine cancer) are ongoing. HYPOTHALAMIC–PITUITARY–THYROID AXIS Thyroid hormones are involved in the regulation of nearly every organ system, particularly those integral to the metabolism of food and the regulation of temperature, and are responsible for optimal development and function of all body tissues. In addition to its prime endocrine function, TRH has direct effects on neuronal excitability, behavior, and neurotransmitter regulation. Thyroid disorders can induce virtually any psychiatric symptom or syndrome, although no consistent associations of specific syndromes and thyroid conditions are found. Hyperthyroidism is commonly associated with fatigue, irritability, insomnia, anxiety, restlessness, weight loss, and emotional lability; marked impairment in concentration and memory may also be evident. Such states can progress into delirium or mania or they can be episodic. On occasion, a true psychosis develops, with paranoia as a particularly common presenting feature. In some cases, psychomotor retardation, apathy, and withdrawal are the presenting features rather than agitation and anxiety. Symptoms of mania have also been reported following rapid normalization of thyroid status in hypothyroid individuals and may covary with thyroid level in individuals with episodic endocrine dysfunction. In general, behavioral abnormalities resolve with normalization of thyroid function and respond symptomatically to traditional

psychopharmacological regimens. The psychiatric symptoms of chronic hypothyroidism are generally well recognized (Fig. 1.5-1). Classically, fatigue, decreased libido, memory impairment, and irritability are noted, but a true secondary psychotic disorder or dementia-like state can also

develop. Suicidal ideation is common, and the lethality of actual attempts is profound. In milder, subclinical states of hypothyroidism, the absence of gross signs accompanying endocrine dysfunction can result in its being overlooked as a possible cause of a mental disorder. FIGURE 1.5-1 Hands of a patient with hypothyroidism (myxedema), illustrating the swelling of the soft parts, the broadening of the fingers, and their consequent stumpy or pudgy appearance. (Reprint from Douthwaite AH, ed. French's Index of Differential Diagnosis. 7th ed. Baltimore: Williams & Wilkins; 1954, with permission.) GROWTH HORMONE Growth hormone deficiencies interfere with growth and delay the onset of puberty. Low GH levels can result from a stressful experience. Administration of GH to individuals with GH deficiency benefits cognitive function in addition to its more obvious somatic effects, but evidence indicates poor psychosocial adaptation in adulthood for children who were treated for GH deficiency. A significant percentage of patients with major depressive disorder and dysthymic disorder may have a GH deficiency. Some prepubertal and adult patients with diagnoses of major depressive disorder exhibit hyposecretion of GHRH during an insulin tolerance test, a deficit that has been interpreted as reflecting alterations in both cholinergic and serotonergic mechanisms. A number of GH abnormalities have been noted in patients with anorexia nervosa. Secondary factors, such as weight loss, however, in both major depressive disorder and eating disorders, may be responsible for alterations in endocrine release. Nonetheless, at

least one study has reported that GHRH stimulates food consumption in patients with anorexia nervosa and lowers food consumption in patients with bulimia. Administration of GH to elderly men increases lean body mass and improves vigor. GH is released in pulses throughout the day, but the pulses are closer together during the first hours of sleep than at other times. PROLACTIN Since its identification in 1970, the anterior pituitary hormone prolactin has been examined as a potential index of dopamine activity, dopamine receptor sensitivity, and antipsychotic drug concentration in studies of CNS function in psychiatric patients and as a correlate of stress responsivity. The secretion of prolactin is under direct inhibitory regulation by dopamine neurons located in the tuberoinfundibular section of the hypothalamus and is, therefore, increased by classical antipsychotic medications. Prolactin also inhibits its own secretion by means of a short-loop feedback circuit to the hypothalamus. In addition, a great number of prolactin-releasing or prolactin-modifying factors have been identified, including estrogen, serotonin (particularly through the 5HT2 and 5-HT3 receptors), norepinephrine, opioids, TRH, T4, histamine, glutamate, cortisol, CRH, and oxytocin, with interaction effects possible. For example, estrogen may promote the serotonin-stimulated release of prolactin. Prolactin is primarily involved in reproductive functions. During maturation, prolactin secretion participates in gonadal development, whereas, in adults, prolactin contributes to the regulation of the behavioral aspects of reproduction and infant care, including estrogen-dependent sexual receptivity and breast-feeding. In female rats, prolactin secretion is strongly stimulated with exposure to pups. In women, basal prolactin levels are elevated in the postpartum period before weaning, and prolactin release is stimulated by suckling. Hyperprolactinemia is associated with low testosterone in men and reduced libido in men and women. In rodents, prolactin level is increased along with corticosterone in response to such stressful stimuli as immobilization, hypoglycemia, surgery, and cold exposure and may be specifically associated with the use of passive coping in the face of a stressor. Prolactin promotes various stress-related behaviors in rats, depending on the condition, such as increasing object-directed exploration while decreasing other exploration. Patients with hyperprolactinemia often complain of depression, decreased libido, stress intolerance, anxiety, and increased irritability.

These behavioral symptoms usually resolve in parallel with decrements in serum prolactin when surgical or pharmacological treatments are used. In psychotic patients, prolactin concentrations and prolactin-related sexual disturbances have been positively correlated with the severity of tardive dyskinesia. Prolactin levels are also positively correlated with negative symptoms in schizophrenia. MELATONIN

Melatonin, a pineal hormone, is derived from the serotonin molecule and it controls photoperiodically mediated endocrine events (particularly those of the hypothalamic–pituitary–gonadal axis). It also modulates immune function, mood, and reproductive performance and is a potent antioxidant and free-radical scavenger. Melatonin has a depressive effect on CNS excitability, is an analgesic, and has seizure-inhibiting effects in animal studies. Melatonin can be a useful therapeutic agent in the treatment of circadian phase disorders such as jet lag. Intake of melatonin increases the speed of falling asleep, as well as its duration and quality. OXYTOCIN Oxytocin, also a posterior pituitary hormone, is involved in osmoregulation, the milk ejection reflex, food intake, and female maternal and sexual behaviors. Oxytocin is theorized to be released during orgasm, more so in women than in men, and is presumed to promote bonding between the sexes. It has been used in autistic children experimentally in an attempt to increase socialization. INSULIN Increasing evidence indicates that insulin may be integrally involved in learning and memory. Insulin receptors occur in high density in the hippocampus and are thought to help neurons metabolize glucose. Patients with Alzheimer’s disease have lower insulin concentrations in the cerebrospinal fluid (CSF) than controls, and both insulin and glucose dramatically improve verbal memory. Depression is frequent in patients with diabetes, as are indexes of impaired hormonal response to stress. It is not known whether these findings represent direct effects of the disease or are secondary, nonspecific effects. Some antipsychotics are known to dysregulate insulin metabolism. ENDOCRINE VARIABLES IN PSYCHIATRIC DISORDERS Although it is clear that alterations in endocrine regulation are involved in the pathophysiology and treatment responses of many psychiatric disorders, incorporating these findings into clinical diagnostic assessment and decision-making remains problematic. Large-scale longitudinal or cost-effectiveness studies are rare, despite indications that baseline alterations in glucocorticoid regulation and thyroid status (two of the best studied abnormalities) may actually be useful in subtyping psychiatric disorders and in prediction of outcome. Alterations in HPA/stress regulation underlie a number of psychiatric diagnoses and may serve as complementary independent variables in assigning treatment response and course of illness to the classical behavioral categories that have thus far defined psychiatric practice. Studying genetic polymorphisms in factors regulating hormonal response may help us better understand the influence of hormonal variability on the illness and also possible underlying differences in the nature of the illness reflected in these genetic subtypes.

06 - 1.6 Immune System and Central Nervous System

1.6 Immune System and Central Nervous System Interactions

REFERENCES Bartz JA, Hollander E. The neuroscience of affiliation: Forging links between basic and clinical research on neuropeptides and social behavior. Horm Behav. 2006;50:518. Dubrovsky B. Neurosteroids, neuroactive steroids, and symptoms of affective disorders. Pharmacol Biochem Behav. 2006;84:644. Duval F, Mokrani MC, Ortiz JA, Schulz P, Champeval C. Neuroendocrine predictors of the evolution of depression. Dialogues Clin Neurosci. 2005;7:273. Goldberg-Stern H, Ganor Y, Cohen R, Pollak L, Teichberg V, Levite M. Glutamate receptor antibodies directed against AMPA receptors subunit 3 peptide B (GluR3B) associate with some cognitive/psychiatric/behavioral abnormalities in epilepsy patients. Psychoneuroendocrinology. 2014;40:221–231. McEwen BS. Physiology and neurobiology of stress and adaptation: Central role of the brain. Physiol Rev. 2007;87:873. Martin EI, Ressler KJ, Binder E, Nemeroff CB. The neurobiology of anxiety disorders: Brain imaging, genetics, and psychoneuroendocrinology. Clin Lab Med. 2010;30(4):865. Phillips DI. Programming of the stress response: A fundamental mechanism underlying the long-term effects of the fetal environment? J Intern Med. 2007;261:453. Strous RD, Maayan R, Weizman A. The relevance of neurosteroids to clinical psychiatry: From the laboratory to the bedside. Eur Neuropsychopharmacol. 2006;16:155. Zitzmann M. Testosterone and the brain. Aging Male. 2006;9:195. 1.6 Immune System and Central Nervous System Interactions Interactions between the immune system and the central nervous system (CNS) play a critical role in the maintenance of bodily homeostasis and the development of diseases, including psychiatric disease. Alterations in CNS function brought about by a variety of stressors have been shown to influence both the immune system as well as diseases that involve the immune system. Moreover, many of the relevant hormonal and neurotransmitter pathways that mediate these effects have been elucidated. Of considerable interest is accumulating data that cytokines, which derive from immune cells and microglia, have profound effects on the CNS. The relative role of cytokines and their signaling pathways in the various psychiatric diseases is an area of active investigation, as is the role of infectious and autoimmune diseases in the pathophysiology of psychiatric disorders.

Taken together, these findings highlight the importance of interdisciplinary efforts involving the neurosciences and immunology for gaining new insights into the etiology of psychiatric disorders. OVERVIEW OF THE IMMUNE SYSTEM The immune system has the capacity to protect the body from the invasion of foreign pathogens, such as viruses, bacteria, fungi, and parasites. In addition, the immune system can detect and eliminate cells that have become neoplastically transformed. These functions are accomplished through highly specific receptors on immune cells for

molecules derived from invading organisms and a rich intercellular communication network that involves direct cell-to-cell interactions and signaling between cells of the immune system by soluble factors called cytokines. The body's absolute dependence on the efficient functioning of the immune system is illustrated by the less than 1-year survival rate of untreated infants born with severe combined immunodeficiency disease and the devastating opportunistic infections and cancers that arise during untreated acquired immunodeficiency syndrome (AIDS). BEHAVIORAL CONDITIONING The fact that learning processes are capable of influencing immunological function is an example of interactions between the immune system and the nervous system. Several classical conditioning paradigms have been associated with suppression or enhancement of the immune response in various experimental designs. The conditioning of immunological reactivity provides further evidence that the CNS can have significant immunomodulatory effects. Some of the first evidence for immunological conditioning was derived from the serendipitous observation that animals undergoing extinction in a taste-aversion paradigm with cyclophosphamide, an immunosuppressive agent, had unexpected mortality. In that taste-aversion paradigm, animals were simultaneously exposed to an oral saccharin solution (the conditioned stimulus) and an intraperitoneal injection of cyclophosphamide (unconditioned stimulus). Because the animals experienced considerable physical discomfort from the cyclophosphamide injection, through the process of conditioning they began to associate the ill effects of cyclophosphamide with the taste of the oral saccharin solution. If given a choice, the animals avoided the saccharin solution (taste aversion). Conditioned avoidance can be eliminated or extinguished if the saccharin is repeatedly presented in the absence of cyclophosphamide. However, it was observed that animals undergoing extinction of cyclophosphamide-induced taste aversion unexpectedly died, leading to the speculation that the oral saccharin solution had a specific conditioned association with the immunosuppressive effects of cyclophosphamide. Repeated exposure to the saccharin-associated conditioned immunosuppression during extinction might explain the unexpected death of animals. To test that hypothesis researchers conditioned the animals with saccharin (conditioned stimulus) and intraperitoneal cyclophosphamide (unconditioned conditioned stimulus) and then immunized them with sheep red blood cells. At different times after immunization the conditioned animals were re-exposed to saccharin (conditioned stimulus) and examined. The conditioned animals exhibited a significant decrease in mean antibody titers to sheep red blood cells when compared to the control animals. Thus, the evidence demonstrated that immunosuppression of humoral immunity was occurring in response to the conditioned stimulus of saccharin alone. STRESS AND THE IMMUNE RESPONSE Interest in the effects of stress on the immune system grew out of a series of animal and human studies suggesting that stressful stimuli can influence the development of immune-related disorders, including infectious diseases, cancer, and autoimmune disorders. Although stress has been historically associated with suppression of immune function, recent data indicate that such a conclusion oversimplifies the complexities of the mammalian immune response to environmental perturbation and that stress may also activate certain aspects of the immune system, particularly the innate immune

response. Stress and Illness Experiments conducted on laboratory animals in the late 1950s and the early 1960s indicated that a wide variety of stressors—including isolation, rotation, crowding, exposure to a predator, and electric shock—increased morbidity and mortality in response to several types of tumors and infectious diseases caused by viruses and parasites. However, as research progressed it became increasingly clear that “stress” is too variegated a concept to have singular effects on immunity and that, in fact, the effects of stress on immunity depend on a number of factors. Chief among these factors is whether a stressor is acute or chronic. Other critical variables include stressor severity and type, as well as the timing of stressor application and the type of tumor or infectious agent investigated. For example, mice subjected to electric grid shock 1 to 3 days before the infection of Maloney murine sarcoma virus-induced tumor cells exhibited decreased tumor size and incidence. In contrast, mice exposed to grid shock 2 days after tumor cell injection exhibited an increase in tumor size and number. The relevance of the effects of stress on immune-related health outcomes in humans has been demonstrated in studies that have shown an association between chronic stress and increased susceptibility to the common cold, reduced antibody responses to vaccination, and delayed wound healing. In addition, stress, as well as depression, through their effects on inflammation have been linked to increased morbidity and mortality in infectious diseases, such as HIV infection, autoimmune disorders, neoplastic diseases, as well as diabetes and cardiovascular disorders, which are increasingly being recognized as diseases in which the immune system, inflammation in particular, plays a pivotal role (Fig. 1.6-1).

FIGURE 1.6-1 Inflammation and disease. IL, interleukin; TNF, tumor necrosis factor; NF-κB, nuclear factor κB; CRP, C-reactive protein. (From Cowles MK, Miller AH. Stress cytokines and depressive illness. In: Squire LR, ed. The New Encyclopedia of Neuroscience. Academic Press; 2009:521, with permission.) Effects of Chronic Stress When challenged with a medical illness or chronic psychological stressor, complex interactions between the immune and nervous systems promote a constellation of immune-induced behavioral changes, alternatively referred to as “sickness syndrome” or “sickness behavior.” These behavioral changes include dysphoria, anhedonia, fatigue, social withdrawal, hyperalgesia, anorexia, altered sleep–wake patterns, and cognitive dysfunction. Although seen in response to infection, the full syndrome can be reproduced in humans and laboratory animals by administration of innate immune cytokines. Blocking cytokine activity diminishes or prevents the development of sickness behavior in laboratory animals, even when such behavior develops as a result of psychological stress. Evidence that cytokine-induced behavioral toxicity is related to major depression comes in part from studies showing that in humans and laboratory animals, antidepressants are able to abolish or attenuate the development of sickness behavior in response to cytokine administration.

RELEVANCE OF IMMUNE SYSTEM–CNS INTERACTIONS TO PSYCHIATRIC DISORDERS Major Depression The neuropsychiatric disorder that has been best characterized in terms of the influence of the brain on the immune system and vice versa is major depression. For many years major depression was seen as the quintessential example of how stress-related disorders may decrease immunocompetence. More recently, however, it has become evident that stress also activates inflammatory pathways, even while suppressing measures of acquired immunity. Not surprisingly, studies now indicate that, in addition to immunosuppression, major depression is also frequently associated with inflammatory activation. Recent research showing that proinflammatory cytokines are capable of suppressing many of the immune measures examined in major depression may provide a mechanism to account for how chronic stress-induced inflammatory activity may

give rise to depression-related suppression of in vitro functional assays, such as lymphocyte proliferation. Bipolar Disorder Patients with bipolar disorder evince many of the immune alterations frequently observed in the context of unipolar depression. Several studies have observed that bipolar patients, especially when manic, demonstrate increased plasma concentrations of inflammatory cytokines. Other studies indicate that treatments for mania, such as lithium, lower plasma concentrations of a number of cytokines. Of interest, the available literature seems to suggest that patients in the manic phase of the disorder may be more likely than depressed patients to demonstrate increased inflammatory markers. It should not be surprising that mania—which seems the phenomenological opposite of depression—should be associated with increased inflammation, given that mania and depression have also been reported to show identical neuroendocrine and autonomic abnormalities, such as dexamethasone nonsuppression and increased sympathetic activity, both of which would be expected to promote inflammatory activity. Schizophrenia There has been growing interest in the idea that infectious agents, particularly viruses, may underlie at least some cases of schizophrenia. Although it is well established that viral encephalitis can present clinically as psychosis, the primary focus of the "viral hypothesis" for schizophrenia has been on infections during neurodevelopment given its congruence with the emerging consensus that prenatal or early postnatal insult is implicated in the causality of schizophrenia. Several lines of indirect evidence suggest that viral infection during CNS development may be involved in the pathogenesis of schizophrenia. The data include: (1) an excess number of patient births in the late

winter and early spring, suggesting possible exposure to viral infection in utero during the fall and winter peak of viral illnesses, (2) an association between exposure to viral epidemics in utero and the later development of schizophrenia, (3) a higher prevalence of schizophrenia in crowded urban areas, which have conditions that are particularly conducive to the transmission of viral pathogens, and (4) seroepidemiological studies indicating a higher infection rate for certain viruses in schizophrenia patients or their mothers. In addition, schizophrenia has been associated with indices of immune activation, including elevations in cytokines. Although these immune findings in patients with schizophrenia may indicate evidence of immune system activation secondary to infection, it should be noted that they might also indicate that an autoimmune process is involved in the disorder. Despite the plethora of studies pointing to abnormalities in cellular and humoral immunity in schizophrenia, the data have not been uniform or conclusive, and there is a need for more studies that account for confounding variables such as medication status and tobacco use. Moreover, attempts to isolate infectious agents from schizophrenic brain tissue or to detect viral nucleic acids in the CNS or peripheral blood of patients with schizophrenia have generally yielded negative results. Because the initial neuronal abnormalities in schizophrenia have been proposed to arise during neurodevelopment, a perinatal viral infection could insidiously disrupt development and then be cleared by the immune system prior to clinical diagnosis. In such a scenario, host factors such as cytokines could be responsible for causing the developmental abnormality by interacting with growth factors or adhesion molecules. Recent animal models have identified that maternal immune activation with resultant production of interleukin 6 (IL-6) critically affects behavioral and transcriptional changes in offspring. Behavioral changes, including deficits in prepulse inhibition and latent inhibition, are consistent with behavioral abnormalities in animal models of both schizophrenia and autism. Various animal models using influenza virus, Borna disease virus, or lymphocytic choriomeningitis virus in rodents have demonstrated that prenatal or postnatal viral infections can lead to neuroanatomical or behavioral alterations that are somewhat

reminiscent of schizophrenia in humans. As mentioned earlier, epidemiological studies also support the link between infection with a teratogenic virus and the development of psychotic disorders later in life. Associations have been observed between maternal infection with rubella or influenza during gestation and the development of a schizophrenia spectrum disorder in the offspring. Similarly, maternal antibodies to herpes simplex virus that develop during pregnancy are correlated with increased rates of psychosis during adulthood in the offspring. Non-HIV retroviruses might also play a role in the pathogenesis of schizophrenia. Retroviruses integrate into host deoxyribonucleic acid (DNA) and can disrupt the function of adjacent genes. Moreover, the genomes of all humans contain sequences of “endogenous retroviruses” that hold the capacity to alter the transcriptional regulation of host genes. If genes controlling the development or function of the brain undergo transcriptional disruption by retroviral effects, then this might lead to a cascade of biochemical abnormalities eventually giving rise to schizophrenia. Autism

Although a convincing case can be made for a significant immune component in autism, the relationship of immune abnormalities to the neurobehavioral symptoms of the disease remains controversial. The claim that autism is triggered by childhood vaccines has not been substantiated by recent epidemiological studies, and immune-based therapies for autism have not been reliably effective. Thus, although it is tempting to speculate that the immune system holds a clue to a cure for autism, there is currently not enough data to determine whether immune anomalies cause autism, are caused by autism, or are just adventitiously associated with the disease. Alzheimer’s Disease Although Alzheimer’s disease is not considered primarily an inflammatory disease, emerging evidence indicates that the immune system may contribute to its pathogenesis. The discovery that amyloid plaques are associated with acute-phase proteins, such as complement proteins and C-reactive protein, suggests the possibility of an ongoing immune response. The idea that inflammatory processes are involved in Alzheimer’s disease has been bolstered by recent studies showing that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) is negatively correlated with the development of Alzheimer’s disease. HIV AIDS AIDS is an immunological disease associated with a variety of neurological manifestations, including dementia. HIV encephalitis results in synaptic abnormalities and loss of neurons in the limbic system, basal ganglia, and neocortex. Multiple Sclerosis Multiple sclerosis (MS) is a demyelinating disease characterized by disseminated inflammatory lesions of white matter. Considerable progress has been made in elucidating the immunopathology of myelin destruction that occurs in MS and in the animal model for the disease, experimental allergic encephalomyelitis. Although the initial step in lesion formation has not been determined, disruption of the blood–brain barrier and infiltration of T cells, B cells, plasma cells, and macrophages appear to be associated with lesion formation. Other Disorders Finally, several disorders are seen in which neural-immune interactions are suspected but not well documented. Chronic fatigue syndrome is an illness with a controversial etiology and pathogenesis. In addition to persistent fatigue, symptoms frequently include depression and sleep disturbances. Tests of immune function have found indications of both immune activation and immunosuppression. Neuroendocrine assessments indicate that patients with chronic fatigue syndrome may be hypocortisolemic because of impaired activation of the hypothalamic–pituitary–adrenal

axis. Although an acute viral infection frequently precedes the onset of chronic fatigue syndrome, no infectious agent has been causally associated with it. In contrast, Lyme disease, in which sleep disturbances and depression are also common, is clearly caused by infection with the tick-borne

spirochete Borrelia burgdorferi, which can invade the CNS and cause encephalitis and neurological symptoms. Lyme disease is remarkable because it appears to produce a spectrum of neuropsychiatric disorders, including anxiety, irritability, obsessions, compulsions, hallucinations, and cognitive deficits. Immunopathology of the CNS may be involved, because symptoms can persist or reappear even after a lengthy course of antibiotic treatment, and the spirochete is frequently difficult to isolate from the brain. Gulf War syndrome is a controversial condition with inflammatory and neuropsychiatric features. The condition has been attributed variously to combat stress, chemical weapons (e.g., cholinesterase inhibitors), infections, and vaccines. Given the impact of stress on neurochemistry and immune responses, these pathogenic mechanisms are not mutually exclusive. THERAPEUTIC IMPLICATIONS The bidirectional nature of CNS–immune system interactions implies the therapeutic possibility that agents known to positively alter stress system activity might benefit immune functioning and, conversely, that agents that modulate immune functioning may be of potential benefit in the treatment of neuropsychiatric disturbance, especially in the context of medical illness. Increasing evidence supports both hypotheses. Antidepressants and the Immune System Emerging data indicate that in animals and humans, antidepressants attenuate or abolish behavioral symptoms induced by inflammatory cytokine exposure. For example, pretreatment of rats with either imipramine or fluoxetine (a tricyclic antidepressant and selective serotonin reuptake inhibitor, respectively) for 5 weeks prior to endotoxin administration significantly attenuated endotoxin-induced decrements in saccharine preference (commonly accepted as a measure for anhedonia), as well as weight loss, anorexia, and reduced exploratory, locomotor, and social behavior. Similarly, several studies in humans suggest that antidepressants can ameliorate mood disturbances in the context of chronic cytokine therapies, especially if given prophylactically before cytokine exposure. For example, the selective serotonin reuptake inhibitor paroxetine significantly decreased the development of major depression in patients receiving high doses of interferon-α (IFN-α) for malignant melanoma. Behavioral Interventions and Immunity It has been known for years that psychosocial factors can mitigate or worsen the effects of stress, not only on immune functioning but also on the long-term outcomes of medical conditions in which the immune system is known to play a role. Therefore, behavioral interventions aimed at maximizing protective psychosocial factors might be predicted to

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have a beneficial effect, not only in terms of mitigating the effect of stress on immune functioning but perhaps also on diminishing emotional disturbances that arise in the context of immune system dysregulation. Two factors that have been repeatedly identified as protective against stress-induced immune alterations are social support and the ability to see stressors as being to some degree under the individual's control. In this regard, a recent study that conducted a genome-wide scan to assess gene expression activity in socially isolated versus nonisolated individuals found that social isolation was associated with increased activation of a number of proinflammatory, cytokine-related pathways and reduced activity in anti-inflammatory cytokine pathways, as well as in the glucocorticoid receptor, which plays an important role in neuroendocrine control of inflammatory processes. Of interest, the two types of psychotherapy most often examined in illnesses associated with immune dysregulation are group therapy, which provides social support, and cognitive behavioral therapy, which provides cognitive reframing techniques aimed at enhancing one's sense of agency (and hence control). REFERENCES Bajramovic J. Regulation of innate immune responses in the central nervous system. CNS Neurol Disord Drug Targets. 2011;10:4. Capuron L, Miller AH. Immune system to brain signaling: Neuropsychopharmacological implications. Pharmacol Ther. 2011;130(2):226. Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch Gen Psychiatry. 2008;65:409. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: When the immune system subjugates the brain. Nat Rev Neurosci. 2008;9:46. Raison CL, Borisov AS, Woolwine BJ, Massung B, Vogt G, Miller AH. Interferon-α effects on diurnal hypothalamicpituitary-adrenal axis activity: Relationship with proinflammatory cytokines and behavior. Mol Psychiatry. 2010;15:535. Raison CL, Cowles MK, Miller AH. Immune system and central nervous system interactions. In: Sadock BJ, Sadock VA, Ruiz P, eds. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th edition. Philadelphia: Lippincott Williams & Wilkins; 2009:175. Ransohoff RM, Brown MA. Innate immunity in the central nervous system. J Clin Invest. 2012;122(4):1164. Steiner J, Bernstein HG, Schiltz K, Müller UJ, Westphal S, Drexhage HA, Bogerts B. Immune system and glucose metabolism interaction in schizophrenia: A chicken–egg dilemma. Prog Neuropsychopharmacol Biol Psychiatry. 2014;48:287–294. Wilson EH, Weninger W, Hunter CA. Trafficking of immune cells in the central nervous system. J Clin Invest. 2010;120(5):1368. Yousef S, Planas R, Chakroun K, Hoffmeister-Ullerich S, Binder TM, Eiermann TH, Martin R, Sospedra M. TCR bias and HLA cross-restriction are strategies of human brain-infiltrating JC virus-specific CD4+ T cells during viral infection. J Immunol. 2012;189(7):3618. 1.7 Neurogenetics

Starting from the rediscovery of Gregor Mendel's basic concepts at the turn of the 20th century, the field of genetics has matured into an essential cornerstone not only of the biological sciences

but of all of medicine. The discovery of the basic structure and properties of deoxyribonucleic acid (DNA) in the middle of the century led to an exponential acceleration in our understanding of all aspects of the life sciences, including deciphering the complete sequence of the human genome, and those of myriad other species. Massive databases of such sequences now provide 21st-century biologists with the task of decoding the functional significance of all this information. In particular, attention has turned to determining how sequence variations contribute to the phenotypic variation between species and between individuals within a species; in humans it is hoped that discoveries about the relationship between genotypes and phenotypes will revolutionize our understanding of why and how some individuals but not others develop common diseases. This hope is particularly strong for psychiatry, as our knowledge of the pathogenic mechanisms of psychiatric disease remains sparse. Genetic mapping studies aim to identify the genes implicated in heritable diseases, based on their chromosomal location. These studies are carried out by investigating affected individuals and their families through two approaches, linkage and association (Fig. 1.7-1). It is now straightforward to genetically map Mendelian traits (traits for which a specific genotype at one particular locus is both necessary and sufficient to cause the trait). Psychiatric diseases, however, do not follow simple Mendelian inheritance patterns but rather are examples of etiologically complex traits. Etiological complexity may be due to many factors, including incomplete penetrance (expression of the phenotype in only some of the individuals carrying the disease-related genotype), the presence of phenocopies (forms of the disease that are not caused by genetic factors), locus heterogeneity (different genes associated with the same disease in different families or populations), or polygenic inheritance (risk for disease increases only if susceptibility variants at multiple genes act in concert). Mapping a complex disorder involves several component steps, including definition of the phenotype to be studied, epidemiological studies to determine the evidence for genetic transmission of that phenotype, choice of an informative study population, and determination of the appropriate experimental and statistical approaches.

FIGURE 1.7-1 Comparison of gene-mapping strategies. Genetic mapping approaches can be divided into those that rely on linkage analysis and those that rely on association analysis. Linkage studies can be further categorized as either focused on investigation of pedigrees or focused on investigation of sib pairs. Association studies can be categorized as either case–control or family-based. Some of the key features as well as advantages and disadvantages of these different approaches are shown. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock’s Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:321.) GENETIC EPIDEMIOLOGICAL APPROACHES Genetic epidemiological investigations provide quantitative evidence regarding the degree to which a given trait aggregates in families and, furthermore, can suggest to what degree such aggregation reflects a genetic contribution to the etiology of the trait. Family studies compare the aggregation of disease among the relatives of affected individuals compared to control samples. Because these studies do not differentiate between genetic and environmental contributions to such familial aggregation, they provide only indirect evidence regarding the heritability of a trait. Often these studies

measure the relative risk (λ), defined as the rate of occurrence of a disease among specified categories of relatives of an affected individual divided by the rate of occurrence of the disease for the general population. A relative risk of >1 suggests a genetic etiology, and the magnitude of the measure gives an estimate of the genetic contribution to the disease. Relative risks can be

calculated for sibling pairs, parent– offspring pairs, and various other types of family relationships. Likely modes of transmission can be assessed by comparing the degree of relative risk for each type of relationship. Multiple family studies have been carried out for many of the major psychiatric disorders, including major depression, bipolar disorder, schizophrenia, and obsessive-compulsive disorder (OCD). Although these studies have consistently reported familial aggregation for all of these disorders, the degree of such aggregation has varied substantially across studies, largely reflecting differences in phenotype definition and how study samples were ascertained and assessed. Twin studies examine the concordance rates of a particular disorder (the percentage of twin pairs where both twins have the disorder) in monozygotic (MZ) and dizygotic (DZ) twins. For a disorder that is strictly determined by genetic factors, the concordance rate should be 100 percent in MZ twin pairs (who share 100 percent of their genetic material) and 25 or 50 percent in DZ twin pairs (who are no more closely related than any siblings), depending on whether the disease is recessive or dominant, respectively. For a disorder where genetic factors play a role in disease causation but are not the exclusive cause of disease, the concordance rates should be greater for MZ twins than those for DZ twins. The higher the degree of concordance of MZ twins, the higher the trait heritability or the evidence for a genetic contribution to disease risk. When genetic factors do not play a role, the concordance rates should not differ between the twin pairs, under the simplifying assumption that the environment for MZ twin pairs is no more similar than that for DZ twin pairs. The several twin studies that have been conducted for traits such as autism, bipolar disorder, and schizophrenia have consistently suggested high heritability and have therefore spurred efforts to genetically map loci for each of these conditions. Different twin studies may however generate varying point estimates for the heritability of any given disorder. When evaluating the results of twin studies, it is therefore important to scrutinize how the phenotype was ascertained because, as with family studies, the different heritability estimates are likely due to differences in the mode of assessing and defining phenotypes. For example, early twin studies of psychiatric disorders often relied for their phenotypes on unstructured interviews by a single clinician. In contrast, modern studies generally utilize standardized assessments and review of diagnostic material by a panel of expert clinicians. Similarly, part of the apparent variation in heritability between different twin studies can be attributed to the fact that some studies employ narrow definitions of affectedness for a given phenotype, while other studies employ broader phenotype definitions (e.g., considering a twin with major depressive disorder to be phenotypically concordant with a co-twin diagnosed with bipolar disorder). Because of such differences in approach across studies it is usually prudent to view such investigations as providing a rough estimate of the genetic contribution to trait variability. Nevertheless, even such estimates are useful in deciding which traits are likely to be mappable. BASIC CONCEPTS OF GENE MAPPING Recombination and Linkage Once genetic epidemiological studies of particular phenotypes have suggested that these phenotypes are heritable, genetic mapping studies are conducted to identify the specific genetic variants that contribute to the risk of the disorder. All genetic mapping methods

aim to identify disease-associated variants based on their chromosomal position and the principle of genetic linkage. All cells contain two copies of each chromosome (called homologs), one inherited from the mother and one inherited from the father. During meiosis, the parental homologs cross over, or recombine, creating unique new chromosomes that are then passed on to the progeny. Genes that are physically close to one another on a chromosome are genetically linked, and those that are farther apart or are on different chromosomes are genetically unlinked.

Genes that are unlinked will recombine at random (i.e., there is a 50 percent chance of recombination with each meiosis). Genetic loci that are linked will recombine less frequently than expected by random segregation, with the degree of recombination proportional to the physical distance between them. The principle of linkage underlies the use of genetic markers, segments of DNA of known chromosomal location that contain variations or polymorphisms (described in more detail later). Strategies to map disease genes are based on identifying genetic marker alleles that are shared—to a greater extent than expected by chance—by affected individuals. It is presumed that such sharing reflects linkage between a disease locus and a marker locus, that is, the alleles at both loci are inherited "identical by descent" (IBD), from a common ancestor, and, furthermore, that this linkage pinpoints the chromosomal site of the disease locus. The evidence for linkage between two loci depends on the recombination frequency between them. Recombination frequency is measured by the recombination fraction (Θ) and is equal to the genetic distance between the two loci (1 percent recombination equals 1 centimorgan [cM] in genetic distance and, on average, covers a physical distance of about 1 megabase [mB] of DNA). A recombination fraction of 0.5 or 50 percent indicates that two loci are not linked but rather that they are segregating independently. A LOD (logarithm of the odds) score is calculated to determine the likelihood that two loci are linked at any particular genetic distance. The LOD score is calculated by dividing the likelihood of acquiring the data if the loci are linked at a given recombination fraction by the likelihood of acquiring the data if the loci are unlinked (Θ = 0.5). This step gives an odds ratio, and the log (base 10) of this odds ratio is the LOD score. A LOD score can be obtained for various values of the recombination fraction, from Θ = 0 (completely linked) to Θ = 0.5 (unlinked). The value of Θ that gives the largest LOD score is considered to be the best estimate of the recombination fraction between the disease locus and the marker locus. This recombination fraction can then be converted into a genetic map distance between the two loci. Linkage Disequilibrium Linkage disequilibrium (LD) is a phenomenon that is used to evaluate the genetic distance between loci in populations rather than in families. When alleles at two loci occur together in the population more often than would be expected given the allele frequencies at the two loci, those alleles are said to be in LD. When strong LD is observed between two loci it usually indicates that the two loci are sited in very close physical proximity to one another on a given chromosome, and is useful in mapping disease susceptibility loci because one locus can be used to predict the presence of another locus. This predictability is important because current gene-mapping strategies are able to sample only a subset of the estimated 10 million common human

polymorphisms. Because of the existence of LD, one can use data from a subset of genotyped polymorphisms to infer genotypes at nearby loci. Clusters of alleles that are in LD and inherited as a single unit are termed haplotypes. Thus LD mapping "consolidates" genomic information by identifying haplotypes in populations that can then be used to infer IBD sharing among unrelated individuals. There are several methods to measure the extent of LD. One of the most commonly used measures of LD is r2, a measure of the difference between observed and expected haplotype probabilities. Unlike D′, another widely used measure of LD, r2 values do not depend on the allele frequencies of the loci being assessed. A large r2 value indicates that the observed frequency of association between two alleles is greater than that expected by chance; that is, the alleles are in LD. LD studies have traditionally been used to complement traditional pedigree analyses, for example, to hone in on a locus that has been mapped by linkage analysis. However, LD-based association analysis has become the method of choice for whole genome screens, particularly for diseases where traditional linkage studies have been unsuccessful. These studies have one great

advantage over a traditional family analysis: because affected individuals are chosen from an entire population rather than from one or a few pedigrees, the number of potential subjects is limited only by the size of the population and the frequency of the disease. Maximizing the potential number of affected individuals that can be included in the analysis is extremely important for disorders where genetic heterogeneity or incomplete penetrance is likely to be a factor. Genetic Markers Mapping studies, regardless of their type, depend on the availability of genetic markers. The most widely used markers are microsatellite markers (also called simple tandem repeats [STRs], or simple sequence length polymorphisms [SSLPs]) and single nucleotide polymorphisms (SNPs). SSLPs are stretches of variable numbers of repeated nucleotides two to four base pairs in length. These markers are highly polymorphic, as the number of repeat units at any given STR locus varies substantially between individuals. SNPs, as the name implies, are single base pair changes at a specific nucleotide; they are the most common form of sequence variation in the genome. SNPs are widely used for genetic mapping studies because they are distributed so widely across the genome and because they can be assessed in a high-throughput, automated fashion. Other forms of genetic variation that have been investigated for use as genetic markers include small insertion or deletion polymorphisms, termed indels, that generally range between 1 and 30 base pairs and copy number variations (CNVs), which can refer to either deletions or duplications. Recent genomewide surveys have revealed that CNVs are common and can range in length from several base pairs to several million base pairs. CNVs may contribute to chromosomal recombination and rearrangements, thereby playing an important role in generating genetic diversity, and also, as many of these variants are sizable, it is hypothesized that they may significantly influence the expression of genes that encompass or are adjacent to the variant. MAPPING STRATEGIES The genetic variants that contribute to disease susceptibility can be roughly categorized

into those that are highly penetrant and those that are of low penetrance. Highpenetrance variants by definition have a large effect on phenotype, and therefore identifying these variants usually provides fundamental insights into pathobiology. Because individuals carrying high-penetrance variants have a high probability of expressing a disease phenotype, such variants tend to be rare and to segregate in families and are generally most powerfully mapped using pedigree-based approaches (see Fig. 1.7-1). In contrast, low-penetrance variants have a relatively weak effect on phenotype, and therefore identification of individual low-penetrance variants may, at least initially, provide relatively little new biological knowledge. However, because of their small effects, such variants are typically common in the population, and therefore identifying them may add to our understanding of disease risk in the population as a whole. Because we do not expect these variants to segregate strongly with the disease phenotype in pedigrees, efforts to identify them focus on population samples. Pedigree Analysis A pedigree analysis, which is conducted in multigenerational families, consists of scanning the genome or a portion of the genome with a series of markers in one or more affected pedigrees, calculating a LOD score at each marker position, and identifying the chromosomal regions that show a significant deviation from what would be expected under independent assortment. The primary goal of pedigree analysis is to determine if two or more genetic loci (i.e., a genetic marker of known location and the unknown disease loci) are cosegregating within a pedigree. Following the successful application of pedigree analysis to map Mendelian disorders such as Huntington’s disease, many investigators adopted this strategy for mapping psychiatric disease genes with, at best, mixed success. In the late 1980s and mid-1990s, several pedigree-based studies reported the mapping of susceptibility loci for

Alzheimer’s disease, bipolar disorder, and schizophrenia. Although the linkage findings for three Alzheimer’s disease loci were relatively quickly replicated, the findings reported for bipolar disorder and schizophrenia were ultimately determined to have been false positives. A number of different explanations have been proposed for the failure of pedigree-based approaches to map psychiatric loci; however, most investigators now recognize that these studies were generally drastically underpowered considering the apparent etiological complexity of psychiatric disorders. Pedigree analysis in psychiatry has increasingly turned toward an application that is more appropriately powered, namely, the mapping of quantitative trait loci (QTLs). QTLs are defined as genetic loci that contribute to the variation in continuously varying traits (as opposed to categorical traits such as disease diagnoses). QTLs are typically loci of small effect that only contribute to a portion of the observed variance of a trait in the population. It is now generally accepted that, using analytical methods developed in the late 1990s, it may be possible to use pedigree studies to map a wide range of quantitative traits that are relevant for understanding psychiatric disorders. Several such studies are now being undertaken, typically with multiple phenotypes being assessed in

each individual in the pedigree. Sib Pair Analysis Affected sib pair (ASP) analysis became widely used during the 1990s for the genetic mapping of complex traits, including many psychiatric disorders. Sib pair analysis examines the frequency with which sibling pairs concordant for a trait share a particular region of the genome compared with the frequency that is expected under random segregation. Sib pair analysis is based on the fact that siblings share approximately 50 percent of their genomes IBD. Therefore, if a set of unrelated sib pairs affected with a given trait shares a particular area of the genome at a frequency significantly greater than 50 percent (the proportion of sharing expected under conditions of random segregation), then that area of the genome is likely to be linked to the trait in question. In this method, siblings are genotyped, and population frequencies and parental genotypes are used to estimate the proportion of genes shared IBD at each site for each sib pair. The linkage analysis then compares those pairs concordant and discordant for each locus. Like pedigree studies, ASP studies have more power to locate genes of large effect than genes of small effect. This limitation can be partially addressed by a two-tiered design that incorporates additional markers or family members after an initial linkage study in affected siblings or by increased sample size. It generally requires less effort to identify and assess even large sets of affected sibs than to identify and assess all members of extended pedigrees, particularly when investigators can take advantage of data repositories that include samples and phenotype data from sib pairs ascertained from multiple sites. For example, the U.S. National Institute of Mental Health (NIMH) maintains such repositories for sizable collections of sib pairs affected with schizophrenia, bipolar disorder, autism, and Alzheimer’s disease. An additional benefit of the ASP design is that it allows for the incorporation of epidemiological information, permitting the simultaneous examination of environmental and gene–environment interactions. Association Studies In the last few years, there has been increasing acceptance of the notion that association studies are more powerful than linkage approaches for mapping the loci of relatively small effect that are thought to underlie much of the risk for complex disorders. Whereas linkage studies attempt to find cosegregation of a genetic marker and a disease locus within a family or families, association studies examine whether a particular allele occurs more frequently than expected in affected individuals within a population. As noted previously in this chapter, mapping of genes using association studies is based on the idea that certain alleles at markers closely surrounding a disease gene will be in LD with the gene; that is, these alleles will be carried in affected individuals more often than expected by random segregation, because they are inherited

IBD. There are two common approaches to association studies (see Fig. 1.7-1), case–control designs and family-based designs, which typically investigate trios (mother, father, and an affected offspring). In a case–control study, allele frequencies are compared between a group of unrelated affected individuals and a matched control sample. This design is

generally more powerful than a family-based design, because large samples of cases and controls are easier to collect than trios and are less expensive, since they require the genotyping of fewer individuals. Case–control samples may be the only practical design for traits with a late age of onset (such as Alzheimer's disease) for which parents of affected individuals are typically unavailable. The main drawback of the case–control approach is the potential problem of population stratification; if the cases and controls are not carefully matched demographically, then they may display substantial differences in allele frequency that reflect population differences rather than associations with the disease. Family-based association studies are designed to ameliorate the problem of population stratification. In this design, the nontransmitted chromosomes (the copy of each chromosome that is not passed from parent to child) are used as control chromosomes, and differences between allele frequencies in the transmitted and nontransmitted chromosomes are examined, eliminating the problem of stratification, as the comparison group is by definition genetically similar to the case group. Although more robust to population stratification than a case–control study, family-based studies are only about two-thirds as powerful using the same number of affected individuals, as noted previously. Until recently, it was not practical to conduct association studies on a genomewide basis, as relatively few SNPs were available. Therefore, association studies focused on testing one or a few markers in candidate genes chosen on the basis of their hypothesized function in relation to a given disease. Recently, however, as a result of international efforts that have identified millions of SNPs distributed relatively evenly across the genome and that have developed technology for genotyping them relatively inexpensively, genomewide association (GWA) studies are now a reality. Such studies hold much promise for the identification of common variants contributing to common diseases. While few GWA studies of psychiatric disorders have been completed, such studies have already reported remarkable findings for complex traits such as rheumatoid arthritis, inflammatory bowel disease, and type 2 diabetes. The successful studies of these diseases have made use of very large samples (in some cases up to several thousand cases and controls), providing further support for the hypothesis that underpowered study designs bear much of the responsibility for the disappointing results to date of psychiatric genetic investigations. Statistical Considerations Scientists in other biomedical research fields are often surprised by the apparently high level of statistical evidence that geneticists require to consider a linkage or association result to be significant. Most simply, this requirement can be thought of in terms of the very low expectation that any two loci selected from the genome are either linked or associated with one another. The likelihood that any two given loci are linked (i.e., the prior probability of linkage) is expected to be approximately 1:50, based on the genetic length of the genome. To compensate for this low prior probability of linkage and bring the posterior (or overall) probability of linkage to about 1:20, which corresponds to the commonly accepted significance level of $P = .05$, a conditional probability of 1,000:1 odds in favor of linkage is required, corresponding to the traditionally accepted LOD score threshold of 3. This generally provides an acceptable false-positive rate (Fig. 1.72), but some false-positive findings have exceeded even this threshold.

FIGURE 1.7-2 Number of false positives expected in a whole genome scan for a given threshold of logarithm of odds (LOD) score. Solid line represents the expectation for a perfect genetic map. Symbols represent the results for 100 sib pairs using genetic maps with markers spaced every .1 cM (circles), every 1 cM (squares), and every 10 cM (triangles). The dotted line indicates the 5 percent genomewide significance level. (Courtesy of Dr. Eric Lander). Geneticists generally assume that the expectation that any two loci in the genome are associated with one another is even lower than that of their being in linkage, and typically a P value of less than about 10−7 is considered to indicate “genomewide significance.” This standard essentially discounts the prior probability that some investigators assign to variants in candidate genes chosen on the basis of their hypothesized functional relevance to a given disorder or trait. GWA studies are now replicating associations with very low P values for a wide range of complex traits, whereas most candidate gene associations (which usually report as significant much higher P values) remain unreplicated. It is therefore increasingly apparent that genomewide levels of significance are appropriately applied to all initial association studies for a given trait. DEFINING PHENOTYPES FOR MAPPING STUDIES The generally disappointing results of psychiatric genetic mapping studies have focused increasing attention on the problem of defining and assessing phenotypes for such studies. Most psychiatric mapping studies to date have relied on categorical disease diagnoses, as exemplified by the Diagnostic and Statistical Manual (DSM-5) classification scheme. Criticisms of this approach rest on two arguments. First, diagnosis of psychiatric disease depends on subjective clinical evaluation, a fact that underscores the difficulty in ascertaining individuals who can be considered definitely affected with a given disease.

Second, even when a psychiatric diagnosis can be established unambiguously, the menubased system used for psychiatric classification provides the possibility that any two individuals affected with a given disorder may display largely nonoverlapping sets of symptoms, likely reflecting distinct etiologies. Concern that the diagnosis-based approach to phenotyping may represent one of the chief obstacles to the genetic mapping of psychiatric phenotypes has generated considerable interest in mapping heritable traits known to demonstrate continuous variation in the population. Continuous measures that are hypothesized to be related to psychiatric disorders include biochemical measures (e.g., serum or CSF levels of neurotransmitter metabolites or hormones), cognitive measures, personality assessments, structural or functional brain images, biophysical markers such as responses to evoked potentials, or molecular assays such as gene expression profiles. Key features of categorical and continuous phenotyping strategies are shown in Figure 1.7-3, and each is discussed in more detail below. FIGURE 1.7-3 Two alternate schemes for conceptualizing psychiatric phenotypes. A. Categorical Traits as conceptualized by the Diagnostic and Statistical Manual (DSM-5) represent a “menubased” approach to psychiatric disorders. Individuals are assessed for a checklist of signs and symptoms that are then used to categorize the individual as “affected” according to a specific diagnosis. Not all symptoms are present in samples of individuals who carry a particular DSM diagnosis, and many of these symptoms occur across diagnostic boundaries, as illustrated in this Venn diagram. DSM phenotypes therefore probably represent etiologically heterogeneous categories, and this fact may help to explain the

limited progress thus far of genetic mapping investigations focused on these phenotypes. B. Alternatively, in the Continuous Traits model, “affectedness” can be conceptualized in terms of an expectation that an individual will demonstrate extreme values on a set of continuous measures

that correlate with psychopathology and thus are hypothesized to underlie the disorder (as illustrated by examples of six different types of measures shown in the hexagon). Such measures may also be associated with particular components of categorical phenotypes, such as those depicted in the Venn diagram in Figure 19-3A. The justification for using continuous measures as the phenotypes for genetic mapping studies is that they are considered etiologically simpler and more reliably assessed compared to categorical phenotypes. In addition, mapping such traits combines information from all members of the study population (affected and unaffected individuals alike), which adds considerably to power. (From Sadock BJ, Sadock VA, Ruiz P. Kaplan & Sadock's Comprehensive Textbook of Psychiatry. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2009:325.) Categorical Phenotypes The most commonly used categorical phenotypes in psychiatry are DSM diagnoses. Some studies focus on a single DSM diagnosis, whereas other studies include individuals with a range of different diagnoses. The latter approach is typically used for disorders that are hypothesized to represent a single disease spectrum, such as mood disorders. Using the categorical approach, it is important to be able to classify subjects as unambiguously as possible. Several strategies are used to accomplish this goal. The first strategy involves deciding on the appropriate diagnostic criteria for the study in question and deciding how these criteria will be applied to individuals in the study. One way of standardizing the procedures used to identify and assess potential study subjects is to use only experienced clinicians in the diagnostic process and to train them in the administration of the instruments and the diagnostic criteria to be employed. In addition, a "best estimate" procedure and/or a consensus diagnosis is frequently used. The best estimate process involves making use of every piece of available information, including medical records, interviews, and videotapes, to arrive at a diagnosis. For a consensus diagnosis, two or more diagnosticians independently review the material and make a diagnosis for each individual. The diagnoses are then compared, and individuals for whom an agreement in diagnosis cannot be reached are not entered as "affected" into the study. A well-designed study makes use of all available information about the genetic epidemiology of the disorder to choose a sample of affected individuals to study. It is often the case that a subset of families carries the disorder in what appears to be a simple Mendelian pattern, whereas the inheritance pattern is less clear for other families or groups. In a disorder where there are likely to be multiple genes contributing to the phenotype, it makes sense to begin with a study sample where there may be major loci. Redefining the disease phenotype can often simplify the mapping process by identifying such groups or families. For example, in the search for a genetic defect for

Alzheimer's disease, the process was advanced enormously by limiting the study population to those individuals who had early age of onset (before age 65); the early onset trait segregated in an autosomal dominant fashion. Other ways of redefining the phenotype include focusing on factors such as ethnic background, age of onset, treatment response, symptom severity, or the presence of comorbid disorders. Narrowing the phenotype using the approaches discussed earlier may increase the chances of finding a genetic defect in complex diseases, but it can also greatly reduce the power of the study by limiting the number of available affected individuals. For this reason, it has been argued that for some disorders broadening the phenotype is an appropriate strategy. The suggestion is that for some complex diseases the phenotype of interest may represent the extreme end of a spectrum and that to have enough power to map genes other phenotypes within the spectrum must also be included. For example, mapping studies of bipolar disorder might include as affected individuals with major depressive disorder as well as those individuals diagnosed with bipolar disorder. Although the two approaches of narrowing the disease phenotype and broadening

the disease phenotype may seem to be mutually exclusive, many groups studying complex disorders have incorporated both approaches into their study designs. One way to do this is to create stratified diagnostic categories, ranging from a narrow diagnostic category to a broad diagnostic category, and test for genetic linkage under each of these schemas. Some investigators argue that for complex diseases that are part of a spectrum, this strategy decreases the rate of false negatives, that is, of missing an existing linkage because of misspecification. Others argue that using several models and picking the one that gives the highest scores greatly increases the rates of false positives, that is, of identifying an area of linkage where none exists. One problem that clearly exists with the use of multiple diagnostic categories is that as more models are used (and therefore more statistical tests are performed), increasingly stringent levels of evidence are required to consider a result significant. While categorical phenotypes remain the mainstay of psychiatric genetic studies, the limitations of DSM nosology as the basis of phenotyping for genetic studies are becoming clear. Genetic investigations are focusing increasingly on traits that may be components of one or more DSM diagnostic categories. For example, there is growing evidence that genetic susceptibility to psychosis, broadly defined, contributes to both severe bipolar disorder and schizophrenia, and a number of investigative approaches are being employed to attempt to identify genes that underlie such susceptibility and even to explore possible etiological relationships between psychiatric and nonpsychiatric disorders. For example, bioinformatics models have been employed to investigate medical records databases and have uncovered extensive pairwise correlations among a diverse list of psychiatric disorders, neurological disorders, autoimmune disorders, and infectious diseases. Eventually, the results of such model-fitting experiments may provide a framework to design more powerful linkage and association studies that can search for alleles that contribute to susceptibility to multiple disorders. Continuous Phenotypes Because of the difficulties experienced in genetic mapping of categorical diagnoses, neurobehavioral geneticists are increasingly focused on investigating quantitative traits that are hypothesized to underlie a particular psychiatric diagnosis and that may be simpler to genetically map. The rationale for efforts to map such alternative phenotypes, or endophenotypes, is that the genes identified through such efforts may provide clues regarding the biological pathways that are relevant to understanding a particular disorder. Several features characterize useful endophenotypes. First, they should be state-independent; that is, they should not fluctuate as a function of the

disease course or medication treatment and should show adequate test–retest stability. Second, they should be heritable; that is, there should be evidence that genetic factors are responsible for a substantial proportion of the variability of the trait within the population. Third, the endophenotype should be correlated with the disease under investigation; that is, different values of the trait measure are observed in patients compared to unrelated control subjects. Measures of brain structure and function provide most of the traits now under investigation as endophenotypes for psychiatric disorders. For example, several features of brain morphometry (as assessed by magnetic resonance imaging [MRI]) are highly heritable (in the range of 60 to 95 percent) including total brain volume, cerebellar volume, gray and white matter density, amygdala and hippocampal volume, and regional cortical volume. Several studies show that brain structural features that are correlated in clinical samples with disorders such as schizophrenia or bipolar disorder are also abnormal in relatives of affected individuals. Physiological measures of brain activity that have been employed as candidate endophenotypes for psychiatric disorders include electroencephalography (EEG) patterns. Several “pencil and paper” assessments have been

employed to measure endophenotypes relating to neurocognitive function and temperament. Animal Models In contrast to categorical phenotypes, endophenotypes can be more straightforwardly related to phenotypes that can be assessed in animal models. Studies of genetic variations that affect circadian rhythms provide a good example. Variations in circadian rhythms have long been recognized as important features of mood disorders, and quantitative assessments of activity patterns have been proposed as endophenotypes for such disorders. Numerous studies in animal models have demonstrated that genetically controlled biological clocks determine circadian activity and that variations in clock genes are associated with variations in such activity from bacteria to humans. Genetic mapping efforts in fruit flies starting in the early 1970s resulted in the identification of at least seven “clock genes,” beginning with period. Subsequent studies showed that the homologs of several of these genes play essential roles in regulating mammalian circadian rhythms. Genetic mapping studies in mice also have identified previously unknown circadian rhythm genes, beginning with the discovery and characterization in the early 1990s of clock. These genetic discoveries have not only explicated the cellular networks and neurophysiological circuits responsible for the control of mammalian circadian rhythms but have also generated animal models that may shed light on the pathobiology of psychiatric syndromes such as bipolar disorder. For example, mice carrying a targeted mutation in clock demonstrate abnormal activity patterns, such as hyperactivity and decreased sleep, which are apparently modified by administration of lithium. PROGRESS IN THE GENETICS OF SPECIFIC DISORDERS Taken as a whole, the progress in identifying susceptibility genes for psychiatric disorders has been disappointing compared to that observed for nonpsychiatric disorders. Alzheimer’s disease represents the most successful application of gene-

mapping strategies to complex neurobehavioral disorders, and the section on this disease provides an example of how genetic linkage studies add to understanding of the pathogenesis of a complex trait. An overview section on autism describes genetic investigations of syndromes that have features of autism but have relatively simple inheritance patterns and discusses how these studies have provided starting points for investigations of more complex autism spectrum disorders. Finally, the frustrating search for unequivocal gene findings for bipolar disorder and schizophrenia is used to illustrate the challenges that are motivating new approaches in the field of neurobehavioral genetics. ALZHEIMER’S DISEASE Alzheimer’s disease provides an excellent example of the power of genetics to elucidate the complex biology of a neuropsychiatric disorder. Alzheimer’s disease is a well-defined form of dementia characterized by progressive impairment of memory and intellectual functioning. The clinical signs and symptoms, although characteristic, are not limited to Alzheimer’s disease; they are also found in several other types of dementia. For this reason, the diagnosis of Alzheimer’s disease can only be confirmed histopathologically at autopsy. The presence of senile plaques (made up of a core of β-amyloid fibrils surrounded by dystrophic neurites), tau-rich neurofibrillary tangles, and congophilic angiopathy in the brain parenchyma and associated blood vessels are pathognomonic for Alzheimer’s disease. A variable age of onset has been noted for Alzheimer’s disease, ranging from as early as age 35 to as late as age 95. The concordance rate for Alzheimer’s disease in MZ twin pairs is about 50 percent, indicating a moderately strong genetic contribution to disease risk. It is now evident from a wide range of genetic studies that Alzheimer’s disease can be divided into two broad categories: familial forms, which account for a tiny minority of Alzheimer’s disease cases and are characterized by early onset and autosomal dominant inheritance with high penetrance; and sporadic forms, in which the genetic contribution is hypothesized to be similar to that characterizing other common

neuropsychiatric diseases. The search for the genetic basis of familial Alzheimer's disease began with traditional linkage studies. First, an investigation of a candidate locus on chromosome 21 in humans identified mutations in the amyloid precursor protein (APP) gene in a small number of families in which significant linkage had previously been observed to markers from this region. Transgenic mice with different APP mutations were created and have been shown to produce β-amyloid deposits and senile plaques as well as to show synapse loss, astrocytosis, and microgliosis, all part of the pathology of Alzheimer's disease. Mutations in the genes that encode β-APP all lead to an increase in the extracellular concentration of longer fragments of β-amyloid (Aβ42). Most of the strains of transgenic mice with mutations in APP exhibit increased rates of behavioral changes and impairment in several memory tasks, indicating dysfunction in object-recognition memory and working memory among others. These findings represent striking evidence that mutations in the β-amyloid gene are indeed responsible for at least some of the histopathological elements of Alzheimer's disease. Even as the preceding findings were being reported, it was clear that mutations in the β-amyloid gene could not

completely explain the etiology and pathology of Alzheimer's disease, not least because it was shown that linkage to chromosome 21 was excluded in most early onset Alzheimer's disease families. In addition, no neurofibrillary tangles are observed in most of the different β-amyloid transgenic mice. The subsequent search for the genetic underpinnings of Alzheimer's disease using genomewide linkage analysis of early onset Alzheimer's disease families resulted in the identification of two additional Alzheimer's disease susceptibility genes: presenilin-1 (PS-1) on chromosome 14q24.3 and presenilin-2 (PS-2) on chromosome 1q. PS-1 and PS-2 are integral transmembrane proteins with at least seven transmembrane domains. Although their function has not yet been completely elucidated, they are clearly involved in the pathogenesis of Alzheimer's disease. Inactivation of presenilins in mice leads to neurodegeneration and behavioral manifestations of memory loss. Biochemical and cellular studies have implicated presenilins in several important pathways, including apoptosis (programmed cell death) and protein processing in the endoplasmic reticulum. These findings emphasize one of the strengths of using family-based linkage analysis. Pedigree-based studies are especially suited to identify highly penetrant disease genes that serve important roles in important biological processes. Although mutations in APP and presenilin are rare, research into the biology of the expressed proteins has provided key insights into the pathophysiology of dementia. Because these highly penetrant mutations elucidate important biological functions, they also provide a firm ground to design therapeutic interventions. For example, amyloid-β "vaccines" designed to induce an immunogenic response to pathogenic amyloid are now in advanced clinical trials. Unlike the current psychopharmacological treatments for Alzheimer's disease that nonspecifically target cholinergic and glutaminergic neuronal systems, the amyloid-β vaccines specifically treat the causes of Alzheimer's disease by generating an immune response that may actually reverse the deposition of senile plaques. Sporadic and Late-Onset Alzheimer's Disease Mutations in APP, PS-1, or PS-2 are present in a majority of familial cases of early-onset Alzheimer's disease but do not account for sporadic or familial late-onset Alzheimer's disease. For this reason, investigators turned to other approaches to search for evidence of linkage in a large number of small families with late-onset Alzheimer's disease. In 1991, the results of a nonparametric linkage study using 36 markers in late-onset Alzheimer's disease families provided evidence for a susceptibility gene on the long arm of chromosome 19. In 1993, association studies revealed that the e4 allele of the apolipoprotein E gene was strongly associated with late-onset Alzheimer's disease and that this association almost certainly was

responsible for the previously observed linkage signal on chromosome 19. There are three known alleles of this gene— e2, e3, and e4. In most populations, the e3 allele is the most common. However, in familial late-onset Alzheimer's disease the incidence of e4 is approximately 50 percent, and in sporadic late-onset Alzheimer's disease it is 40 percent, compared with about 16 percent in normal controls. Epidemiological studies suggest that between 30 and 60 percent of late-onset Alzheimer's disease cases have at least one apoE-e4 allele. The e4 genotype appears to be a more important risk factor for Alzheimer's disease in populations of European and Asian origin when compared with populations of African origin. Overall, the association of apoE-e4 with Alzheimer's disease remains probably the strongest association yet identified for a common human disease. The establishment of apoE-e4 as a susceptibility allele for late-onset Alzheimer's disease has led to the search for additional alleles that might interact with apoE-e4 to

modify disease risk. In 2007, investigators used genomewide association strategies (in histologically confirmed cases and controls) to identify GAB2 (GRB-associated binding protein 2) as an additional risk allele in apoE-e4 carriers (but not in Alzheimer's disease patients who were not e4 carriers). Initial studies suggest that carriers of both apoE-e4 and GAB2 risk alleles have an almost 25-fold greater risk for Alzheimer's disease than individuals who do not carry either risk allele. Larger-scale GWA studies of Alzheimer's disease are in progress and will likely yield further associations; however, it is unlikely that any will have as strong an effect as apoE. AUTISM Autism is a severe neurodevelopmental disorder that is characterized by three primary features: impaired language and communication; abnormal or impaired social interaction; and restricted, repetitive, and stereotyped patterns of behavior. Understanding of the etiology of autism has proceeded slowly, but there is now convincing evidence that alterations in specific cellular and molecular neurodevelopmental pathways are important in its etiology. In comparison with other neuropsychiatric disorders, there is particularly strong evidence for a genetic contribution to the risk of autism and autism spectrum disorders (ASDs). The sibling recurrence risk for autism and/or ASD is between 2 and 6 percent. Given a population prevalence of about 1 in 2,000 (.04 percent), this means that the siblings of autistic individuals are approximately 50 to 100 times more likely to develop autism than a person in the general population. Twin studies of autism show an extraordinarily high heritability (as demonstrated by MZ twin concordance of 80 to 92 percent) but also demonstrate the genetic complexity of these disorders, with the DZ twin concordance rate of 1 to 10 percent suggesting a highly multigenic mode of inheritance. Increasing interest is now focused on the possibility that individuals affected with autism may display larger numbers of large-scale chromosomal aberrations (5 to 10 percent in some studies) than unaffected individuals. In addition to such gross abnormalities, several recent studies have suggested that autism is associated with an unusually high prevalence of submicroscopic CNVs. For example, in 2007, the Autism Genome Project Consortium applied microarray strategies to almost 8,000 individuals from about 1,500 families, each with at least two affected family members, and found that about 10 percent of the ASD families carried CNVs, with an average size of more than 3 million base pairs, mostly consisting of duplications rather than deletions. Although the design of this study did not permit assessment of whether the frequency of CNVs is greater in patients with autism than that in controls, another study found a de novo CNV incidence of 10 percent in sporadic (no family history) cases of autism compared to an incidence of 1 percent in controls. These results, while exciting, are still considered preliminary. Even before the demonstration of high rates of de novo mutations in autism, epidemiological studies had strongly suggested that the genetic basis of this disorder is likely complex. For example, although the risk of autism in firstdegree relatives of autistic probands

is high, there is a substantial falloff for second-

degree and third-degree relatives of such probands, suggesting that multiple genetic variants must interact to increase susceptibility to this syndrome. Segregation analyses of autism also support the hypothesis that it is a heterogeneous disorder that reflects the actions of multiple genetic variants of small effect. A latent class analysis performed to study possible modes of transmission suggested an epistatic model with up to about 10 interacting loci, whereas other studies have estimated that as many as 15 such loci may be involved. Genetic studies of autism have included whole genome screens, candidate gene studies, chromosome rearrangement studies, mutation analyses, and, most recently, comparative genomic hybridization studies. Taken together and recognizing that most findings still await adequate replication, these studies have contributed to an emerging picture of autism susceptibility that includes genes involved in three major systems: those involving synapse formation and maintenance, those involving cell migration, and those involving the excitatory/inhibitory neurotransmitter networks. Figure 1.7-4 shows a schematic of the currently known potential candidate genes for autism and their molecular relationships with one another. FIGURE 1.7-4 Schematic of the cell biology of proteins expressed from genes identified through mapping studies of autism spectrum disorders. The function of each gene product falls into three broad functional categories. Proteins involved in synapse formation and

maintenance include FMR1, TSC1, TSC2, MeCP2, NLGN 3 and 4, and SHANK3. Another set of proteins is involved in neuronal migration and cell fate including REELIN, WNT2, LAMB1, and NrCAM. Proteins involved in neurotransmitter systems are also altered in some individuals with autism and include 5-HTT (serotonin transporter encoded by SLC6A4), GABAR, and the NMDA subunit encoded by GRIN2A. See text for details. (From Persico AM, Bourgeron T. Searching for ways out of the autism maze: Genetic, epigenetic and environmental clues. Trends Neurosci. 2006;29:349, with permission.) Synapse Formation and Maintenance Perhaps the biggest breakthroughs in identifying susceptibility genes for autism have come from studies of disorders that display clinical features associated with autism or ASDs but with simpler inheritance patterns, including fragile X syndrome, tuberous sclerosis, and Rett syndrome. In general, the genetic defects associated with these disorders affect synapse formation and maintenance. Fragile X, which accounts for 3 to 4 percent of autism cases, is caused by an unstable trinucleotide repeat in the 5′ region of the fragile X mental retardation 1 (FMR1) gene at Xq27.3. This repeat expands as it is transmitted to succeeding generations, resulting in abnormal methylation and inhibition of expression of FMR1. FMR1 produces a ribonucleic acid (RNA)-binding protein that acts as a chaperone for the transport of RNA from the nucleus to the cytoplasm and is involved in messenger RNA (mRNA) translation at the synapse. Abnormalities in dendritic spine density (increased over normal) and anatomy (longer and thinner than normal) have been reported in individuals with fragile X as well as in mouse models of this disorder. Tuberous sclerosis, which accounts for perhaps 2 to 10 percent of autism cases (the rate of tuberous sclerosis is higher among autistic individuals with seizure disorders), results from mutations in one of two tumor suppressor genes, TSC1 on 9q34, and TSC2 on 16p13, both of which are involved in guanosine triphosphatase (GTPase) inactivation. Loss of a single copy of TSC1 in mice has been shown to disrupt cytoskeletal dynamics and dendritic spine structure. Although somewhat less well understood, the genetics of Rett syndrome, an X-linked pervasive developmental disorder (the first with a known genetic etiology) that occurs only in girls and is associated with normal early development followed by loss of skills—particularly social engagement and purposeful hand skills by age 4—also point to

abnormalities in synapse formation and maintenance in ASD and ASD-like disorders. Rett syndrome is caused by mutations in MeCP2, which makes a methylated-DNA-binding protein that regulates gene expression and chromatin structure. Although little is known about the exact role of MeCP2 in the development of Rett syndrome, the pattern of normal early development and later regression suggests that this gene is more likely to be involved in synapse maintenance and remodeling than in synapse development. Neuroligin (NLGN) 3 and 4 and SHANK3, additional genes that appear to play a role in synapse formation, may be affected by chromosomal rearrangements observed in some individuals affected with autism. The neuroligin genes, sited on the X chromosome, produce cell adhesion molecules that are located on postsynaptic glutamatergic neurons. When

mutated in rodents, these genes show defective trafficking and synapse induction. In nonmutated form, their expression induces the formation of normal, presynaptic terminals in axons. SHANK3 is a binding partner of the neuroligins and regulates the structural organization of dendritic spines. Mutations in SHANK3 have been identified in ASD-affected members of at least three families to date, and a comparative genomic hybridization study of autistic individuals, their family members, and controls recently identified a large deletion in chromosome 22q13, the region containing SHANK3, in at least one individual with autism. Cell Migration Of the regions highlighted by a genome screen in autism families, chromosome 7q has provided the most consistent evidence for linkage, albeit over a very broad region. Known chromosomal rearrangements in this region in individuals affected with autism add to its interest. The linkage region on chromosome 7q contains several genes that are strong candidates for autism, most notably RELN, which maps to chromosome 7q22. RELN codes for reelin, a signaling protein secreted by Cajal-Retzius cells located in the marginal zone of the developing brain. It plays an important role in neuronal migration as well as in the development of neural connections. Reeler mice, which have spontaneous deletions of RELN, have cytoarchitectonic alterations in their brains during development that are similar to those that have been described in autistic brains. The complete absence of RELN in humans leads to a more severe phenotype with lissencephaly and severe mental retardation but not autism. Individuals with autism show reduced levels of reelin mRNA and protein in brain and blood serum, suggesting that mutations leading to reduced expression of RELN rather than its absence may be important in ASD. Genetic association studies with RELN have been equivocal, suggesting that if RELN does contribute to the development of autism, then it may play such a role in a small subset of affected individuals. WNT2 (wingless-type MMTV integration site family member 2) is another gene identified as a potential candidate for autism based on linkage studies. WNT2 is located on 7q31 and is part of a family of genes that encode secreted signaling proteins implicated in several developmental processes, including the regulation of cell fate and patterning during embryogenesis. At least two families have been identified in which nonconservative coding sequence variants in WNT2 segregate with autism. LD between a SNP in the 3′ untranslated region of WNT2 and autism is also present in families with severe language abnormalities that accounted for most of the evidence for linkage on chromosome 7q in one of the original genome screens. Excitatory/Inhibitory Neurotransmitter Systems Although there is little current evidence that mutations in genes encoding neurotransmitter transporters and/or receptors are directly responsible for the development of autism, there is some evidence that such genes might act as modifiers or susceptibility factors for an autism spectrum phenotype. The evidence is perhaps strongest for the role of the γ-aminobutyric acid (GABA) receptors in the development and expression of autistic disorders. These receptors occur in a cluster on chromosome

15q11–13, and duplications of this region are the most common cytogenetic abnormalities seen in autism cases (up to 6 percent of cases). GABA is an important inhibitory neurotransmitter in the central nervous system and is responsible for controlling excitability in mature brains. Chromosome 15q11–13 is one of the most complex regions of the genome. It has a high rate of genomic instability, including frequent duplication and deletion events, and imprinting plays an important role in the expression of genes in this region. The 15q11–13 region is the critical region for Angelman and Prader-Willi syndromes, neurological disorders due to deletions or mutations in this region that occur on maternally and paternally inherited chromosomes, respectively. Despite the high rate of duplications of 15q11–13 among autistic individuals, genome screens have not shown strong support for linkage or association to this region. Candidate gene studies continue, however, in part because a rate of 6 percent of autistic individuals with duplications in this region is hard to ignore. BIPOLAR DISORDER The search for the genetic basis of bipolar affective disorder has been fraught with missteps and partial answers. The history of genetic mapping attempts for bipolar disorder illustrates not only the extreme complexity of psychiatric disorders but also the evolution of genetic approaches to such diseases. Bipolar disorder is an episodic illness characterized by recurrent periods of both mania and depression. Psychotic symptoms are often a part of the clinical picture, particularly in more severely affected individuals. Numerous genetic epidemiological investigations conducted over several decades have strongly supported a genetic contribution to risk for bipolar disorder. As with other psychiatric disorders, however, the definition of the bipolar disorder phenotype in these studies has varied substantially, and this in turn has resulted in a wide range in estimates of its heritability. For example, many early studies into the genetic basis of mood disorders did not distinguish between unipolar and bipolar mood disorders. Furthermore, the diagnostic methodology used in such early studies differs substantially from that employed in current-day genetic studies. For example, a Danish twin study that suggested a very high heritability for bipolar disorder and thereby had a heavy influence on the design of initial genetic mapping studies of mood disorders employed only unstructured diagnostic interviews by a single clinician rather than the structured assessments used in current studies, which have suggested somewhat lower heritabilities. Current estimates of concordance for bipolar disorder range between 65 and 100 percent in MZ twins and between 10 and 30 percent in DZ twins, indicating that the disorder is highly heritable (between about 60 and 80 percent). Several studies have shown that bipolar disorder is substantially more heritable than unipolar major depression, which has an estimated heritability between 30 and 40 percent. Early family studies suggested that bipolar disorder segregation patterns were compatible with single gene inheritance of a locus of major effect. However, although it is possible that some bipolar disorder pedigrees segregate such a locus,

mounting evidence indicates that if such pedigrees exist they must be quite rare. Furthermore, the fact that genetic linkage studies have failed to uncover such a locus with unequivocal evidence in any pedigrees argues against this possibility. The observed rapid decrease in recurrence risk for bipolar disorder from monozygotic co-twins to first-degree relatives is also not consistent with single gene inheritance models but rather suggests models of multiple interacting genes. Early Linkage Studies Tremendous excitement followed the first reports of linkage to bipolar disorder on chromosomes X and 11 in 1987. Investigators noted that in several families, bipolar disorder and other affective disorders appeared to be inherited in an X-linked fashion. Likewise, these disorders appeared to cosegregate in several Israeli families with color blindness and G6PD deficiency, which map to the X chromosome. Linkage studies in these pedigrees, using color blindness or G6PD

deficiency as marker loci, gave LOD scores between 4 and 9. Early studies of chromosome 11 were similar to those for chromosome X in that they reported significant linkage after testing only a few markers in a single region, in this case in an extended Old Order Amish pedigree heavily loaded for bipolar disorder. Not surprisingly, these findings generated a great deal of interest. Both studies showed high LOD scores and seemed to provide clear evidence for linkage. However, replication studies in other populations failed to produce positive results for either the X chromosome or chromosome 11, and evidence for linkage essentially disappeared in both chromosomal regions in the samples in which linkage was originally reported when the pedigrees were extended to include additional affected individuals and when additional markers were typed in the putative linkage regions. The most likely explanation in each case is that the original linkage results were false-positive findings and may have reflected overoptimistic interpretation of evidence that, in retrospect, was relatively scanty. Genomewide Screens The early linkage studies of bipolar disorder evaluated only a few markers because they were all that were available. With the construction of genetic linkage maps of the genome in the 1990s, linkage studies of most complex traits, including bipolar disorder, began to search genomewide. The advantage of genomewide mapping studies is that they do not require a priori knowledge of the biological underpinnings of a particular phenotype. Complete genome screens provide an opportunity to evaluate the evidence of linkage at all points in the genome without bias (see Color Plate 1.7-5). Although genomewide studies clearly had greater power to detect true linkage than studies focused on only a few markers in arbitrary locations or around a few candidate genes, these investigations have also generally had disappointing results. The challenge of achieving replicated significant linkage results for bipolar disorder and other complex traits is apparent when one reviews the many gene-mapping studies that have suggested —but not demonstrated unequivocally—bipolar disorder susceptibility loci on chromosome 18.

Chromosome 18 The first report of linkage came from a partial genome screen that examined 11 markers on chromosome 18 and identified suggestive linkage near the centromere. Because the inheritance patterns for bipolar disorder are unknown, the results were analyzed using both recessive and dominant models. Some of the markers were positive under a recessive model in some families, some were positive under a dominant model in other families, and some markers gave positive LOD scores in a subset of families under both models. Attempts to replicate this finding in other populations have been mixed. So far at least two groups have found no evidence for linkage to the pericentromeric region of chromosome 18 in their samples, although one other group has found evidence to support linkage to this region. Other studies have found suggestive evidence for linkage on chromosome 18, including a complete genome screen in two large Costa Rican pedigrees that gave evidence for linkage on chromosome 18q22–23 as well as in an area on 18p. The combined evidence of these several studies, although somewhat contradictory and confusing, points to at least two different susceptibility loci on chromosome 18: one on 18p and one on 18q. Improving Study Power The equivocal findings represented by the attempts to pinpoint susceptibility loci on chromosome 18 have led investigators to implement several new strategies to map bipolar disorder genes. One such strategy is meta-analysis, which involves combining data across multiple individual investigations to increase statistical power, and in some cases the combined analysis points to loci not originally found in the individual studies. Several meta-analytical techniques have been used to explore gene-mapping studies for bipolar disorder. The multiple scan probability (MSP) and genome scan meta-analysis (GSMA) methods require only linkage statistics and P-values from each study to examine combined data. MSP was used to

combine chromosomal regions with P-values less than .01 from 11 independent bipolar disorder studies and provided evidence for susceptibility loci on chromosomes 13q and 22q. Although the MSP and GSMA methods have the advantage of requiring only linkage significance data, they are not able to account for study-specific issues that will limit the extent to which multiple studies can be compared. Combining original genotype data from multiple studies can circumvent this problem. With this method, the largest meta-analysis to date combined 11 bipolar disorder genomewide linkage scans consisting of 5,179 individuals from 1,067 families. Access to the original genotype data allowed the construction of a standardized genetic map in which the markers of each respective study were mapped onto one common gender-averaged map. The results of this meta-analysis identified two susceptibility loci with genomewide significance on 6q and 8q. Another strategy that has been used to increase the power of gene-mapping studies is the formation of consortia that combine data across multiple clinical sites. A consortium combining data from the UK and Ireland led to support for linkage at 9p21 and 10p14–21. Likewise, combining data from Spanish, Romanian, and Bulgarian families provided

additional support for findings on chromosomes 4q31 and 6q24. Investigators can also increase power by standardizing marker sets and clinical evaluation protocols between independent studies to permit direct comparisons between such studies. This approach was used to identify a bipolar disorder susceptibility locus on chromosome 5q31–33. The region showed suggestive nonparametric linkage results in pedigrees from the Central Valley of Costa Rica. With identical genetic markers and diagnostic criteria, the same region was highlighted in an independent analysis of a set of Colombian families who have a genetic background similar to that of the Costa Rican families. A follow-up study using additional markers in an expanded set of Colombian and Costa Rican families confirmed genomewide significant evidence to a candidate region of 10 cM in 5q31–33. This finding is especially interesting given that the linkage peak in the bipolar studies overlaps with linkage regions for schizophrenia and psychosis, identified in a previous study of 40 families from the Portuguese Islands. These results contribute to a growing opinion that there may be substantial genetic overlap between different DSM disorders. SCHIZOPHRENIA As with bipolar disorder, investigations of the genetic basis of schizophrenia exemplify the frustrations still characteristic of psychiatric genetics, and the field still struggles to interpret the significance of initially promising linkage and association results that began to emerge over a decade ago. Unlike with bipolar disorder, however, candidate genes have emerged from each of the regions highlighted from these studies. Thus, although none of these findings have been validated unequivocally, they have spawned a diverse range of basic and clinical investigations aiming to elucidate their functional significance, for example, using mouse gene targeting and functional MRI. Here we discuss some of the more extensively investigated loci for purposes of illustration; it could be argued that roughly equivalent evidence supports schizophrenia candidate loci that we do not discuss in detail, for example, AKT1 on chromosome 14 or COMT on chromosome 22. Chromosome 6p24–22 was among the first regions to be implicated by a complete genome screen for schizophrenia, in this case from a study of Irish families heavily loaded for schizophrenia. The linkage results were strongest under a broad diagnostic definition that included schizophrenia spectrum disorders, such as schizotypal personality disorder. Six additional linkage studies have shown positive results over approximately the same region, but at least three studies have found no linkage to the region. Fine-scale mapping of this region using association analysis in the original Irish kindreds led to the proposal of Dysbindin (DTNB1) as a candidate gene for schizophrenia. Additional association studies of Dysbindin have been equivocal. Although multiple association

studies in a variety of populations have shown positive results, interpretation of the results has been difficult. Different association studies have not used the same SNP marker sets. Meta-analysis of five “positive” association studies using a high-resolution haplotype map designed to compare the five studies showed significant inconsistencies with regard to the identified disease-associated Dysbindin allele. Although it is possible that several different variants in the same gene could each contribute to disease susceptibility in different families or populations, this possibility does not explain the inconsistencies between the several Dysbindin association studies.

Linkage studies subsequently pointed to a region on chromosome 1 containing the candidate genes DISC 1 and DISC 2 (disrupted in schizophrenia 1 and 2) located on chromosome 1q21–22 and 1q32–42. These genes were initially identified in a large Scottish pedigree in the early 1990s. A balanced translocation between chromosomes 1 and 11 segregated in this pedigree and was possibly associated with serious mental illness. DISC 1 and 2 were identified in the original Scottish family because of their location near the chromosomal translocation breakpoint. As with Dysbindin, follow-up studies of DISC 1 and 2 have been equivocal. Genome screens, including a screen focused on extended Icelandic kindreds, have identified a schizophrenia candidate region on chromosome 8p21–22. Fine mapping of the region narrowed the search and eventually led to the proposal of neuregulin 1 (NRG1) as a schizophrenia candidate gene. Association studies again provided equivocal and difficult-tointerpret results. Meta-analysis of 14 separate studies using the SNP marker that demonstrated an association in the original study showed significant heterogeneity between the follow-up studies. It also showed that there is no consistent association between the specific risk allele “tagged” by the marker SNP and schizophrenia in different populations. However, after taking account of the statistical power of each association study, the meta-analysis showed a positive association between NRG1 at the level of the gene (as opposed to the SNP or haplotype level). Despite the equivocal genetic studies, significant resources have been channeled into molecular and neurophysiological investigations of the functional products of dysbindin, DISC 1 and 2, and neuregulin. Mutant mice for each of the three genes are now available and have been used to demonstrate interesting biological findings. For example, dysbindin is expressed in the hippocampus and dorsolateral prefrontal cortex. The dysbindin protein binds to B-dystrobrevin and has been implicated in synaptic structure and signaling. DISC 1 has been shown to influence neurite formation in cellular studies, and mutant mice for DISC 1 show impairments in a wide variety of tests including learning, memory, and sociability. Neuregulin belongs to a family of growth factors that mediate numerous functions including synapse formation, neuronal migration, and neurotransmission. Targeted disruption of erbB4, the postsynaptic target of neuregulin, leads to synaptic glutamatergic hypofunction. Despite the interesting biology uncovered, it remains unclear whether and to what extent any of these genes contribute to the etiology of schizophrenia in humans, and many geneticists have been cautious in their endorsement of the legitimacy of the mutant mice generated from the current list of candidate genes as models of psychiatric disorders. As with bipolar disorder, the genetic mapping findings for schizophrenia are promising but equivocal. Unlike for bipolar disorder, these mapping studies have generated a set of candidate genes that have stimulated a wide range of functional investigations, many of which have biologically interesting findings. As with bipolar disorder and other psychiatric disorders, the primary challenge in elucidating the genetic basis of schizophrenia is assembling adequate richly phenotyped samples for wellpowered genomewide mapping studies. REFERENCES Craddock N, O’Donovan MC, Owen MJ. Phenotypic and genetic complexity of psychosis. Invited commentary on Schizophrenia: A common disease caused by multiple rare

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electrical potential measured between any two EEG electrodes fluctuates or oscillates rapidly, usually many times per second. It is this oscillation that produces the characteristic “squiggly line” that is recognized as the appearance of “brain waves.” Brain waves reflect change by becoming faster or slower in frequency or lower or higher in voltage, or perhaps some combination of these two responses. A normal EEG can never constitute positive proof of absence of brain dysfunction. Even in diseases

with established brain pathophysiology, such as multiple sclerosis, deep subcortical neoplasm, some seizure disorders, and Parkinson’s disease and other movement disorders, a substantial incidence of patients with normal EEG studies may be encountered. Nonetheless, a normal EEG can often provide convincing evidence for excluding certain types of brain pathology that may present with behavioral or psychiatric symptoms. More often, information from the patient’s symptoms, clinical course and history, and other laboratory results identifies a probable cause for the EEG findings. EEG studies are often ordered when a pathophysiological process is already suspected or a patient experiences a sudden, unexplained change in mental status. Electrode Placement The electrodes normally used to record the EEG are attached to the scalp with a conductive paste. A standard array consists of 21 electrodes. Placement of the electrodes is based on the 10/20 International System of Electrode Placement (Fig. 1.8-1). This system measures the distance between readily identifiable landmarks on the head and then locates electrode positions at 10 percent or 20 percent of that distance in an anterior–posterior or transverse direction. Electrodes are then designated by an uppercase letter denoting the brain region beneath that electrode and a number, with odd numbers used for the left hemisphere and with even numbers signifying the right hemisphere (the subscript Z denotes midline electrodes). Thus, the O2 electrode is placed over the right occipital region, and the P3 lead is found over the left parietal area (Fig. 1.8-2). FIGURE 1.8-1

International 10–20 Electrode Placement System. (Courtesy of Grass, Astro-Med, Inc. Product Group.) FIGURE 1.8-2 A left-lateral diagram of the head showing the locations of the routine 10–20 electrodes (left-side electrode locations F7 and T3 and the new electrode placement [T1]) in relation to the temporal pole. (Modification of figure reprinted courtesy of Grass, AstroMed, Inc. Product Group.) In special circumstances, other electrodes may be used. Nasopharyngeal (NP) electrodes can be inserted into the NP space through the nostrils and can be closer to the temporal lobe than scalp electrodes. No actual penetration of tissue occurs. These electrodes may be contraindicated with many psychiatric patients displaying behaviors, such as confusion, agitation, or belligerence, which could pull the leads out, possibly lacerating the nasal passage. Sphenoidal electrodes use a hollow needle through which a fine electrode that is insulated, except at the tip, is inserted between the zygoma and the sigmoid notch in the mandible, until it is in contact with the base of the skull lateral to the foramen ovale. Activated EEG Certain activating procedures are used to increase the probability that abnormal discharges, particularly spike or spike-wave seizure discharges, will occur. Strenuous hyperventilation is one of the most frequently used activation procedures. While

remaining reclined with the eyes closed, the patient is asked to overbreathe through the open mouth with deep breaths for 1 to 4 minutes, depending on the laboratory (3 minutes is common). In general, hyperventilation is one of the safest EEG-activating procedures, and, for most of the population, it presents no physical risk. It can pose a risk for patients with cardiopulmonary disease or risk factors for cerebral vascular pathophysiology, however. Photic stimulation (PS) generally

involves placing an intense strobe light approximately 12 inches in front of the subject's closed eyes and flashing at frequencies that can range from 1 to 50 Hz, depending on how the procedure is carried out. Retinal damage does not occur, because each strobe flash, although intense, is extremely brief in duration. When the resting EEG is normal, and a seizure disorder or behavior that is suspected to be a manifestation of a paroxysmal EEG dysrhythmia is suspected, PS can be a valuable activation method to use. EEG recording during sleep, natural, or sedated, is now widely accepted as an essential technique for eliciting a variety of paroxysmal discharges, when the wake tracing is normal, or for increasing the number of abnormal discharges to permit a more definitive interpretation. It has been shown that the central nervous system (CNS) stress produced by 24 hours of sleep deprivation alone can lead to the activation of paroxysmal EEG discharges in some cases. NORMAL EEG TRACING The normal EEG tracing (Fig. 1.8-3) is composed of a complex mixture of many different frequencies. Discrete frequency bands within the broad EEG frequency spectrum are designated with Greek letters. FIGURE 1.8-3 Normal electroencephalogram (EEG) tracings in an awake 28-year-old man. (Reprinted from Emerson RG, Walesak TS, Turner CA. EEG and evoked potentials. In: Rowland LP,

ed. Merritt's Textbook of Neurology. 9th ed. Baltimore: Lippincott Williams & Wilkins; 1995:68, with permission.) Awake EEG The four basic wave forms are alpha, beta, delta, and theta. Highly rhythmic alpha waves with a frequency range of 8 to 13 Hz constitute the dominant brain wave frequency of the normal eyes-closed awake EEG. Alpha frequency can be increased or decreased by a wide variety of pharmacological, metabolic, or endocrine variables. Frequencies that are faster than the upper 13 Hz limit of the alpha rhythm are termed beta waves, and they are not uncommon in normal adult waking EEG studies, particularly over the frontal– central regions. Delta waves (≤3.5 Hz) are not present in the normal waking EEG, but are a prominent feature of deeper stages of sleep. The presence of significant generalized or focal delta waves in the awake EEG is strongly indicative of a pathophysiological process. Waves with a frequency of 4.0 to 7.5 Hz are collectively referred to as theta waves. A small amount of sporadic, arrhythmic, and isolated theta activity can be seen in many normal waking EEG studies, particularly in frontal– temporal regions. Although theta activity is limited in the waking EEG, it is a prominent feature of the drowsy and sleep tracing. Excessive theta in awake EEG, generalized or focal in nature, suggests the operation of a pathological process. With maturation, EEG activity gradually goes from a preponderance of irregular medium- to high-voltage delta activity in the tracing of the infant, to greater frequency and more rhythmic pattern. Rhythmic activity in the upper theta–lower alpha range (7 to 8 Hz) can be seen in posterior areas by early childhood, and, by mid-adolescence, the EEG essentially has the appearance of an adult tracing. Sleep EEG The EEG patterns that characterize drowsy and sleep states are different from the patterns seen during the awake state. The rhythmic posterior alpha activity of the waking state subsides during drowsiness and is replaced by irregular low-voltage theta activity. As drowsiness deepens, slower frequencies emerge, and sporadic vertex sharp waves may appear at central electrode sites, particularly among younger persons. Finally, the progression into sleep is marked by the appearance of 14-Hz sleep spindles (also called sigma waves), which, in turn, gradually become replaced by high-voltage delta waves as deep sleep stages are reached. EEG ABNORMALITIES Apart from some of the obvious indications for an EEG study (i.e., suspected seizures), EEG studies are not routinely performed as part of a diagnostic work-up in psychiatry. EEG, however, is a valuable assessment tool in clinical situations in which the initial presentation or the clinical course appear to be unusual or atypical (Table 1.8-1). Table 1.8-2 summarizes some common types of EEG abnormalities.

Table 1.8-1 Warning Signs of the Presence of Covert Medical or Organic Factors Causing or Contributing to Psychiatric Presentation Table 1.8-2 Common Electroencephalography (EEG) Abnormalities Some psychotropic medications and recreational or abused drugs produce EEG changes, yet, with the exception of the benzodiazepines and some compounds with a

propensity to induce paroxysmal EEG discharges, little, if any, clinically relevant effect is noted when the medication is not causing toxicity. Benzodiazepines, which always generate a significant amount of diffuse beta activity, have EEG-protective effects, so that they can mask alterations caused by concomitant medications (Table 1.8-3). Table 1.8-3 Electroencephalography (EEG) Alterations Associated with Medication and Drugs Medical and neurological conditions produce a wide range of abnormal EEG findings. EEG studies, thus, can contribute to the detection of unsuspected organic pathophysiology influencing a psychiatric presentation (Fig. 1.8-4). Table 1.8-4 lists EEG alterations in medical disorders and Table 1.8-5 lists EEG alterations associated with psychiatric disorders.

FIGURE 1.8-4 Diffuse slowing in a 67-year-old patient with dementia. Six- to seven cycles per second (cps) activity predominates over the parieto-occipital regions. Although reactive to eye closure, the frequency of this rhythm is abnormally slow. (Reprinted from Emerson RG, Walesak TS, Turner CA. EEG and evoked potentials. In: Rowland LP, ed. Merritt’s Textbook of Neurology. 9th ed. Baltimore: Lippincott Williams & Wilkins; 1995:68, with permission.) Table 1.8-4 Electroencephalography (EEG) Alterations Associated with Medical Disorders

Table 1.8-5 Electroencephalography (EEG) Alterations Associated with Psychiatric Disorders

TOPOGRAPHIC QUANTITATIVE ELECTROENCEPHALOGRAPHY (QEEG) Unlike standard EEG interpretation, which relies on waveform recognition, QEEG involves a computer analysis of data extracted from the EEG. Findings are compared with a large population database of subjects without any known neurological or psychiatric disorder as well as QEEG profiles that may be characteristic of some defined diagnostic group. In QEEG, the analog-based electrical signals are processed digitally and converted to graphic, colored topographical displays. These images are sometimes called “brain maps.” Color Plate 1.8-5 illustrates topographic QEEG images of a patient with a closed head injury. QEEG remains primarily a research method, but it holds considerable clinical potential for psychiatry, mainly in establishing neurophysiological subtypes of specific disorders and for identifying electrophysiological predictors of response. Examples of some of the more promising results of QEEG research include the identification of subtypes of cocaine dependence and the subtype most likely to be associated with sustained abstinence; identification of subtypes of obsessive–compulsive disorder (OCD) that predict clinical responsiveness or lack of responsiveness to selective serotonin reuptake inhibitors (SSRIs); and the differentiation between normals, attention–deficit disorder and attentiondeficit/hyperactivity disorder (ADHD), and learning disability subpopulations. QEEG findings in ADHD show that increased theta abnormality frontally may be a strong predictor of response to methylphenidate and other psychostimulants and that favorable clinical responses may be associated with a normalization of the EEG abnormality. CEREBRAL EVOKED POTENTIALS Cerebral EPs are a series of surface (scalp) recordable waves that result from brain visual, auditory, somatosensory, and cognitive stimulation. They have been shown to be abnormal in many psychiatric conditions, including schizophrenia and Alzheimer’s disease, thus creating difficulty in using cerebral EPs for differential diagnosis purposes.

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treatment outcomes in major depressive disorder. Psychiatr Clin North Am. 2007;30:105. Jarahi M, Sheibani V, Safakhah HA, Torkmandi H, Rashidy-Pour A. Effects of progesterone on neuropathic pain responses in an experimental animal model for peripheral neuropathy in the rat: A behavioral and electrophysiological study. Neuroscience. 2014;256:403–411. Winterer G, McCarley RW. Electrophysiology of schizophrenia. In: Weinberger DR, Harrison PJ. Schizophrenia. 3rd ed. Hoboken, NJ: Blackwell Publishing Ltd; 2011:311. 1.9 Chronobiology Chronobiology is the study of biological time. The rotation of the Earth about its axis imposes a 24-hour cyclicity on the biosphere. Although it is widely accepted that organisms have evolved to occupy geographical niches that can be defined by the three spatial dimensions, it is less appreciated that organisms have also evolved to occupy temporal niches that are defined by the fourth dimension—time. Much like light represents a small portion of the electromagnetic spectrum, the 24-hour periodicity represents a small time domain within the spectrum of temporal biology. A broad range of frequencies exist throughout biology, ranging from millisecond oscillations in ocular field potentials to the 17-year cycle of emergence seen in the periodic cicada (Magicicada spp.). Although these different periodicities all fall within the realm of chronobiology, circadian (Latin: circa, about; dies, day) rhythms that have a period of about one day are among the most extensively studied and best understood biological rhythms. A defining feature of circadian rhythms is that they persist in the absence of time cues and are not simply driven by the 24-hour environmental cycle. Experimental animals housed for several months under constant darkness, temperature, and humidity continue to exhibit robust circadian rhythms. Maintenance of rhythmicity in a “timeless” environment points to the existence of an internal biological timing system that is responsible for generating these endogenous rhythms. The site of the primary circadian oscillator in mammals, including humans, is the suprachiasmatic nucleus (SCN), located in the anterior hypothalamus. The mean circadian period generated by the human SCN is approximately 24.18 hours. Like a watch that ticks 10 minutes and 48 seconds too slowly per day, an individual with such a period gradually comes out of synchrony with the astronomical day. In slightly more than 3 months, a normally diurnal human would be in antiphase to the day–night cycle and thus would become transiently nocturnal. Therefore, a circadian clock must be reset on a regular basis to be effective at maintaining the proper phase relationships of behavioral and physiological processes within the context of the 24-hour day. Although factors such as temperature and humidity exhibit daily fluctuations, the environmental parameter that most reliably corresponds to the period of Earth’s rotation around its axis is the change in illuminance associated with the day–night cycle. Accordingly, organisms have evolved to use this daily change in light levels as a time cue or zeitgeber (German: zeit, time; geber, giver) to reset the endogenous circadian clock. Regulation of the circadian pacemaker through the detection of changes in

illuminance requires a photoreceptive apparatus that communicates with the central oscillator. This apparatus is known to reside in the eyes, because surgical removal of the eyes renders an animal incapable of resetting its clock in response to light. The circadian clock drives many rhythms, including rhythms in behavior, core body temperature, sleep, feeding, drinking, and hormonal levels. One such circadianregulated hormone is the indoleamine, melatonin. Melatonin synthesis is controlled through a multisynaptic pathway from the SCN to the pineal gland. Serum levels of melatonin become elevated at night and return to baseline during the day. The nocturnal rise in melatonin is a convenient marker of circadian phase. Exposure to light elicits two distinct effects on the daily melatonin profile. First, light acutely suppresses elevated melatonin levels, immediately decreasing them to baseline levels. Second, light shifts the phase of the circadian rhythm of melatonin synthesis. Because melatonin can be assayed easily, it provides a convenient window into the state of the circadian pacemaker. Any perturbation of the clock is reflected in the melatonin profile; thus melatonin offers an output that can be used to study the regulation of the central circadian pacemaker. SLEEP AND CIRCADIAN RHYTHMS Sleep Regulation Restful consolidated sleep is most appreciated when sleep disturbances are experienced. Sleep is the integrated product of two oscillatory processes. The first process, frequently referred to as the sleep homeostat, is an oscillation that stems from the accumulation and dissipation of sleep debt. The biological substrates encoding sleep debt are not known, although adenosine is emerging as a primary candidate neuromodulator of the sleep homeostat. The second oscillatory process is governed by the circadian clock and controls a daily rhythm in sleep propensity or, conversely, arousal. These interacting oscillations can be dissociated by housing subjects in a timeless environment for several weeks. The circadian cycle in arousal (wakefulness) steadily increases throughout the day, reaching a maximum immediately before the circadian increase in plasma melatonin (Fig. 1.9-1). Arousal subsequently decreases to coincide with the circadian trough in core body temperature. Experiments imposing forced sleep schedules throughout the circadian day have shown that an uninterrupted 8-hour bout of sleep can only be obtained if sleep is initiated approximately 6 hours before the temperature nadir. This nadir typically occurs at approximately 5:00 AM to 6:00 AM. In healthy individuals, initiating sleep between 11:00 PM and 12:00 AM affords the highest probability of getting 8 solid hours of sleep.

FIGURE 1.9-1 Relative phase relationship of sleep in young adults to other circadian phase markers. (From Dijk D-J, Lockley SW. Invited review: Integration of human sleep-wake regulation and circadian rhythmicity. J Appl Physiol. 2002;92:852, with permission.) It should be stressed that diurnal preference varies among individuals as a function of age, endogenous circadian periods, and other factors. This variability is paralleled by physiology. Clinically, diurnal preference can be quantified using the Horne–Östberg (HO) Morningness-Eveningness Questionnaire (MEQ). In qualitative terms, morning people or morning larks tend to awaken earlier and experience the core body temperature minimum at an earlier clock time relative to night people or night owls. Sleep deprivation studies have shown that the homeostatic component of sleep is remarkably similar among individuals of similar age. (It should be noted that there is a well-established agedependent decline in sleep need.) Therefore, diurnal preference is dictated almost exclusively by the circadian component of sleep regulation. Circadian Sleep Disorders Advanced sleep phase syndrome (ASPS) is a pathological extreme of the morning lark

phenotype. An autosomal-dominant familial form of ASPS (FASPS) recently has been genetically characterized. Afflicted family members exhibit a striking 4-hour advance of the daily sleep–wake

rhythm. They typically fall asleep at approximately 7:30 PM and spontaneously awaken at approximately 4:30 AM. Affected individuals have a single nucleotide polymorphism in the gene encoding hPER2, the human homolog of the mouse Per2 clock gene. This adenine-to-guanine nucleotide polymorphism results in serine-toglycine amino acid substitution that causes the mutant protein to be inefficiently phosphorylated by casein kinase Iε, an established component of the circadian molecular clockwork. Similarly, delayed sleep phase syndrome (DSPS) has been shown to be influenced by genetics. A length polymorphism in a repeat region of the hPER3 gene appears to be associated with diurnal preference in patients with DSPS, the shorter allele being associated with evening preference. The advent of the light bulb has extended the human day into the natural night. This encroachment on the night, although increasing productivity, has affected human sleep patterns (Fig. 1.9-2). Typical use of artificial lights results in a single, consolidated bout of sleep lasting approximately 8 hours. This pattern of sleep is uncommon among most other mammals, which typically experience more fractured sleep. Human sleep under more natural photoperiods, where the duration of the night is longer, becomes decompressed. Specifically, a bimodal distribution of sleep is observed; bouts of sleep occur in early and late night. Periods of quiet wakefulness are interspersed between the two primary bouts of sleep. This natural sleep pattern is more similar to the sleep patterns of other mammals.

FIGURE 1.9-2 Change in sleep structure in response to artificial lighting. Total sleep time is reduced, and periods of quiet wakefulness are abolished by extending daytime into nighttime through artificial lighting. (From Wehr TA, Moul DE, Barbato G, et al. Conservation of photoperiod-responsive mechanisms in humans. Am J Physiol. 1993;265:R846, with permission.) SEASONALITY The 24-hour period of the Earth’s rotation around its axis is unchanging. However, the Earth’s axis is tilted 23.45 degrees from the plane of its own orbit around the sun (the

ecliptic). As a result, the relative proportion of daytime to nighttime within the 24-hour astronomical day varies as the Earth proceeds through its orbit of the sun. Many organisms are capable of synchronizing physiology to the seasonal cycle to maximize survival. For example, precise seasonal cycles in reproduction are seen throughout the plant and animal kingdoms. Large mammals that typically have long gestation periods, such as sheep, conceive in the fall when the nights are long and the days are short, so birth occurs during the relatively mild season of spring. These animals are referred to as short-day breeders. Conversely, mammals with gestation periods of only a few weeks, such as hamsters, conceive and give birth during spring and summer, when the days are long and the nights are short. Hence, these animals are referred to as long-day breeders. Like circadian rhythms, many of these yearly (circannual) rhythms tend to persist in the absence of seasonal cues with endogenous periods of approximately 1 year. Melatonin and Seasonality The most reliable environmental parameter providing a faithful representation of the solar day is the day–night cycle. Similarly, the most reliable environmental parameter reflecting the progression through the seasons is the change in day length, the fraction of the 24-hour day between sunrise and sunset. In seasonally breeding animals, day length is physiologically encoded through the melatonin profile. As described previously, melatonin levels are elevated during the night. A long night, such as that experienced during the short day lengths of winter, results in an elevated melatonin profile of a relatively long duration. A short summer night, by contrast, results in a short duration of elevated melatonin. This seasonal signal is interpreted by the reproductive axis, resulting in an appropriate reproductive response. Melatonin’s role in transducing day length was elucidated by pinealectomizing seasonally breeding animals, thereby removing the primary

endogenous source of melatonin. Melatonin was then infused in profiles mimicking long days or short days. The duration of elevated melatonin was the primary determinant of seasonal reproductive status, even when the infused profile was administered under a conflicting day length. Variations in other parameters, such as the amplitude of the melatonin profile, the amount of total melatonin synthesized, or the phase relationship of the profile to the light–dark cycle, are of limited importance in producing a humoral signal that transduces day length. Reproductive responses to changing day length can be dramatic. A male Siberian hamster (Phodopus sungorus) maintained in long days is reproductively competent and typically has a testicular weight of approximately 250 mg per testis. Under short days, however, the testes regress to approximately 15 mg per testis, representing a 94 percent decrease in testicular mass. The same degree of regression is observed in response to melatonin infusions that mimic short days. Communication of the hormonally transduced day length to the reproductive axis is likely to be mediated, at least partially, through melatonin receptors in the pars tuberalis of the pituitary gland. The exact mechanism remains unknown, but activation of these receptors is hypothesized to indirectly regulate an unidentified factor putatively named tuberalin. Tuberalin, in turn,

controls gene expression and prolactin release from lactotrophs in the adenohypophysis of the pituitary. Seasonality in Humans Whether humans are truly seasonal is still a point of considerable debate. Several lines of evidence exist that suggest the presence of a residual tendency toward seasonality. A peak in the rate of suicide occurs in the summer; this peak is cross-cultural. Birth rates also tend to show a seasonal variation; a small but distinguishable peak in the rate of births occurs in spring and summer. This pattern, however, is itself variable and is heavily influenced by unknown cultural and geographic factors. Of interest, the amplitude of the spring–summer birth rate peak has diminished as societies have become industrialized. The decompressed bimodal structure of human sleep during long nights indicates that the length of natural sleep is related to the length of the night. Potentially, a twooscillator system could function to maintain proper sleep patterns during changing photoperiods. Such a proposed system would consist of an evening oscillator that tracks the transition from day to night (dusk) and a morning oscillator that tracks the transition from night to day (dawn). The relative phase differences between these oscillators may encode the changing day lengths associated with the passing of the seasons. Biological evidence for a two-oscillator system exists in rodents and humans. The melatonin profile of many vertebrates, including some humans, is bimodal, with evening and morning peaks. In rodents, metabolic and electrophysiological studies of the SCN typically have been done in brain slices cut in the coronal plane. Results of electrophysiological studies conducted in brain slices cut in the horizontal plane have provided new insights. The action potential frequency in SCN neurons from horizontally cut preparations is bimodal, with peaks in the early and late subjective day. Furthermore, the interpeak interval varies as a function of the photoperiod in which the animal was housed. These studies lend credence to long-standing suspicions that the SCN of seasonally breeding mammals and, perhaps, nonseasonal mammals harbor a morning and evening oscillator that interact to convey day-length information. Effect of Aging In general, as humans age, the circadian period shortens, the circadian phase advances resulting in earlier waking times and bedtimes, the amplitudes of most circadian rhythms decrease, and dramatic phase shifts such as those caused by jet-lag are less tolerated. Again, a mouse model has provided interesting insight into the interaction of the aging process and the circadian clock. The effect of chronic jet-lag on aged mice has dramatic consequences on mortality. About half of aged mice forced to phase advance 6 hours once per week survive this treatment compared with an 83 percent survival rate

in unshifted age-matched mice. Aged mice subjected to weekly 6-hour phase delays show an intermediate survival of 68 percent. These profound effects of phase shifting are not observed in younger mice. The pathogenesis of chronic jet-lag remains to be determined.

Of interest, these mice did not have an increased rate of tumorigenesis. It is likely that in humans, as in mice, the internal desynchrony of oscillators that result from a rotating light schedule may have dire consequences that may be exacerbated by aging. CIRCADIAN RHYTHMS AND PHARMACOTHERAPY Circadian rhythmicity can be affected by drugs, and conversely, the circadian clock can modulate the efficacy of drugs throughout the course of the day. A better understanding of these interactions will lead to more effective pharmacotherapies. Some of the beststudied interactions between medications and the circadian clock have included the circadian effects of antidepressants. Elevated nocturnal body temperature is a common feature among depressed patients. This effect may be due to a reduced amplitude of the master circadian oscillator in the hypothalamus that drives body temperature. Tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) reduce elevated nocturnal body temperature while simultaneously enhancing circadian amplitude. Similarly, many depressed patients exhibit a dampened amplitude in daily activity rhythms. Like body temperature, the amplitude in daily activity cycles of depressed individuals may be augmented by TCA or SSRI therapy. The use of lithium to treat bipolar disorder has been long established. However, lithium also affects the circadian system, resulting in a lengthening of circadian period. The molecular mechanism by which this occurs remains unknown. Glycogen synthase kinase 3β (GSK3β) has been implicated in participating within the molecular clock mechanism. Of interest, GSK3β is inhibited by lithium. In cell culture, GSK3β has been shown to stabilize the negative clockwork regulator REV-ERBα via phosphorylation. REV-ERBα typically represses the transcription of the BMAL1 gene. In the presence of lithium, however, GSK3β is inhibited, thereby preventing the phosphorylation and stabilization of REV-ERBα, which as a consequence is targeted for proteasomal degradation. The degradation of REV-ERBα results in the de-repression of BMAL1 transcription. Whether lithium’s influence on circadian behavior is attributable to its inhibitory effect on GSK3β-mediated stabilization of REV-ERBα remains to be determined. Short-acting benzodiazepines (e.g., triazolam [Halcion] and brotizolam [Lendormin]) also exert chronobiological effects. In hamsters, triazolam or brotizolam administered during the middle of the subjective day induces circadian phase advances in activity. Brotizolam has been shown to reduce the light-induced expression of clock genes Per1 and Per2 in the SCN. Although benzodiazepines are allosteric modulators of γaminobutyric acid A receptors (GABAA), several lines of evidence indicate that the circadian effects of short-acting benzodiazepines require an intact serotonergic system. When the 5-HT1A/7 receptor agonist 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OHDPAT) is injected into hamsters at subjective midday, phase advances in locomotor behavior and SCN neuronal activity are observed in addition to a reduction in Per1 and Per2 gene expression in the SCN. Recreational drugs of abuse also affect the circadian system. 3,4-Methylenedioxymethamphetamine (MDMA), or “ecstasy,” can act as a

serotonin neurotoxin. Hamsters treated with MDMA showed reduced triazolam-induced phase shifts in circadian locomotor activity and a diminished ability to reentrain rhythms posttreatment. MDMA-treated animals exhibited a reduction of serotonergic axonal terminals in the SCN, again emphasizing the importance of an intact serotonergic system in the regulation of the circadian axis. Recreational use of methamphetamine has increased dramatically. Chronic administration of methamphetamine disorganizes rodent activity rhythms. However, administration of

methamphetamine to rodents rendered arrhythmic through ablation of the SCN results in a reemergence of rhythmicity. The mechanism involved in the rescue of rhythmicity or site of action remains unknown. The efficacy and toxicity of many pharmacotherapeutics vary as a function of circadian phase. Daily variations in fixed-dose lethal toxicity have been appreciated in rodents for years. Many anticancer drugs, ranging in mechanism from antimetabolites to deoxyribonucleic acid (DNA) intercalators to mitotic inhibitors, have been shown to have 2- to 10-fold changes in tolerability in rodents over the course of the day. Much of this difference is attributed to circadian variations in the body's ability to absorb, distribute, metabolize, and eliminate toxic compounds. These four processes are affected by underlying circadian rhythms in physiological processes such as daily variations in gastric pH, gastrointestinal mobility, glomerular filtration rate, and membrane viscosity. The rhythmic intake of food during traditionally timed meals also influences how therapeutic drugs are handled by the body. It is becoming clear that to maximize efficacy and minimize toxicity of drugs, the circadian phase of administration must be considered. Appropriate circadian timing of the administration of multiple drugs can be a daunting challenge to infirmed individuals or their caretakers. The development of small implanted programmable pumps that can be directed to administer anticancer drugs or other therapeutics at particular times of day may provide a limited solution to this challenge. The emergence of the field of chronotherapy is a reflection of our increased understanding of the impact of the circadian system on the effectiveness of pharmacological treatments. REFERENCES Delezie J, Challet E. Interactions between metabolism and circadian clocks: Reciprocal disturbances. Ann N Y Acad Sci. 2011;1243:30. Dridi D, Zouiten A, Mansour HB. Depression: chronophysiology and chronotherapy. Biol Rhyth Res. 2014;45:77–91. Eckel-Mahan K, Sassone-Corsi P. Metabolism and the circadian clock converge. Physiol Rev. 2013;93(1):107. Glickman G, Webb IC, Elliott JA, Baltazar RM, Reale ME, Lehman MN, Gorman MR. Photic sensitivity for circadian response to light varies with photoperiod. J Biol Rhythms. 2012;27(4):308. Gonnissen HK, Rutters F, Mazuy C, Martens EA, Adam TC, Westerterp-Plantenga MS. Effect of a phase advance and phase delay of the 24-h cycle on energy metabolism, appetite, and related hormones. Am J Clin Nutr. 2012;96:689. Lanzani MF, de Zavalía N, Fontana H, Sarmiento MI, Golombek D, Rosenstein RE. Alterations of locomotor activity rhythm and sleep parameters in patients with advanced glaucoma. Chronobiol Int. 2012;29(7):911. Loddenkemper T, Lockley SW, Kaleyias J, Kothare SV. Chronobiology of epilepsy: Diagnostic and therapeutic implications of chrono-epileptology. J Clin Neurophysiol. 2011;28:146.

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