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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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