Critical Periods of Vulnerability for the Developing Nervous System: Evidence from Humans and Animal Models

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Vulnerable periods during the development of the nervous system are sensitive to environmental insults because they are dependent on the temporal and regional emergence of critical developmental processes (i.e., proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis). Evidence from numerous sources demonstrates that neural development extends from the embryonic period through adolescence. In general, the sequence of events is comparable among species, although the time scales are considerably different. Developmental exposure of animals or humans to numerous agents (e.g., X-ray irradiation, methylazoxymethanol, ethanol, lead, methyl mercury, or chlorpyrifos) demonstrates that interference with one or more of these developmental processes can lead to developmental neurotoxicity. Different behavioral domains (e.g., sensory, motor, and various cognitive functions) are subserved by different brain areas. Although there are important differences between the rodent and human brain, analogous structures can be identified. Moreover, the ontogeny of specific behaviors can be used to draw inferences regarding the maturation of specific brain structures or neural circuits in rodents and primates, including humans. Furthermore, various clinical disorders in humans (e.g., schizophrenia, dyslexia, epilepsy, and autism) may also be the result of interference with normal ontogeny of developmental processes in the nervous system. Of critical concern is the possibility that developmental exposure to neurotoxicants may result in an acceleration of age-related decline in function. This concern is compounded by the fact that developmental neurotoxicity that results in small effects can have a profound societal impact when amortized across the entire population and across the life span of humans. Key words: abnormal neurological development, apoptosis, behavioral testing methodology, delayed neurotoxicity, differentiation, migration, myelination, neurobiological substrates of function, neuronal plasticity, neurotrophic factor, primate, rodent. — Environ Health Perspect 108(suppl 3):511–533 (2000).
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The vulnerability of the developing brain is dependent on two main exposure issues. The first factor relates to whether an agent or its active metabolite(s) reaches the developing nervous system, and the second factor relates to the period of exposure. Exposure to environmental toxicants coincident with the ontogeny of developmental processes is more likely to cause adverse effects if they interfere with the cascade of these developmental processes. In general, if exposure occurs before or after an organ develops, it is less vulnerable to perturbation than if exposure occurs during development of that organ.

It is critical for the risk assessment of environmental chemicals that we have a good understanding of the time lines of normal neural development in humans and other species used in toxicological testing. This understanding should include both pharmacokinetic and pharmacodynamic factors that may differ across species. Both species- and age-related differences in pharmacokinetic parameters are out of the scope of this review paper, but other papers have attempted to describe these parameters in a limited way with pesticides (1–5), metals (6–10), and a solvent (11).

There is general recognition that the developing nervous system is qualitatively different from the adult nervous system. The normal ontogeny of neural development in rodents is different from humans because rodents have considerable postnatal development and humans have considerably more prenatal maturation of their nervous systems. These differences may be confounded with differences in the route of exposure during critical periods of nervous system development and thus differences in vulnerability between developing animals and humans (e.g., lactational transfer during the first postnatal week in rodents and transplacental transfer during the third trimester in humans).

Key aims of this article are to characterize the profiles of developmental processes, describe similarities and differences in windows of vulnerability between rodents and humans, and describe similarities and differences in effects after perturbations of developmental processes in humans and animal models, including rodents and monkeys. The brain areas subserving various behavioral functions are described for human infants and the ontogeny of these specific structures is discussed in the context of the effects of

lesions in nonhuman primates with altered performance on these tasks. This will further our understanding of developmental neurotoxicology, improve our ability to predict adverse outcomes in animals, and assist in the extrapolation of these adverse outcomes to risks to human health.

Development of the Brain in Utero

The cell precursors of the brain and spinal cord, which compose the central nervous system (CNS), begin to develop early in embryogenesis through the process called neurulation. The notochord, which is a cellular rod that defines the primitive axis of the embryo, will be incorporated into the vertebral system. The notochord induces the overlying ectodermal tissue to form the neural plate at approximately 2 weeks of gestation in humans. This overlying ectodermal tissue gives rise to the CNS. In humans on approximately gestational day (GD) 18, the neural plate invaginates along its central axis to form the neural groove with neural folds on each side. By the end of the third week of gestation in humans, the neural folds have begun to move together and fuse, forming the neural tube near the anterior end of the notochord; this fusion progresses both cranially and caudally in a zipperlike manner. The neural tube then separates from the overlying ectoderm, which becomes a contiguous surface over the back of the embryo and differentiates into the epidermis of the skin. As the neural tube forms, a population of cells separates from the

surface ectoderm at the apex of the neural folds to form the neural crest, which will give rise to the sensory ganglia of spinal and cranial nerves, Schwann cells (the cells covering peripheral nerves), the meningeal covering of the brain and spinal cord, and some skeletal and muscle components of the head, among other structures. The neural tube begins to close in the area of the hindbrain above the origin of the notochord and proceeds anteriorly and posteriorly, creating a caudal-to-rostral gradient in development of the brain. Neural tube formation is complete at approximately GD 10.5–11 in rats and from GD 26 to 28 in humans; the anterior neuropore closes first (rats GD 10.5, humans GD 24–26) and the posterior neuropore closes later (rats GD 11.3, humans GD 25–28) [reviewed by DeSesso (12)].

Interruption of neural development during this early period can result in severe abnormalities of the brain and spinal cord. Spina bifida (divided spine) results from defective induction of mesoderm around the notochord that forms the osseous bone of the spine. There are several types of spina bifida, which range from anomalies in the vertebrae of no significance to severe defects in the spinal cord or brain. Extreme cases of spina bifida (i.e., anencephaly) lead to failure in the closure of the neural tube and severe defects in the spinal cord or brain. For example, failure of neural tube closure results in extroversion of the neural tissue, which then degenerates as in anencephaly, wherein the brain is represented by a mass of degenerated neural tissue exposed on the surface of the head. It has recently been established that an increased intake of folic acid during early gestation or prenatally decreases the prevalence of neural tube defects in offspring (13). Increased risk for spina bifida may depend on the mother and/or fetus being homozygous for specific forms of enzymes involved in folate metabolism (14).

Beginning early in the second week of gestation in rodents (GD 7 in mouse, GD 9.5 in rats) and the first month of gestation in humans, specific areas of the CNS begin to form with the neurogenesis and migration of cells in the forebrain, midbrain, and hindbrain. There follows a sequence of developmental processes including proliferation, migration, differentiation, synaptogenesis, apoptosis, and myelination [Figures 1 (15) and 2 (16)].

Alterations in these processes can result in severe congenital abnormalities of the nervous system of humans, with a frequency of 0.74–1.89 cases per 1,000 births according to a recent survey (17). These overt abnormalities include conditions that produce extremely severe functional deficits and which may be incompatible with life, including anencephaly, hydrocephaly, and herniation of the spinal cord. Significant risk factors associated with these conditions include parental age, toxemia in the mother, threatened interruption of the pregnancy, and prematurity or intrauterine hypotrophy. A literature survey of > 70 studies from various countries (18) found that the incidence of mental retardation is approximately 4 per 1,000 births, although, as stated by the authors, the true prevalence is difficult to determine.
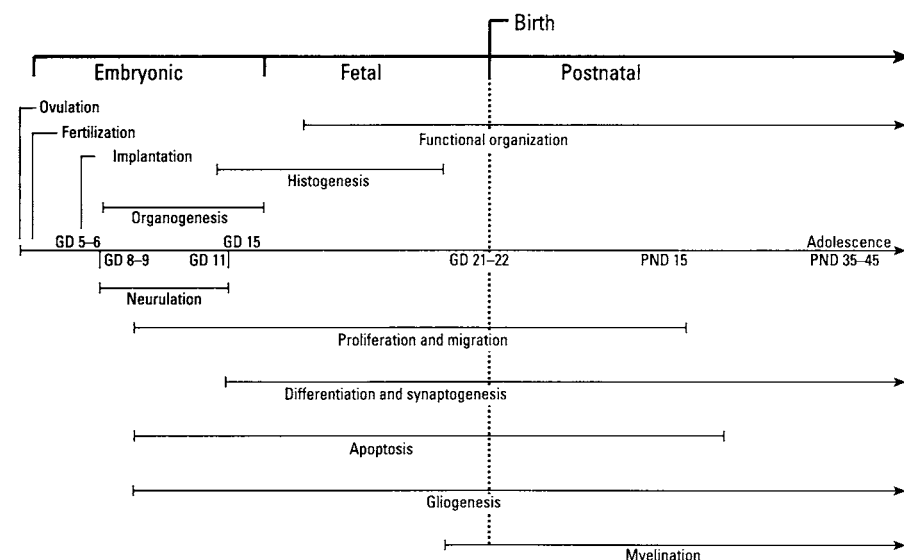

Figure 1. Timelines of developmental processes in the nervous system of rats compared to timing of fertilization, organogenesis, and histogenesis. Modified from Vorhees (15) and reprinted with permission of Plenum Press.
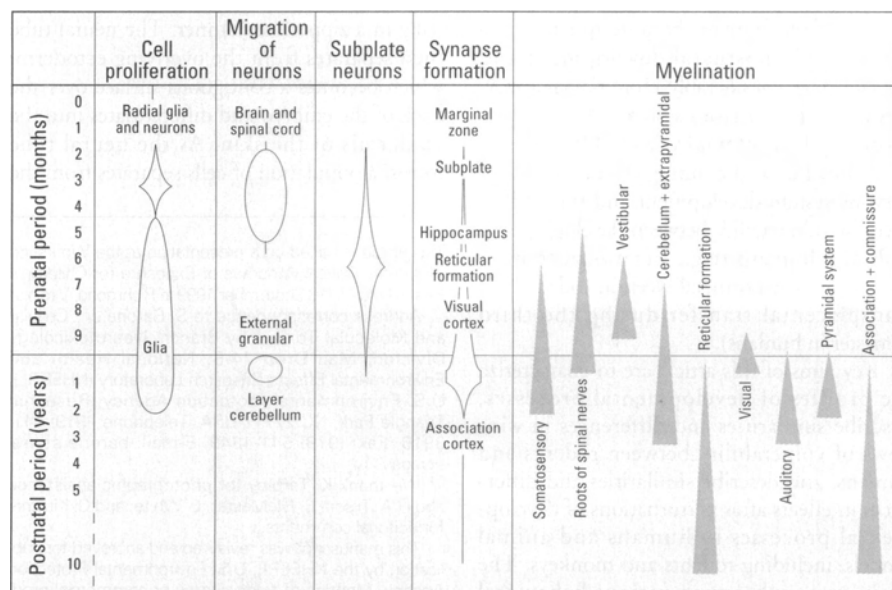

Figure 2. Comparison of timelines for developmental processes in humans. The prenatal period is scaled in months and the postnatal development is scaled in years. Adapted from Herschkowitz et al. (16) and reprinted with permission of Hippokrates Verlag GmbH.

Regional Development of the Rodent and Primate Brain

In general, regional development of the rodent brain proceeds on a timeline of days versus weeks to months in humans, although gross regional development of the brains of rodents and humans is similar. In the case of specific structures, however, there may be differences in the relative mass and/or volume of a specific structure between species. Examples include the relatively larger mass of the neocortex and visual system in humans versus that of rats. Conversely, the relative mass of the olfactory system is larger in rodents than in humans. The gradients of maturation of developing regions of the nervous system in rats and humans follow the same general sequence, with more caudal regions like the hindbrain {metencephalon and myelencephalon [Figure 3 (19)]} developing earlier than the more rostral areas like the forebrain (telencephalon and diencephalon) and with the more medial
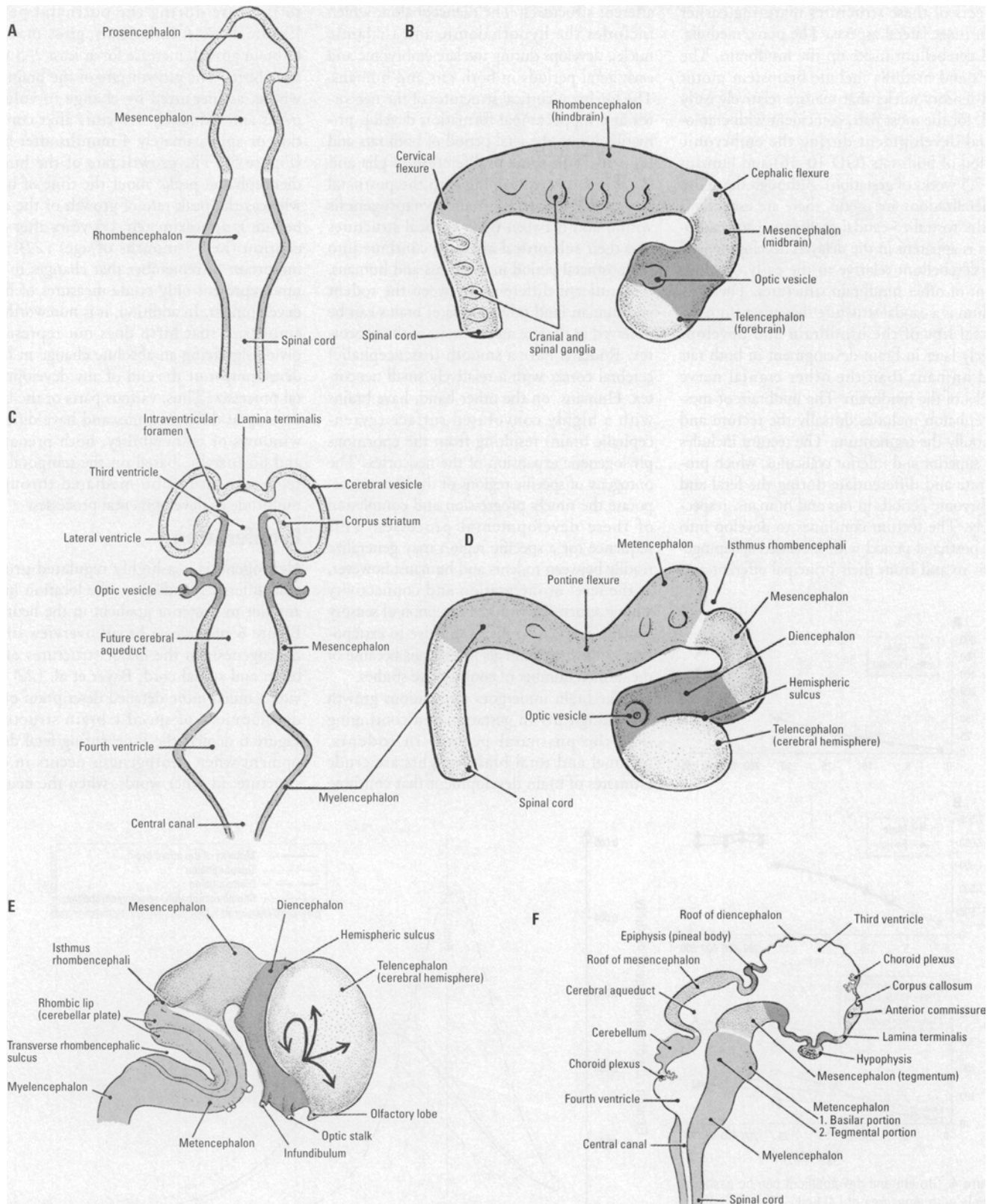
Figure 3. Development of the brain vesicles and subsequent brain divisions of the mammalian brain. (*A*) and (*B*) The development of the three primary brain vesicles on GD 10.5 in rats and GD 26 ± 1 in humans. The corresponding shading between panels illustrates the earlier origins of different regions from the three original brain vesicles with a horizontal and lateral view, respectively. (*C*) and (*D*) The more mature brain with five brain vesicles: the horizontal and lateral views correspond to GD 11.5 in rats and GD 33 ± 1 in humans. (*E*) The lateral view shows the migratory paths from the more central ventricular zone and gradients maturation of the neocortex (see arrows). (*F*) The midsagittal view of the brain and spinal cord, with the major divisions delineated and the continuity of the ventricles noted; the formation of the choroid plexus corresponds to GD 13.5 in rats and GD 48–51 in humans. Adapted from Carpenter and Sutin (*19*) and reprinted with permission of Williams and Wilkins.

aspects of these structures maturing earlier than more lateral aspects. The pons, medulla, and cerebellum make up the hindbrain. The pons and medulla include brainstem motor and sensory nuclei that mature relatively early and, for the most part, coincident with cranio-facial development during the embryonic period of both rats (GD 10–16) and humans (3–7.5 weeks of gestation). Although the above generalizations are useful, there are exceptions to the rostral-to-caudal gradients. One exception is apparent in the delayed development of the cerebellum relative to the early development of other hindbrain structures. The cerebellum is a caudal structure that arises from the dorsal lips of the hindbrain and develops largely later in brain development in both rats and humans than the other cranial nerve nuclei of the hindbrain. The midbrain or mesencephalon includes dorsally the tectum and ventrally the tegmentum. The tectum includes the superior and inferior colliculus, which proliferate and differentiate during the fetal and embryonic periods in rats and humans, respectively. The tectum continues to develop into the postnatal period with increased synaptogenesis to and from their principal efferent and afferent structures. The diencephalon, which includes the hypothalamic and thalamic nuclei, develops during the late embryonic and early fetal periods in both rats and humans. The forebrain cortical structures of the neocortex and hippocampal formation develop primarily during the fetal period of both rats and humans, with some proliferation of glia and microneurons continuing into the postnatal period. Differentiation and synaptogenesis within and between these cortical structures and their subcortical afferents continue into the postnatal period in both rats and humans. A significant difference between the rodent and human (and other primate) brains can be observed in the size and contour of the neocortex. Rodents have a smooth (lissencephalic) cerebral cortex with a relatively small neocortex. Humans, on the other hand, have brains with a highly convoluted surface (gyrencephalic brain) resulting from the enormous phylogenetic expansion of the neocortex. The ontogeny of specific regions of the brain incorporate the timely progression and completion of these developmental processes. This sequence for a specific region may generalize readily between rodents and humans; however, at the level of integration and connectivity among structures, with the exception of sensory systems, it is often only speculative to extrapolate results from animals to humans because of the limited number of comparative studies.

The brain undergoes tremendous growth beginning early in gestation and continuing into the postnatal period. In rodents, regional and total brain weights are crude estimates of brain development that continue to increase during the postnatal period [Figure 4 (20)]. In humans, gross measures of brain growth increase for at least 2–3 years after birth. The growth rate of the brain as a whole, as measured by change in volume, peaks approximately 1.1 years after conception or approximately 4 months after birth (Figure 5). The growth rate of the human diencephalon peaks about the time of birth, whereas the peak rate of growth of the cerebellum is approximately 1.3 years after conception (i.e., 7 months of age) (21). It is important to remember that changes in volume represent only crude measures of brain development. In addition, it is noteworthy to emphasize that birth does not represent a divide signifying an absolute change in brain development or the end of any developmental processes. Thus, various parts of the brain develop at different times and have different windows of vulnerability, both prenatally and postnatally, based on the temporal and regional maturation mediated through a multitude of developmental processes.

Proliferation

Neurogenesis is a highly regulated process that differs depending on the location in the anterior to posterior gradient in the neuraxis. Figure 6 provides a broad overview of the neurogenesis in the major structures of the brain and spinal cord. Bayer et al. (22) provide a much more detailed description of the development of specific brain structures. Figure 6 depicts the time during fetal development when neurogenesis occurs in each structure: in other words, when the neurons
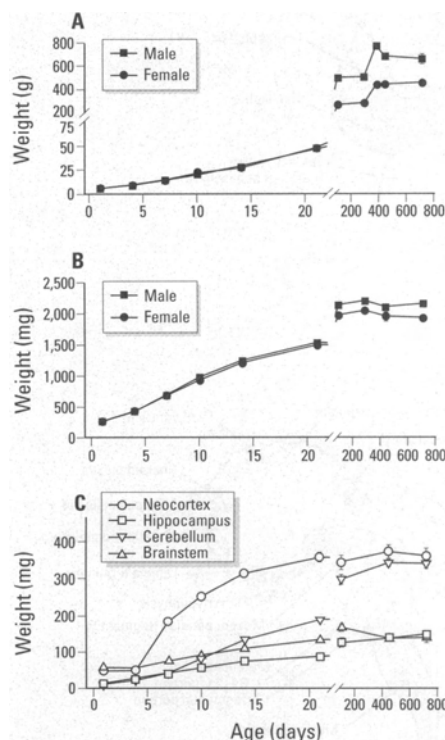
Figure 4. Growth and development can be assessed crudely with measures of (A) body, (B) brain, and (C) regional weights. The data depicted illustrates weights (mass) of female Long-Evans rats during postnatal development. Regional weights are from freehand dissections of each region (brainstem is defined as the pons and medulla). Cerebellum includes both the hemisphere and the flocculonodular lobes. Data are expressed as means ± SE. Adapted from Barone et al. (20) and reprinted with permission of Intox Press, Inc.
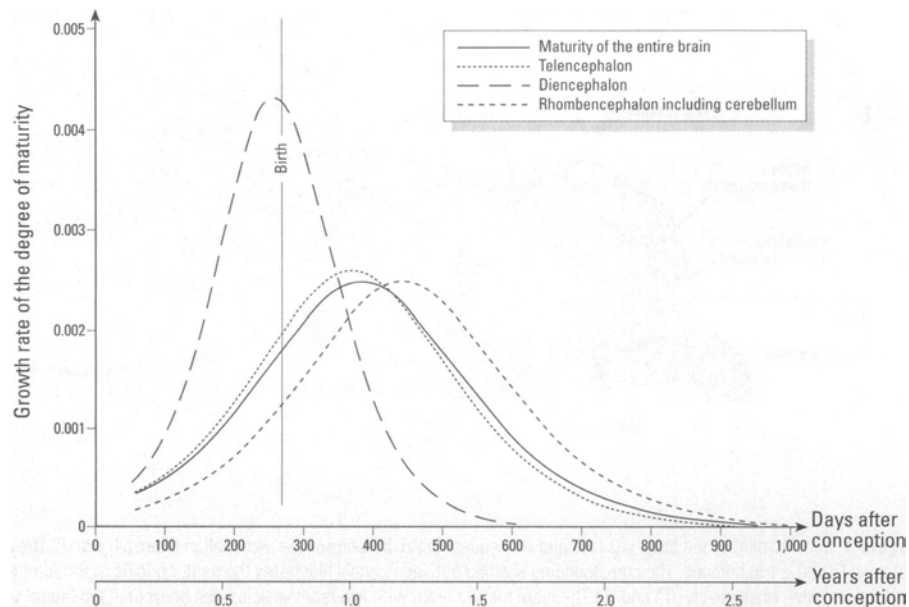
Figure 5. The regional growth rate of the human brain and different regions during prenatal and postnatal development scaled for both days and years after conception. Growth rate of the entire brain, telencephalon, diencephalon, and rhombencephalon including cerebellum during the development of humans. These curves were derived from four-parametric logistic functions. Adapted from Koop et al. (21) and reprinted with permission of S. Karger AG.

which will eventually comprise that structure are born. Within each broad brain region various nuclei and other substructures also develop according to a predictable timetable.

Both the amount and temporal windows of neurogenesis vary within different regions of the brain. These incongruities can be exemplified by comparing the development of three cortical regions: the neocortex, hippocampus, and cerebellum. Cytogenesis in the neocortex is dependent on the exponential proliferation of a multipotent population of pseudostratified cells localized in the ventricular zone, as concluded by the Boulder Committee study of proliferation and neural development (23). In the rat, this ventricular zone is histologically distinct beginning around GD 12. Shortly thereafter, the anatomically superficial subventricular zone is distinguishable. This is a secondary proliferative population of cells that also contributes appreciably to the ultimate cell population of the mature neocortex (24). The most expansive phase of proliferation in the rat ventricular and subventricular zones occurs roughly between GD 13 and 18, although some neocortical cells may remain mitotic until near parturition in rodents [GD 21/22; reviewed by Bayer and Altman (25) and Rakic and Caviness (26)]. In addition, interspecies comparisons between rodent and human proliferation and migration demonstrate that the patterns of these processes and the progression of regional development are relatively parallel, although the time scales are substantially different (days for rodents and weeks to months for humans) (22).

The hippocampal formation arises from the morphogenetic folding of the medial proliferative zone of the cortical plate to form the subiculum, pyramidal cell fields, and subsequently the dentate gyrus. The dentate gyrus, which forms prenatally, displays continued postnatal proliferation of granule cells in the hippocampal formation of both rodent and humans (27). The proliferation of the cerebellum takes place in different phases, with the primary proliferative cells migrating to the rhombic lips of the primordial cerebellum during the fetal period and then migrating to a secondary proliferative zone in the external granule layer of the cerebellum. This secondary wave of proliferation in the cerebellar cortex occurs postnatally in rodents and during the last trimester in humans but also continues postnatally.

Numerous environmental agents can alter neural proliferation. Classic experimental tools for studying the effects of altered developmental proliferation have included administration of antimitotic agents like ionizing radiation [reviewed by Bayer and Altman (25)] and methylazoxymethanol (MAM) (28) during critical windows of development. Studies with antimitotic agents have clearly demonstrated that when proliferation is actively occurring in a given region of the brain it is vulnerable, but when cell proliferation ceases, the brain is more resilient to these agents. More recently, a number of reports have focused on the effects of ethanol on proliferation in the fetal brain of experimental animals [reviewed by Guerri (29)]. The capacity of ethanol to alter proliferation in the ventricular and subventricular zones has been clearly demonstrated in rodents (30). The organophosphorus pesticide chlorpyrifos appears to alter proliferation after developmental exposure in rodents (31–34). In the case of methyl mercury it appears that proliferation is affected in both experimental animals (35,36) and humans (37).

Migration

Migration of recently proliferated cells (G_0 cells) from the ventricular zone and other germinal layers occurs radially in the medial/dorsal neocortex and tangentially in other regions of the forebrain such as the olfactory bulb and lateral neocortex (38). In the neocortex, for example, the mature cortical plate has histologically distinct laminae, classified as layers I
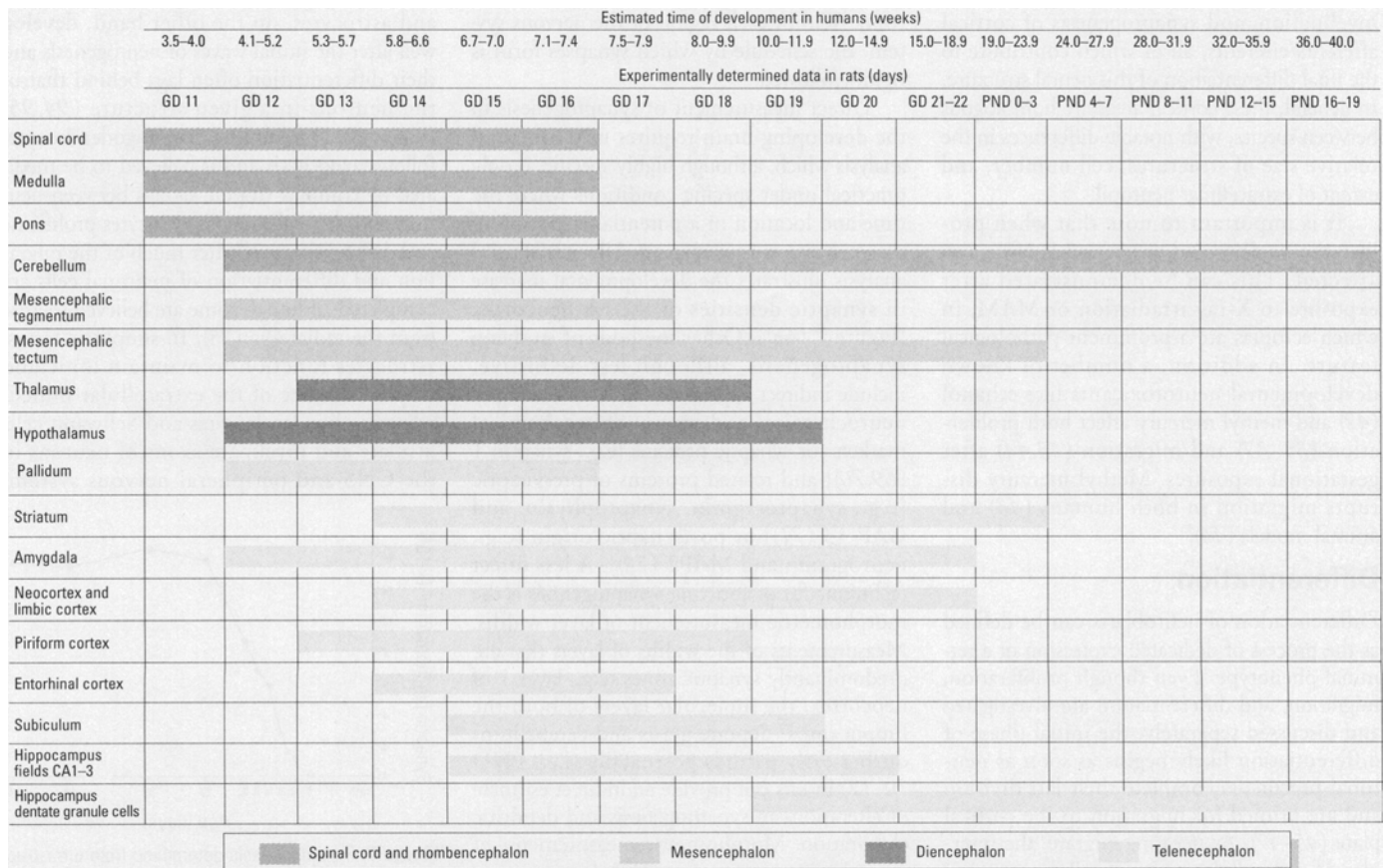
	3.5–4.0	4.1–5.2	5.3–5.7	5.8–6.6	6.7–7.0	7.1–7.4	7.5–7.9	8.0–9.9	10.0–11.9	12.0–14.9	15.0–18.9	19.0–23.9	24.0–27.9	28.0–31.9	32.0–35.9	36.0–40.0

Estimated time of development in humans (weeks)

Experimentally determined data in rats (days)

	GD 11	GD 12	GD 13	GD 14	GD 15	GD 16	GD 17	GD 18	GD 19	GD 20	GD 21–22	PND 0–3	PND 4–7	PND 8–11	PND 12–15	PND 16–19
Spinal cord																
Medulla																
Pons																
Cerebellum																
Mesencephalic tegmentum																
Mesencephalic tectum																
Thalamus																
Hypothalamus																
Pallidum																
Striatum																
Amygdala																
Neocortex and limbic cortex																
Piriform cortex																
Entorhinal cortex																
Subiculum																
Hippocampus fields CA1–3																
Hippocampus dentate granule cells																

Spinal cord and rhombencephalon ■   Mesencephalon ■   Diencephalon ■   Telencephalon

Figure 6. Estimated timelines of regional neurogenesis in rats and humans; shading corresponds to brain vesicle in Figure 3 The scale for rats is in days and the scale for humans is in weeks. Adapted from Bayer et al. (22) and reprinted with permission of Intox Press, Inc.

through VI, and a subplate (layer VII). Layer VI is the deepest layer of the cortical plate; i.e., it is closest to the geometric center of the brain. Layer I is the most superficial layer; it is the outward-most subpial layer. The formation of layers VII and I is distinct from the cell migrations that populate layers VI through II (39). Cells from the ventricular zone populate layers VI–II in an inside-out fashion, meaning that the deeper layers form first (40). By GD 16 and 17 in the rat, the first cells are arriving in the area that will ultimately form the laminae of the cortical plate. Through the remainder of gestation, the cortical plate gets thicker as more cells migrate from the ventricular zone. In the days before parturition, layer VI is histologically distinct from the remainder of the maturing cortical plate. On postnatal day (PND) 5 of the rat, layers VI and V are readily distinguishable, but layers IV–II are not as clearly defined. These superficial layers (IV–II) are more distinct in adulthood but never constitute more than one-third of the cortical width. The remaining two-thirds of the cortical width is split approximately equally between layers VI and V, depending on the neocortical region of study (41). In addition to the migration of neurons to the cortical plate, other factors modify the lamination of the neocortex including cell packing density, cell size, extracellular matrix, gliogenesis, myelination, and synaptogenesis of cortical afferents/efferents, all of which contribute to the final differentiation of this neural structure. In general, these cortical areas are homologous between species, with notable differences in the relative size of structures, cell number, and extent of extracellular neuropil.

It is important to note that when proliferation is disrupted, migration is often also affected. This can be demonstrated after exposure to X-ray irradiation or MAM, in which ectopias are a prominent pathological feature. In addition, a number of known developmental neurotoxicants like ethanol (42) and methyl mercury affect both proliferation (35–37) and migration (43,44) after gestational exposures. Methyl mercury disrupts migration in both humans (43) and animal models (44).

Differentiation

Differentiation of neuroblasts can be defined as the process of dedicated expression of a terminal phenotype. Even though proliferation, migration, and differentiation are investigated and discussed separately, the initial phase of differentiation likely begins as soon as neuronal precursors complete their last division and are primed for migration to the cortical plate (45–47). As neurons migrate, the interplay of extracellular and intracellular cues initiates the expression of particular genes that will influence the neuronal or glial phenotype.

Particular phenotypic characteristics such as shape, size, polarity, and expression of neurotransmitters and receptors, among other features, allow distinction among differentiated neurons and glia. Numerous examples appear to support the contention that environmental influences affect this developmental process. Environmental agents that affect differentiation of the nervous system run across a broad gamut of chemical classes including ethanol (48–51), nicotine (52–57), methyl mercury (58), and lead (59–61). It is important to note that perturbations that alter neural proliferation and migration often result in altered differentiation. This has been illustrated in studies of punctate exposure to X-ray irradiation in rodent models (62,63).

Synaptogenesis

Synapses, the neurobiological substrates of almost all cell-to-cell communication (64), mature over the first 3 weeks during the postnatal period in rats (65) and through adolescence in monkeys (66) and humans (67). Synaptogenesis consists of biochemical and morphological changes in both the pre- and postsynaptic elements. Physicochemical compatibility of these elements and correct timing with competitive exclusion of inappropriate connections are essential for the maturation of synaptic connections [reviewed by Jacobson (65)]. Within each region of the nervous system, the schedule by which synapses form is rigidly followed.

Direct measurement of synaptogenesis in the developing brain requires ultrastructural analysis which, although highly specific, is only practical under specific conditions where the time and location of a potential alteration of this process is in question. Ultrastructural analysis illustrates the developmental increase in synaptic densities of the rat neocortex [Figure 7 (68)]. Other methods of studying synaptogenesis, although less definitive, include indirect approaches such as looking at neurochemical and immunohistochemical markers for synaptic proteins [e.g., synapsin 1 (69,70)] and related proteins of presynaptic [e.g., synaptotagmin, synaptophysin, and GAP-43 (71)] or postsynaptic origin [e.g., neurogranin and MAP2 (72)]. A less direct technique for quantifying synaptogenesis is the morphometric measurement of layer widths. Measurements of the widths of layers that are predominantly synaptic zones (e.g., layer 1 of neocortex, the molecular layers of both the hippocampal dentate gyrus and cerebellum) during early periods postnatally (i.e., PND 10–12) in rats can provide an indirect estimate of alterations in synaptogenesis and dendritic elaboration. Morphometric measurements of synaptic zones in neocortex (58), hippocampus (73), and cerebellum (74) can reveal perturbations of this developmental process.

Exposure during critical periods of development to a number of agents, including MAM (75), X-ray irradiation (76), ethanol (77,78), lead (79–82), methyl mercury (83), triethyltin (84), inhibitors of serotonin synthesis (85), parathion (86), permethrin, diisopropyl fluorophosphate, and polychlorinated biphenyl (PCB) mixtures (87), have all been implicated in altered synaptogenesis. In general, findings from these rodent studies indicate that the effects of these agents are more restricted to synaptogenesis when they are administered postnatally during the first and second week of brain development when this process is occurring rapidly.

Gliogenesis and Myelination

There are numerous supportive cells in the nervous system called glia, including microglia, radial glia, astroglia, oligodendroglia, and Schwann cells, that are integral for the normal development and function of the nervous system (88,89). Radial glial and microglial development occurs in parallel with neurogenesis in most structures of the brain, and in the case of microglia may be partially derived from extrabrain sources (90,91). Radial glia provide a scaffold for radial migration of neuroblasts (92,93). Microglia function as brain macrophages to clean up dead cells and cellular debris after apoptosis (91). Oligodendrocytes and astrocytes, on the other hand, develop well after the initial waves of neurogenesis and their differentiation often lags behind that of the neurons in a given structure (94,95) (Figure 8). Differentiation of oligodendrocytes follows axogenesis and is believed to be mediated by complex trophic signals between neurons and these glia. Most astrocytes proliferate and differentiate well after much of the migration and differentiation of neuronal cells are completed, although some are believed to arise from the radial glia (88). In simplistic terms, astrocytes function to maintain ionic and trophic balance of the extracellular milieu, whereas oligodendrocytes and Schwann cells insulate and myelinate axons of neurons in the CNS and peripheral nervous system,
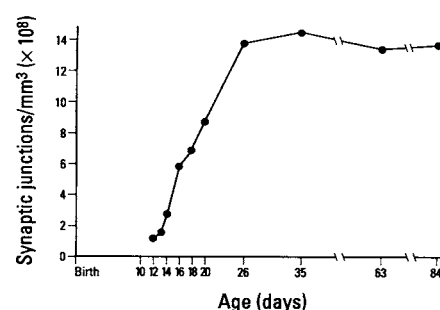

Figure 7. Synaptogenesis determined from ultrastructural analysis of layer 1 of the rat parietal cortex. The x-axis scale is in days. Adapted from O'Callaghan (69) and reprinted with permission of Raven Press.

respectively. The functional role of astrocytes and microglia may be transformed after nervous system damage (96,97); both cell types become reactive after insults to the nervous system. This reactive gliosis is unquestionably a pathological index when observed in the adult nervous system. In the developing nervous system, neural damage may not initiate the same degree of reactive gliosis depending on the timing of the insult which, in the case of astrocytes, could precede their major phase of proliferation and differentiation. Perturbations of the development of glial proliferation, migration, and differentiation can also result in adverse consequences (98,99).

Elaboration of myelin occurs later in development than proliferation and migration of neuronal populations (100). Malnutrition can affect myelination, and this process is especially vulnerable during late gestational development in humans and postnatally in rodents. Alterations in myelination can have a persistent adverse outcome resulting from postnatal malnutrition and alterations in thyroid hormone homeostasis even though somatic growth may recover [reviewed by Wiggins (101)]. Effects on myelination are readily observed after malnutrition or endocrine disruption during the ontological peak of this process, which occurs in the second week of postnatal development in rats and during the last trimester in humans. However, myelination continues to progress through adolescence in both rodents (101) and humans (102–104). The protracted time course for this process has

also been demonstrated to be vulnerable to perturbation in both rodents and humans. Prenatal and postnatal exposure of rats (105,106), and probably late prenatal exposure in humans, to ethanol (107) produces significant decreases in myelination and these effects can persist throughout the postnatal period. Similar findings have also been observed after postnatal lead exposure in rats (108–110) and developmental exposure in humans (111), where delayed myelination was observed during the postnatal period.

Apoptosis

Proper development of the nervous system requires apoptosis, a form of programmed cell death that systematically removes large numbers of neurons in some structures produced during ontogeny. Apoptosis occurs during both pre- and postnatal development of the CNS in two waves. The earlier wave occurs in proliferative zones; the second occurs in postmitotic cells that are both neuronal and glial in phenotype (112,113). Apoptosis is distinguished from the other general category of cell death, necrosis, by specific characteristics including maintenance of membrane, chromatin condensation, cell shrinkage, oligonucleosomal fragmentation of DNA, and a general lack of inflammation [reviewed by Bredesen (114)]. Cells undergoing apoptosis in the nervous system are targeted asynchronously and removed from the surrounding tissue without stimulation of an inflammatory response. In a region sustaining massive

developmental apoptosis, only a small percentage of the cells may be morphologically identifiable as apoptotic at a given time (115,116). Thus, characterization of developmental apoptosis may be necessary to understand normal nervous system development and could elucidate how neurotoxic agents perturb this process and result in altered cell number and neural function after exposure (117,118). This form of cell death results in less overt pathology than that which would be observed after damage to the adult nervous system but still leads to alterations in cell number and connectivity within the nervous system. Anomalous patterns of this form of cell death may be a consequence of exposure to environmental neurotoxicants. During critical periods, exposure to environmental agents may shift the tightly regulated balance of neurotrophic signals that regulate apoptosis, thereby causing alterations that result in undesirable increases or decreases in cell number in a particular region of the nervous system. Exposure to a number of agents, including ethanol (119,120), lead (121,122), methyl mercury [reviewed by Nagashima (123)], and chlorpyrifos (33), promotes this process during development in rodents. Alterations induced by xenobiotics can result in the progressive loss of neurons and may result in compromised neurological function later in life, as is the case in some neurodegenerative diseases (124–128). Although apoptosis has been studied in neurodegenerative disease, there has been little work examining the effects of environmental agents on apoptosis in the developing nervous system in humans.

Neurotrophic Signals

Cascading events define the entirety of brain development. The number of molecules involved in modulating neuronogenesis is enormous. The signal transduction of these trophic molecules uses both second and third messengers, which may lead to alterations in gene expression or epigenetic changes via phosphorylation and/or glycosylation of existing signaling and structural proteins. Changes in gene expression and signaling are reflected in cellular processes and events leading to differentiation. The intervening factors that can potentially modulate every step of a signaling cascade further complicate investigation of neuronal differentiation. What follows is a brief discussion of a few individual molecules important in proliferation, differentiation, and apoptosis. Nerve growth factor is the prototypical neurotrophic ligand. This soluble peptide is a ligand for tyrosine kinase receptors and p75 receptors throughout the developing brain. In vivo and in vitro investigations have demonstrated that this ligand [reviewed by Klein (129) and Zhou and Bradford (130)] and other structurally
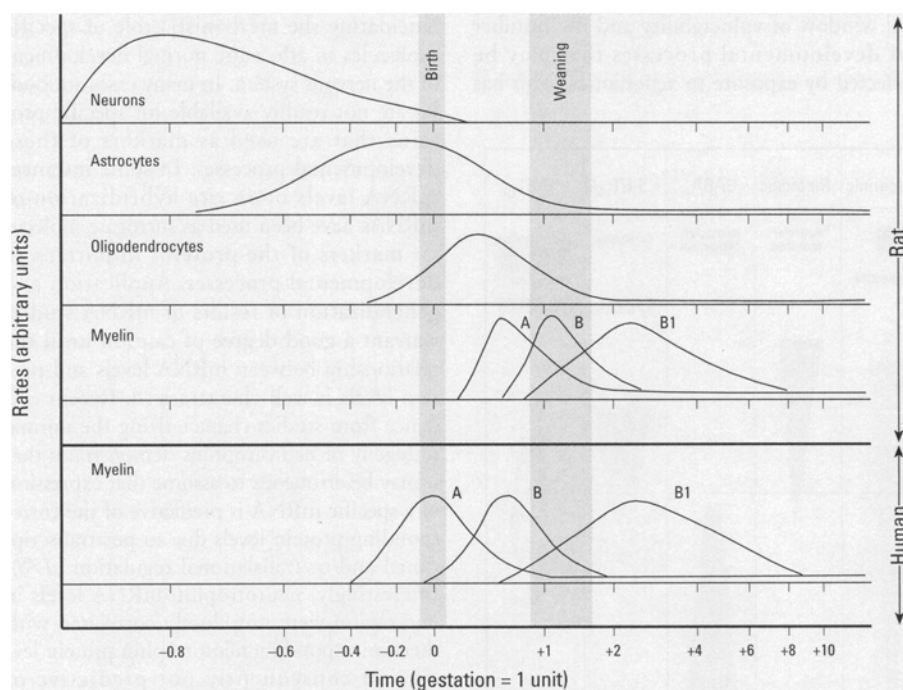

Figure 8. Proliferation of neurons and glia are depicted with the temporal and regional pattern of myelination which occurs later in development. Abbreviations: A, sciatic nerve; B, whole brain; B1, corpus callosum. The schedule of brain development for rats and humans is in arbitrary units (x-axis). Adapted from Wiggins (94) and reprinted with permission of Intox Press, Inc.

homologous neurotrophic factors, including brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4, are important to many processes in neural development [reviewed by Barone (*131*)]. Alterations in the expression or signaling of these trophic molecules result in adverse consequences for both the structure and function of the nervous system (*131*). A number of studies have demonstrated that changes in cell excitability resulting from pharmacological manipulations of many neurotransmitter systems, including glutaminergic (*132–137*), cholinergic (*138–142*), catecholaminergic (*143*), gabaergic (*144*), and peptidergic [opioid agonists and antagonists (*145*)] lead to pronounced changes in neurotrophic factor expression. In addition, a number of environmental chemicals and pharmaceutical agents alter the expression and/or signal transduction of these trophic molecules, including ethanol (*146–152*), methyl mercury (*153,154*), aluminum (*155*), and cholinesterase inhibitors (*138,139,156*). Although neurotrophic factors are perturbed in experimental animals after exposure to environmental agents, the involvement of these factors in developmental disorders has only recently been suggested in humans (*157*).

Neurotransmitters as Morphogenetic Signals

Neurotransmitters and other ligands have qualitatively different functions during development of the nervous system than in the adult organism. In the adult nervous system neurotransmitters are classically thought of as mediating or modulating synaptic transmission across a cleft between two cells. However, during development these same factors may interact with their cognate receptors over a much greater distance and form a morphogenetic gradient that is important in pattern formation of different regions of the nervous system and differentiation of different neurotransmitter systems [reviewed by Buznikov et al. (*158*)]. Developmental ontogeny of the neurotransmitter systems for humans is detailed in Figure 9 (*16*). Some factors that are important for initiating cascades that influence neural development include the neurotransmitters [reviewed by Cameron et al. (*159*) and Chausovsky et al. (*160*)], γ-aminobutyric acid (*161,162*), excitatory amino acids (*163*), opioids (*164–168*), acetylcholine (*169*) and acetylcholinesterase (*170,171*), catecholaminergic agonists and antagonists (*172*), and serotonin (*173*). Because many of these factors are pleiotropic and play fundamentally different roles during development than during adulthood (*174–176*), it would seem prudent to investigate whether pharmaceutics and pesticides (e.g., insecticides, herbicides, and fungicides) that are neuroactive or designed to target specific neurotransmitter systems may have qualitatively different effects than their pharmacological effects observed in adults. For example, effects of the organochlorine pesticide dieldrin on morphogenesis have been observed after exposures in whole embryo culture conditions (*177*).

It is apparent from the summary of these specific developmental processes that ontogeny of different parts of the brain occurs at different times during the prenatal and postnatal period, thus broadening the temporal window of vulnerability and the number of developmental processes that may be affected by exposure to xenobiotics. This has important implications concerning the anatomical, neurochemical, and functional consequences of the timing of exposure to a neurotoxic agent and effects that may be manifested later in life. For example, short-term exposure to a neurotoxicant during week 4 of gestation in humans (or on GD 11 in rats) would be expected to interfere with neurogenesis in the spinal cord and hindbrain structures but not the more anterior structures of the brain. In contrast, toxic insult at the end of gestation in humans or in the first postnatal week in rats might affect neurogenesis in cerebellum and hippocampus, as well as possibly other processes (cell migration, myelination, and synaptogenesis) in areas that have already undergone neurogenesis. Specific effects would depend not only on the timing and duration of the exposure but the processes affected by a specific neurotoxicant. Numerous neurotoxicants can affect the same developmental processes depending on dose and duration. In many cases, these effects on these developmental processes are mediated through very different mechanisms. By inference the effects on the developmental processes reflect downstream convergence on modes of action critical to the normal development and function of the nervous system.

Application of Molecular Biology to Developmental Neurotoxicology

The application of molecular techniques and transgenic technology has many advantages in elucidating the mechanistic role of specific molecules to affect the normal development of the nervous system. In many cases antibodies are not readily available for specific proteins that are used as markers of these developmental processes. In some instances mRNA levels or *in situ* hybridization of mRNAs have been used as surrogate molecular markers of the proteins important to developmental processes. Application and generalization of results of mRNA studies warrant a good degree of caution until the relationship between mRNA levels and protein levels is well characterized. Recent evidence from studies characterizing the normal ontogeny of neurotrophins demonstrates that it may be erroneous to assume that expression of a specific mRNA is predictive of the corresponding protein levels due to posttranscriptional and/or translational regulation (*178*). Interestingly, neurotrophin mRNA levels in one region were not closely correlated with their corresponding neurotrophin protein levels and consequently not predictive of regional neurotrophin protein levels (*178*). In short, molecular approaches may be useful in mechanistic studies and in the context of gene discovery, but unless they are used in
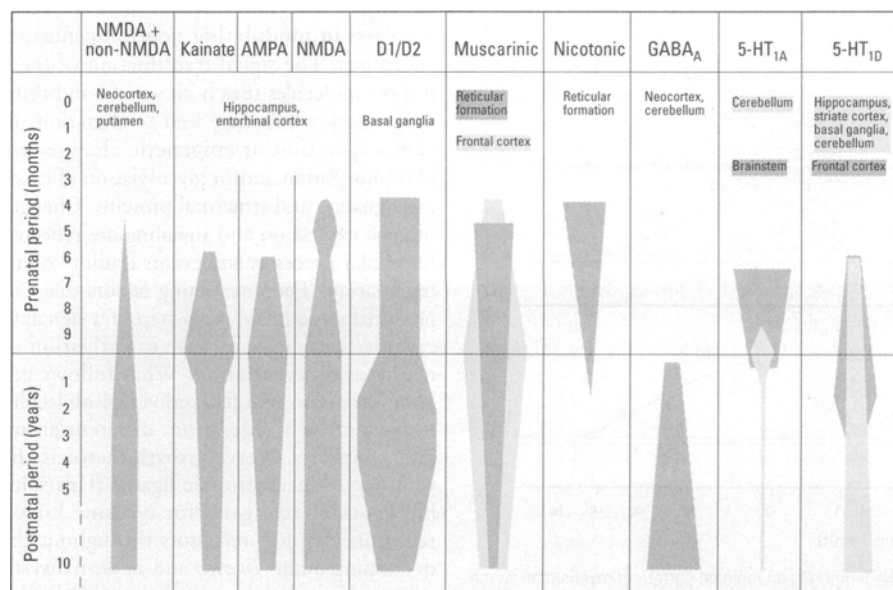

Figure 9. Comparison of regional timelines in the receptors for classical neurotransmitter is depicted for humans. Adapted from Herschkowitz et al. (*16*) and reprinted with permission of Hippokrates Verlag GmbH.

conjunction with an examination of the protein levels, they may not always prove useful in predicting an adverse effect.

Another approach worthy of attention is the use of knockout and transgenic animal models. These technologies may be useful in demonstrating the effects of regulation of specific molecules during development. Transgenic technologies may also prove useful in understanding the role of genetic polymorphism in response variability when molecular targets are identified. Recent research has identified genetic polymorphisms in a number of enzymes that both metabolize and detoxify xenobiotics, including pesticides (179). A particularly salient example is the role of genetic polymorphisms for paraoxonase (179,180), an esterase that detoxifies many organophosphorus pesticides. These polymorphisms appear to play a role in response variability in the degree of cholinesterase inhibition. In addition, there appears to be ontogenic development in both rodents and humans that may in part play a role in age-related sensitivity (2). However, interpretation of these molecular approaches requires caution when extrapolating the results to developmental toxicology because knockout technology may result in an all-or-none response, which may not be relevant to xenobiotic exposure. Conversely, the toxicological exposure may perturb a molecular mechanism or group of related molecular pathways and thus be more adverse than knocking out or altering one gene product during development.

Neuronal Plasticity

It is important to recognize that the nervous system continues to remodel and change not just early in development but throughout the entire period of development (and even during adulthood) in response to environmental influences as well as genetically programmed events. One mechanism for this remodeling is the normal genetically programmed overproduction and subsequent elimination of neurons and neuronal processes. For example, there has been extensive research on this issue in the rhesus monkey by Pasko Rakic and colleagues (181). Synaptic density in primary visual cortex increases until approximately the third postnatal month, then decreases until about 2 years of age, followed by a slower decrease between 2.7 and 5 years of age (puberty) (181). The timeframe of synaptic development in monkey cortex is the same for areas including somatosensory, motor, and prefrontal cortex (182), suggesting that cell-to-cell communication in the neocortex may be orchestrated by a single genetic or humoral signal. The overproduction and subsequent elimination of neurotransmitter receptors in disparate areas of the monkey neocortex is synchronized with synaptogenesis (183,184). In contrast, elimination of neurons in the

lateral geniculate nucleus of the thalamus, which is up-system from neocortex, occurs during the middle third of gestation (185). These examples demonstrate the elegant and orderly sequence of elimination of neuronal elements during development, which continues at least until puberty. In motor cortex of the monkey, synaptogenesis continues for several months postnatally, and then decreases until approximately 10 years of age (well after puberty) (186). Similarly, synaptogenesis in prefrontal (66) and somatosensory cortex (187) continues into the early postnatal period followed by a slow decline into adulthood. Approximately 70% of the axons in the corpus callosum are eliminated between birth and 4 months of age (188).

Environmental input plays a crucial role in the development of the architecture of the brain after birth. The seminal work of David Hubel and Torsten Wiesel, for which they won the Nobel Prize in Physiology and Medicine in 1981, demonstrated significant remodeling of the visual system of kittens in response to deprivation of input to one eye (189). The visual cortex was remodeled such that the primary visual cortex consisted of representation from only the unoccluded eye. Further, the kittens appeared essentially blind in the deprived eye when the lid suture was reversed at 3 months of age. Similar results were observed in a series of experiments with monkeys (190). Subsequently, numerous studies have documented remodeling of other sensory systems in a number of species in response to experimental manipulation of input into that system (191–195). Interestingly, when kittens are deprived of visual input from birth, an area of the parietal cortex normally associated with vision is taken over almost completely by auditory and somatosensory inputs (196). Auditory neurons in this area exhibited sharper spatial tuning in visually deprived cats, and these cats could localize sound sources more precisely than nondeprived cats.

Other brain areas also respond to early experience. For example, rearing rodents in enriched environments compared to impoverished or typical laboratory environments resulted in significant increases in cortical thickness, dendritic branching, and synaptic density (197–200). Exposure to enriched environments early in development also results in improved performance on learning tasks in rats (201). The environment during the first several years of life in humans may also have profound effects on structure and function of the limbic system (202), which is critical for social and emotional function.

Neuronal plasticity continues at least throughout childhood and into adolescence. Computational analysis of the brains of children 4–17 years of age revealed an age-related

increase in the size of fiber tracts supporting motor and speech functions (104) (Figure 10). In an example of remodeling in the somatosensory cortex in response to experience, magnetic resonance imaging (MRI) revealed that the cortical representation of the left but not the right hand of string players was larger than in nonplayers, and that the increase in area was correlated with the age at which individuals began to play (203) (Figure 11). Other researchers have also identified continued development of brain structures through late childhood, adolescence, and young adulthood using imaging techniques (204–206).

Assessment of Nervous System Function in Animals and Humans

There are a number of standardized clinical tests for assessment of the integrity of nervous system function in the developing human from infancy through the school years. Standardized test batteries such as the Neonatal Behavioral Assessment Scale (NBAS) are available for assessing intactness of the nervous system in young infants, measuring such functions as neuromuscular and motor reflexes, reaction to sensory stimuli, habituation to repeated stimuli, and autonomic function (207,208). Assessment of neurological and cognitive development may be performed in children by the use of age-adjusted scales such as the Bayley Scales of Infant Development in very young children, the McCarthy Scales of Children's Ability (MSCA), and the Kaufman Assessment Battery for Children in older children. These instruments include tests for psychomotor, memory, verbal, perceptual, and other components depending on the instrument and age range being assessed. In older children, the Weschler Intelligence Scales for Children - Revised (WISC-R) is typically used to assess cognitive function; this instrument is divided into a number of subscales to partition out various aspects of cognitive performance. Performance on early childhood tests is not predictive of performance on the WISC-R at school age for the general population of children (209) but may have better predictive power for low-functioning children (210). There has also been some research on the development of play behavior in young children (211–214), including the correlation between play and language development (215) and coping behavior (216). A test that has proved extremely useful in the assessment of the cognitive abilities of infants is the preferential-looking test [Fagan test (217)]. This test takes advantage of the fact that an infant looks longer at a novel stimulus (e.g., a picture of a face) than at a familiar one, and so the test can be used to assess short-term memory. If the infant looks longer at the novel

stimulus than at the one previously presented (preferential looking), it is assumed that the infant remembers the previously presented stimulus. This task is more highly correlated with later performance on intelligence quotient (IQ) tests than sensorimotor tests such as the Bayley Scales (218). Preterm infants do less well on tests of preferential looking at 6 and 12 months of age than full-term infants (219), a finding that has been replicated in infant monkeys tested at corresponding ages (220). Monkeys exposed to methyl mercury *in utero* perform more poorly on this task than do control monkeys (221), as do human infants exposed prenatally to PCBs (222).

There are other cognitive behaviors that may be tested in very young infants, such as imitation of facial expressions (223), a form of cross-modal matching (i.e., visual and sensorimotor). Young infants are capable of cross-modal matching in other modalities as well, such as visual tactile (224). Infants also progress through a series of stages of

understanding that an object that is no longer in sight still exists (225), a cognitive awareness labeled object permanence. A comparison of tests of early cognitive function and later IQ (224) revealed a good correlation between preferential looking, cross-modal matching, and object permanence with IQ at 6 years of age. Additionally, results of preferential-looking tests at 7 months of age and cross-modal matching tests at 1 year of age predicted children considered to be at risk for learning disabilities at 6 years of age. Development of object permanence is also retarded in infant monkeys exposed prenatally to methyl mercury (226). An assessment of concurrent predictors of IQ in children 6 years of age (227) found a high correlation between IQ and tasks that may be performed in animals, including delayed match to sample (DMTS) and conditional discrimination. Another test that is highly correlated with IQ is speed of information processing, including complex reaction time (228–231). This test has been used in

monkeys to assess cognitive effects of developmental methyl mercury exposure (232).

Tests of sensory function, motor function, reflex ontogeny, and learning have been incorporated into a number of behavioral teratology rodent screening batteries like the Cincinnati Psychoteratogenicity Screening Test Battery (233), Barlow and Sullivan Screening Battery (233), and the U.S. Environmental Protection Agency testing guidelines for developmental neurotoxicity (234). These screening batteries were designed with considerable emphasis on apical tests of function during development. Specific tests have been developed to assess the ontogeny of sensorimotor function in the rat, including habituation, reactivity, exploratory behavior, and motor coordination and integrity (235–238). Such tests assess many of the same functions as the NBAS and Bayley Scales used with human infants and toddlers. Other early tests of nervous system integrity in rodents may include homing behavior (returning to the nest or siblings), cliff avoidance, placing reaction, passive avoidance, and escape latency on a swimming task. Whereas most of these behaviors show a smooth pattern of development some time during the first 21 days of life (236) (e.g., Figure 12), the ontogeny of motor activity and exploration activity displays a more complicated pattern (235) (Figure 13). Locomotor activity increases and then decreases during the first 21 days of life, and then increases again after weaning. The decrease in locomotor activity is mirrored by an increase in exploratory behavior during that developmental period. The emergence of locomotor behavior reflects the development of mesencephalic and spinal function, from crawling at PND 10 to an adultlike pattern at PND 15 in rats (235). Similarly, the increase and later decrease in exploratory activity is presumed to be reflective of the brain circuits
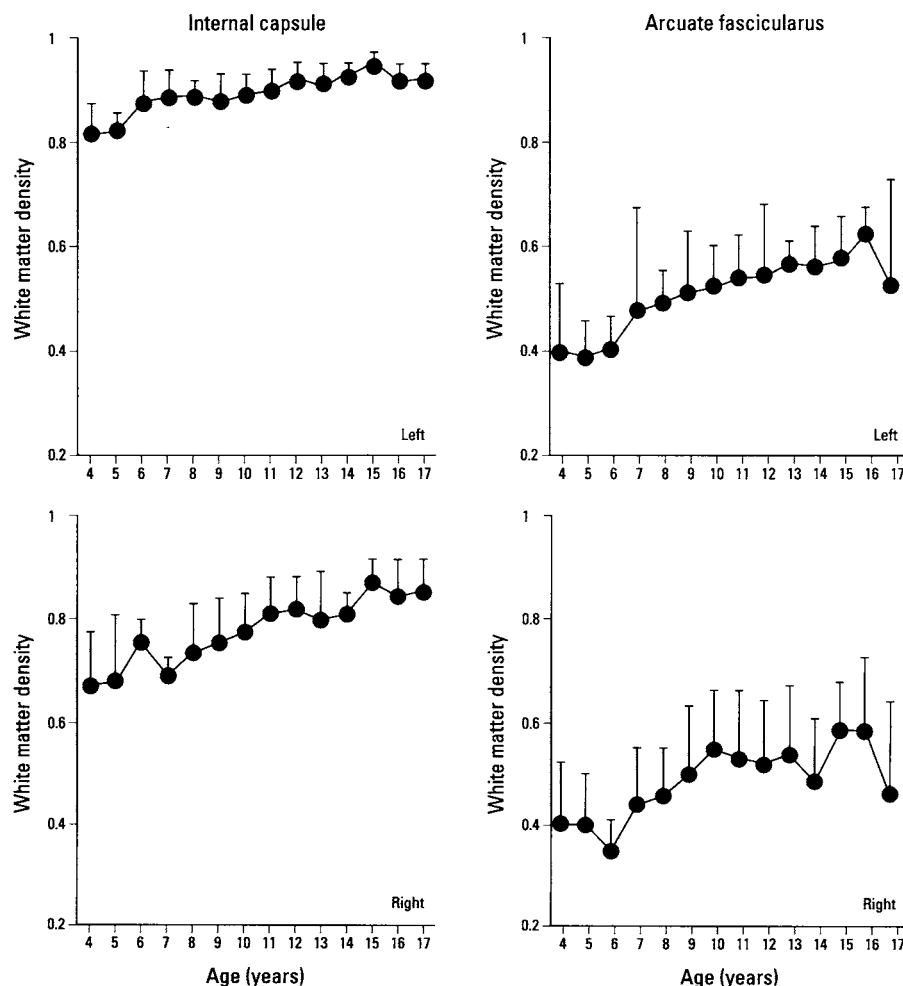

Figure 10. Postnatal myelination in humans, with increasing white matter density for the internal capsule and arcuate fasciculars for 111 children and adolescents ranging in age from 4 to 17 years of age. The internal capsule is a fiber tract involved in motor function; the left arcuate fasciculars subserves speech function. The late maturation of these pathways suggest ongoing increases in diameter and/or myelination of axons at least through adolescence. Adapted from Paus et al. (104).
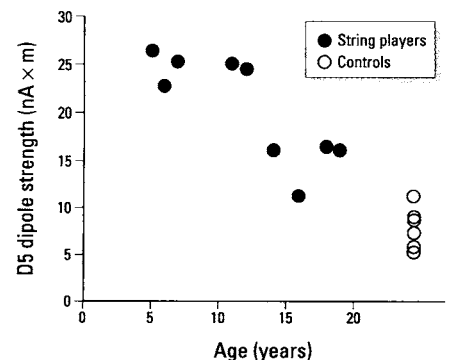

Figure 11. Center of cortical responsivity for tactile stimulation to the fifth (little) finger of the left hand in string players and controls, as measured by MRI. String players had an expansion of the cortical area devoted to the hand area that was correlated with the age at inception of musical practice but not the amount of practice. Adapted from Elbert et al. (203).

"coming online" for attention to and integration of sensory information, followed by the ability to cease to attend to irrelevant stimuli. The ontogeny of specific tasks that rely on hippocampal function and integration with other neural circuits has also been a specific focus for research in the rat (Table 1) (239).

A number of tasks may be used in both animals and humans to assess cognitive function. Although some of these are used mostly with monkeys, a number of them can also be used with rodents. Simple visual discrimination tasks, in which the subject must learn which of two stimuli is correct, can be used with many species. An additional requirement that is often imposed is termed discrimination reversal. After the subject learns the task to some predetermined criterion, the positive

(correct) stimulus becomes the negative one, and vice versa. This requirement is often more sensitive to disruption by exposure to a toxicant than is the initial acquisition of the discrimination task. Developmental lead exposure produces impaired performance on discrimination reversal tasks in rats (240), monkeys (241), and children (242). Another task that has proved sensitive to developmental neurotoxicant exposure is the delayed alternation task, in which the subject is required to simply alternate responses between two positions on successive trials to be reinforced. Delays between opportunities to respond may be imposed to assess short-term spatial memory. Performance on this task has proved sensitive to developmental lead exposure in rats (243) and monkeys (244,245). Developmental PCB

exposure also produces impaired performance in both species (246,247).

The delayed response (DR) task used with monkeys and infant humans is a variant of the delayed alternation task. In this task, the subject watches one of two positions being baited (with food or a preferred toy, for example) and then a delay is interposed during which the subject is prevented from viewing the stimuli. After the delay, a correct response (reach) results in attainment of the reward. As long as the subject's responses are correct, the correct stimulus alternates between two possible positions from trial to trial. In the typical DR task, the correct position continues to alternate irrespective of whether a particular response is correct. Thus, a subject could always respond at one position, left or right, and be correct 50% of the time. In an alternative version of the task, incorrect responses are "corrected"; i.e., the correct position remains on the same side until the subject responds on that side. This task has been much studied in human infants, and is referred to as A not B. Delayed alternation can also be tested as a corrected or noncorrected task.
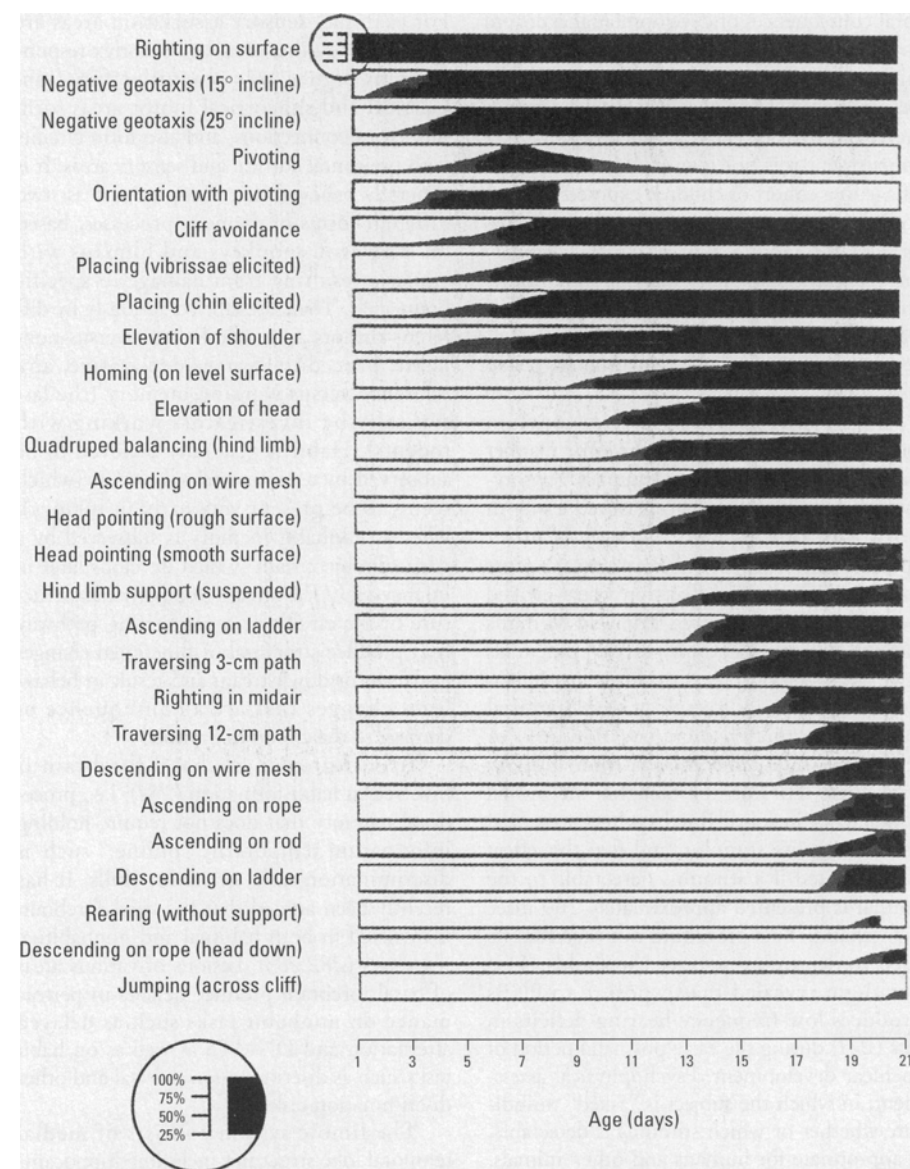

Figure 13. Ontogeny of motor activity and exploratory behavior in rats. Locomotion was measured by crossing of photocell beams in an area 36 × 36 cm. Exploration was measured by number of nose pokes into holes in the floor of the same apparatus. Activity patterns display a complex ontogenetic development, with exploratory and locomotor activity being more or less inversely related. Adapted from Bâ and Seri (235) and reprinted with permission of Elsevier Science Ltd.

Table 1. Timetable of hippocampally mediated functions in rats.

Behavioral tasks	Emergence of adult levels of proficiency on PND
Partial reinforcement inhibition	12–14
Olfactory reversal	18
Morris water maze	21–23
Delayed alternation	21–27
Spatial navigation	22–25
Pavlovian trace fear conditioning	25
Habituation to novel stimuli	25–30
Passive avoidance	25–30
Spontaneous alternation	27–30
Latent inhibition	32

Adapted from Bachevalier and Beauregard (239).

Figure 12. Summary diagram of the emergence of different postural, locomotor, and other related skills in rats. In the majority of instances performances levels (25, 50, 75, and 100 percent) refers to the percentage of rats successful in the display of the response. In a few instances the reference is to a level of performance with respect to asymptotic response frequency. Redrawn from Altman and Sudarshan (236) and reprinted with permission of Academic Press, Ltd.
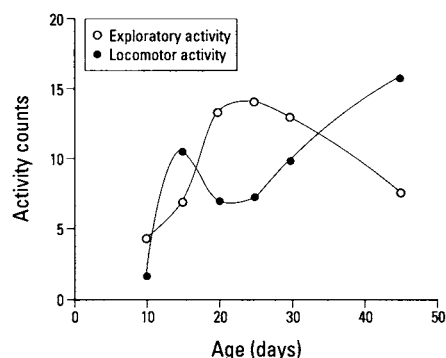
Tasks that have been used extensively in monkeys are DMTS and delayed nonmatching to sample (DNMTS). These tasks are also suitable for use with rats and have been used to study the ontogeny of specific brain regions in human infants. In the DMTS, the subject is presented with a stimulus, which is then withdrawn. After a delay, which may vary from trial to trial, the subject is presented with the previous stimulus plus one or more additional stimuli and must choose (match) the previously presented stimulus. In the DNMTS, the subject must choose the novel (nonmatch) stimulus from a pair of stimuli. The DNMTS is typically easier to learn because test subjects prefer a novel to a familiar stimulus. This task has been used in both children (227) and monkeys [reviewed by Webster et al. (248) and Murray and Mishkin (249)]. The DMTS task has proved sensitive to disruption by developmental lead exposure in monkeys (250).

Intermittent schedules of reinforcement have been used extensively in behavioral pharmacology and toxicology to assess the effects of environmental agents on behavioral function (251). On an intermittent schedule of reinforcement, the subject is reinforced according to a set of rules: for example, after emitting a certain number of responses (fixed ratio), or for the first response after a specified period of time that remains constant from one reinforcement opportunity to the next [fixed interval (FI)]. Although only one response at the end of the interval is required for reinforcement, FI performance is typically characterized by a gradually accelerating rate of response terminating in reinforcement; both the temporal pattern of responding and the rate of response may be modified by exposure to a toxicant. The FI schedule has proved particularly sensitive to developmental exposure to such agents as lead, PCBs, methyl mercury, and pesticides in both rats and monkeys (252–260). Performance on other intermittent schedules has also proved sensitive to disruption by developmental toxicant exposure (261).

An issue that is of particular importance in the design of animal behavioral studies is whether each task is assessed independently in experimentally naive animals or whether a series of tasks is presented sequentially to the same subjects. Children are of course required to build knowledge and skills sequentially, necessitating transfer of learning in virtually all aspects of life. In contrast, many developmental toxicology studies of learned behavior in rodents use a separate set of subjects for each task or the same set of animals for at most a couple of tasks. Primate research, in contrast, necessitates testing the same cohort of subjects on many tasks to maximally utilize an expensive and valuable resource. Whether

failure to assess cumulative learning misses an important aspect of childhood experience is an important question. In a direct comparison using a rat model of maternal phenylketonuria, it was determined that the requirement for cumulative learning on a series of related tasks revealed treatment-related deficits that were absent when each task was assessed in a naive group of animals (262). This is consistent with the finding that one of the characteristics of humans with low IQs is an impairment in the ability to transfer learning from one situation to another (263). Therefore, low performance on an IQ test may be viewed as reflecting an accumulated deficit as a result of years of failure to transfer and integrate previous experiences. This issue is also relevant to the findings from longitudinal prospective studies assessing the behavioral consequences of developmental exposure to toxicants. For example, the apparent cognitive decrement produced by lead may become more apparent as the child gets older [reviewed by Rice (264)]. Similarly, cognitive impairment was not identified at 18 months of age in a cohort of children exposed in utero to PCBs (265) but was manifest at 42 months in the same cohort (266). Some of the apparent increased sensitivity undoubtedly results from the more informative assessments possible as the child develops; however, an inability to build on previous learning may also play a part.

Assessment of sensory system function may be performed in much the same manner in humans and experimental animals. Sensory-evoked potentials can be measured easily in both rats and humans, as well as other species. Exposure to environmental agents may produce similar effects on visual-evoked potentials in pigmented rats and humans (267). Changes in auditory-evoked potentials have been demonstrated in different populations of children as a result of developmental exposure to methyl mercury (268,269) or lead (270). The reflex modification auditory procedure relies on the fact that an animal will startle (jump or flinch) when presented with an intense stimulus, and that this effect is attenuated if a stimulus detectable to the animal is presented approximately 100 msec beforehand. This procedure can therefore be used to determine sensory thresholds. This paradigm revealed that exposure to PCBs produces low-frequency hearing deficits in rats (271) during the early postnatal period of cochlear development. Psychophysical assessment, in which the subject is "asked" to indicate whether or which stimulus is detectable, is appropriate for humans and other animals. Such procedures have been used to assess function in visual, auditory, or somatosensory systems in rats, monkeys, and humans after toxicant exposure (244,272–275).

Neurobiological Substrates of Function

All of the major brain structures in humans are also present in rodents and subserve roughly the same functions. The brain stem is mostly responsible for autonomic functions, arousal, and sleep–wake cycles. Some mesencephalic structures may also be involved in cognitive processes in addition to attentional ones (276,277). The cerebellum subserves motor function and motor learning (278); its role in cognitive processes has only recently been characterized (279,280). Specific areas of the basal forebrain are involved in motor function as well as some types of learning.

It must be borne in mind that all brain areas have multiple reciprocal connections, forming circuits subserving various functions. For example, sensory association areas are connected to other areas of neocortex responsible for higher order cognitive function. Cortical and subcortical motor areas form numerous connections, and also form circuits with prefrontal cortical and sensory areas. It is generally believed that there are at least two different forms of memory processes, based on studies in monkeys and humans with amnesia resulting from damage to specific brain areas. These are labeled variously by different authors and include habit versus nonhabit, procedural versus declarative, and reference versus working memory (the last typically by investigators working with rodents). Habit is generally believed to be subserved by a corticostriatal circuit (which seems to be present very early in infancy), whereas nonhabit memory is subserved by a corticolimbic circuit [which develops later in infancy (239)]. Damage to a particular structure in the circuit or a connecting pathway may produce structural or functional changes upstream or downstream and result in behavioral changes that are a consequence of damage to the circuit as a whole.

It is clear that the basal forebrain is involved in habit formation (281): i.e., procedural memory that does not require holding information temporarily "online," such as discrimination tasks or motor skills. It has recently been argued that the basal forebrain is involved in both habitual and nonhabitual responses (282,283). Lesions of various areas of basal forebrain produce deficits in performance on nonhabit tasks such as delayed alternation and DNMTS as well as on habit tasks such as discrimination reversal and other discrimination tasks.

The limbic system consists of medial temporal lobe structures including hippocampus, amygdala, and surrounding cortical structures. Researchers using the rodent as the experimental subject have probably studied the function of the hippocampus more than

any other brain structure involved in learning and memory, perhaps because the hippocampus in the rodent is relatively large and easy to identify cytoarchitectonically. The hippocampus is involved in spatial memory in both rodents and primates and is also critical for very short-term memory storage, at least in monkeys (284). It is unclear whether the deficits observed on such nonhabit tasks as delayed alternation (DA), DR, and DNMTS are the result of lesions of only the hippocampus, the hippocampus plus amygdala, and/or the surrounding entorhinal and perirhinal cortex (281,285–288).

The neocortex is divided into areas subserving a number of functions in both rats and primates, including humans [Figure 14, (289,290)]. Specific areas are devoted to motor function and the various sensory systems, including visual, auditory, and various somatosensory modalities. These functional areas are further subdivided into primary cortex, which receives input directly from the corresponding thalamic nucleus, and various areas upstream that are responsible for integration and higher order processing. This explanation is a simplification: there is some thalamic input to nonprimary sensory cortex as well, but the bulk of thalamic input is to primary cortical areas. Perhaps the biggest difference between rodents and primates (including humans) in terms of sensory system structure and function is the fact that primates are highly visual animals, whereas the rodent relies heavily on auditory and olfactory cues (as well as tactile cues from the vibrissae) for information about the world. Consequently, approximately half of the primate brain is devoted to visual areas, including higher order processing and integration areas. In contrast, only a small part of rodent cortex is devoted to vision. The visual function of primates is also more highly developed than that of the rodent, with excellent color discrimination and spatial resolution, of which the rodent has neither [Figure 15 (291)]. The rodent therefore has considerable limitations with respect to utility for the assessment of visual function, although there are certain aspects of visual structure and function shared between the species (e.g., low-light-sensitive rod-mediated visual pathways). The presumed relative unimportance of visual information compared to information from other sensory modalities in rodents also has implications for testing paradigms: the stimuli used for assessing cognitive function in rodents are typically visual stimuli, probably in part because they are easy to control and in part because we are visual animals. It would seem preferable to use olfactory cues when assessing cognitive abilities in rodents (262). It should also be emphasized that both the visual and auditory systems of albino animals of all species are abnormal

(e.g., Figure 15); therefore, albino rats or mice are a poor choice for assessment when the nervous system is of interest.

Undoubtedly, the most obvious and important difference between the rodent and human brain, structurally and functionally, is the tremendous relative size and functional importance of the prefrontal cortex (PFC) in humans. Homologous areas of PFC between species are typically defined by projections from specific thalamic nuclei. By that criterion, the rat has a defined PFC that may be subdivided into a number of areas (292). The primate cortex, including that of the human, is also divided structurally and functionally into a number of specific areas (293,294). The PFC in primates is considered necessary for so-called executive functions (283,295,296), including organization of behavior in time and space, planning, inhibition of inappropriate actions (impulse control), ability to change strategy (set-shift), and emotional control. It has been suggested that attention deficit hyperactivity disorder is largely a dysfunction of prefrontal cortical function (297). It has also been postulated that damage to prefrontal cortical areas underlies the behavioral impairment produced by developmental lead exposure (298). Lesions of various specific areas of PFC in monkeys produce deficits in DA and DR, DMTS and DNMTS, and discrimination tasks. Such lesions also result in impairment in

the ability to inhibit inappropriate responding, perseveration, and distractibility. At least some of these same functions (e.g., inappropriate responding and perseveration) are impaired after PFC lesions in rats (299). However, behavioral toxicologists using rats as the experimental model have generally not focused on PFC as a possible site of toxicant-induced damage.

An approach that has proved extremely fruitful in the study of the development of cognitive processes and the brain structures subserving these processes in human infants is the assessment of infants on tests used in adult and infant monkeys, and for which the brain structures and circuits necessary for performance of the specific tasks have been delineated. One brain area that has received a considerable amount of attention with respect to ontogenetic development is the PFC. Lesion studies in monkeys have identified a number of tasks that are heavily dependent on various areas of prefrontal cortex, including DR, A not B, DA, and DMTS and DNMTS (300–302). Both infant humans and infant monkeys develop the ability to perform these tasks relatively late: over the course of the first year of life in monkeys (303,304) and over the first 3 years or so in humans (305,306), thereby suggesting a relatively long maturation time for prefrontal cortex. In fact, development of PFC may
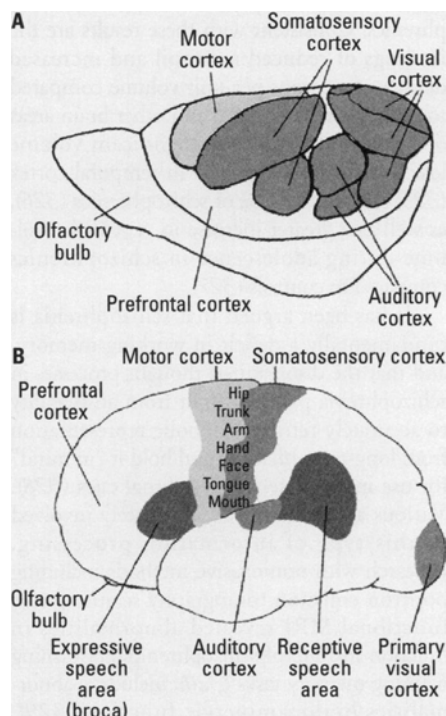

Figure 14. Lateral view of functional areas of the (A) rodent and (B) human cerebral cortex. The lateral view of the rodent neocortex is redrawn from Kolb and Tees (289) and the lateral view of the human neocortex is redrawn from Heimer (290). Reprinted with permission of Springer-Verlag.
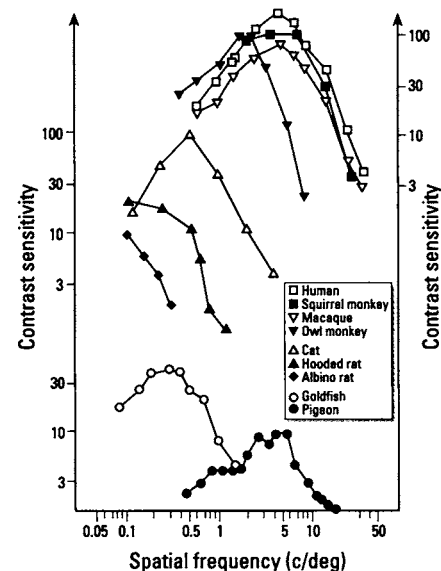
Figure 15. Comparison of spatial visual function in various species. Abscissa: spatial frequency. Ordinate: contrast sensitivity = reciprocal of contrast, which is a function of the difference between the light and dark bars of a series of parallel bars. The visual function of the albino rat is least like that of humans (note three scales of the ordinate), whereas the contrast sensitivity of many nonhuman primates are virtually identical to humans. Adapted from Uhlrich et al. (291) and reprinted with permission of The MIT Press and Elsevier Science B.V.

continue beyond puberty in both species. In contrast to performance on tasks dependent on PFC, habit formation tasks such as discrimination tasks, which are dependent on corticostriatal circuits, are performed at adult levels of competence in both monkeys and humans during early infancy (*304,307,308*).

Another brain circuit for which ontogeny has been studied is the limbic system, including hippocampus, amygdala, and surrounding entorhinal and perirhinal cortex. The hippocampus develops perinatally in the rat but prenatally in humans or monkeys during the last trimester. For example, 15% of cells in the dentate gyrus of a rat are present by birth, compared to 80% of the cells present in the monkey at birth (*308*). In humans, the volume of the hippocampus is fully mature at 15 months, whereas the PFC does not reach adult volume until 10 years of age. Preferential-looking performance is developed at 4 months of age in humans and 2 weeks of age in monkeys (*307,308*). Lesions of the hippocampus result in impaired performance on this task, evidence that this structure matures early in primates (*309*). Lesions of hippocampus in young monkeys also result in deficient social interactions early in life, which become more severe during adulthood (*310*). There is evidence of differences between the sexes in the development of different brain areas as revealed by differential performance on specific behavior tasks in young monkeys (*311–313*) and humans (*312*) that are not present later.

Clinical Consequences of Errors in Developmental Processes

It is well established that being small for gestational age (SGA) is a risk factor for behavioral and other health problems. For example, in a prospective cohort study of 1,037 children, SGA children scored lower on tests of intelligence when tested repeatedly between 3 and 13 years of age (*314,315*). Parents reported more behavioral problems at 15 years of age in the SGA group (*315*). Being SGA presumably results from a suboptimum prenatal environment, and may be an indication of poor overall nutrition, specific nutritional deficiencies, or other factors including exposure to a toxicant. Neurotoxic agents including alcohol, maternal cigarette smoking, PCBs, and perhaps lead all may produce intrauterine growth retardation. It has been argued, at least with respect to lead, that controlling this factor in the statistical analysis represents over-controlling. Prematurity may also produce retarded development. In a study following a group of children from birth to 5 years of age (*316*), premature infants initially lagged behind full-term infants on age-appropriate measures including the Bayley Scales, Stanford-Binet, and

MSCA, even when results were age-corrected. By 5 years of age, the premature infants had caught up on most, but not all, measures.

Functional abnormalities of the nervous system and their underlying structural and neurochemical abnormalities may provide important information relevant to normal development, as well as perturbations that may be produced by neurotoxic agents. Schizophrenia is a devastating mental illness that probably has both genetic and environmental causes. Although the identical twin of a person with schizophrenia has a greatly increased risk of developing the disease, the risk has an odds ratio < 1.0, indicating that environmental influences also play a part in the etiology. For example, obstetric complications or maternal influenza during pregnancy are associated with an increased risk of schizophrenia (*317–319*).

There is compelling evidence that schizophrenia is a consequence of errors in developmental processes (*320*) based on the types of abnormalities observed. Many major psychoses, including schizophrenia, become manifest at the end of adolescence and in early adulthood. The period of adolescence is a time of high synaptic density in all higher primates, including humans (*321*) (Figure 16). Thereafter synaptic density decreases in all areas of cortex. Recent studies using MRI (*322*) suggest excessive elimination of dendrites and axons of prefrontal cortex of schizophrenics. Consistent with these results are the findings of reduced neuropil and increased density of neurons per unit volume compared to controls in PFC (*323*) and other brain areas of schizophrenics (*324*). Significant volume loss has also been reported in temporal cortex (*325*) and frontal lobe of schizophrenics (*326*), as well as a greater increase in ventricular volume during adolescence in schizophrenics compared to controls (*327*).

It has been argued that schizophrenia is fundamentally a deficit in working memory, and that the disorganized thought processes in schizophrenia patients result from an inability to accurately retrieve symbolic representation from long-term memory and hold it "in mind" for use in the absence of external cues (*328*). Various areas of PFC are intimately involved in this type of information processing. Research with noninvasive methods including positron emission tomography scanning and functional MRI revealed abnormalities in function in PFC of schizophrenics performing working memory tasks (*320*), including abnormalities in dopaminergic function (*329*). Alternatively, it has been argued that abnormalities of medial temporal lobe, particularly hippocampus, are intimately involved in the pathogenesis of schizophrenia (*330*).

Dyslexia is another mental disability in which specific abnormalities of development

are observed. Dyslexia is associated with neuronal ectopias and architectonic dysplasias in various areas of the cerebral cortex (*331,332*) as well as other structural abnormalities. In addition, people with dyslexia have a lack of asymmetry in the planum temporale, a language region on the superior surface of the temporal lobe that is asymmetrical in nondyslexic individuals (*331*). Dyslexics are also more likely to have autoimmune disorders and an increased incidence of left-handedness (*333*). Autoimmune strains of mice have been developed that also have cerebral ectopias and aberrant cortical lateralization (*334,335*), which are of genetic origin. These mice also show differences in various tests of spatial learning, which may be modified by environmental enrichment (*336*).

Epilepsy is a nervous system abnormality that in some cases has a developmental etiology. A large program studying the causes of epilepsy has been ongoing in Rochester, Minnesota, since 1975 (*337*). From that large database it is estimated that approximately 5% of epilepsies have a developmental origin. One-third of children with severe mental retardation or cerebral palsy (CP) also have epilepsy. Perinatal events such as repeated or prolonged febrile seizures may result in epilepsy, and there is evidence for genetic causes for epilepsy in some families. CP is associated with abnormalities of pregnancy and birth, particularly birth asphyxia and low birth weight (prematurity) (*338*). Cranial ultrasound
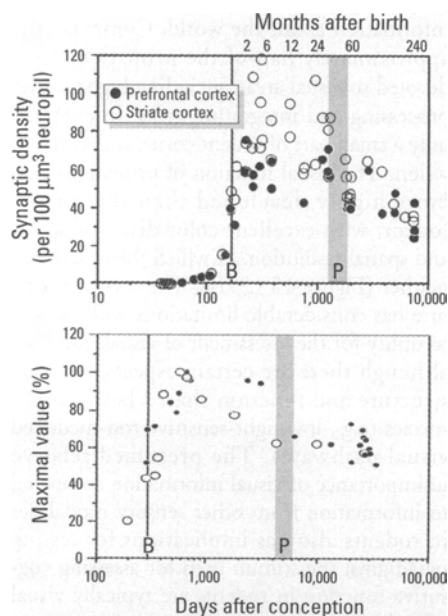

Figure 16. Developmental ontogeny of synaptic density for monkey (top) and human primary visual cortex (area 17 or striate cortex) or prefrontal cortex (area 46 in monkeys). Abbreviations: B, birth; P, puberty. Data in the bottom panel are normalized as a percentage of the maximal value of the curve. Adapted from Rakic et al. (*321*) and reprinted with permission of Elsevier Science B.V.

imaging in preterm infants may reveal patterns of neonatal brain damage that are highly predictive of later CP: specifically, echodense regions that presumably reflect ischemic or infarctive lesions of the white matter. CP may in some instances result from disorders of neuronal migration (339,340) or periventricular white matter damage that may arise during weeks 28–35 of gestation (339,340).

Autism is a developmental abnormality that is characterized by impairment of social interaction, limited activities or interests, and deficiency or abnormality of speech development. Autism occurs with a prevalence of 1.3 per 1,000 births (341). Although there is a strong genetic factor in autism, it is not heritable in a simple fashion, and environmental factors also play a role. In an insightful piece of detective work, Strömland et al. (342) observed that in the Swedish thalidomide registry, there were several patients with autism who had all been exposed to thalidomide between GD 20 and 24. This time period corresponds with closure of the neural tube and production of the first neurons that form the motor nuclei of the cranial nerves. Moreover, these individuals also had ear malformations and hearing deficits, providing further evidence for damage in this short window during gestation. Rodier et al. (343) reproduced the major features of the neuropathological syndrome of autism using valproic acid in mice because mice are not sensitive to thalidomide-induced teratogenicity. These abnormalities are also observed in mice in which the hox-A1 gene is lacking (344–346). The hox-A1 gene is present in humans (347), although the consequences of alterations in expression and/or signaling through either genetic mutations or exposure to environmental agents is an area in need of further research.

Long-Latency Delayed Neurotoxicity

An issue that is of considerable concern to neurotoxicologists, and which has great relevance to risk assessment, is the potential for agents to induce delayed neurotoxicity years after cessation of exposure, including exposure during development. The possibility of delayed neurotoxicity stems from at least two scenarios. In one scenario, the ontogeny of a particular function occurs late in development and the manifestation of pathological changes is not revealed until the function in normal animals is apparent. A second scenario involves a developmental insult in which both anatomical and/or functional effects may be masked or attenuated due to neuronal compensation or plasticity, resulting in apparently transient effects that may nonetheless have later sequelae. The possibility of an interaction between aging and exposure to neurotoxic agents is also of critical

concern: this possibility was postulated 2 decades ago (348) and has been raised repeatedly since (349,350). As the normal brain ages, there is a decrease in the number of cells in certain regions, as well as a decline in neurotransmitter levels and repair mechanisms. If this process were accelerated by chronic or historic exposure to a neurotoxicant, the effect as the individual aged would be a further decrease in functional capacity from that typically observed during aging. Alternatively, damage that decreased the reserve capacity of the brain at any point in life might also hasten the appearance of functional deficits.

This phenomenon has been demonstrated in both animal models and in humans. In rodents, latent neurotoxicity has been demonstrated after developmental exposure to X-ray irradiation (351), methyl mercury (352), and triethyltin (73). Developmental exposure to triethyltin produced damage to cortical structures of the nervous system and transient deficits in spatial learning. Apparent compensation at the anatomical level was evident, with some reactive synaptogenesis from surviving cells in the entorhinal cortex and a recovery from spatial learning deficits during adulthood. However, when littermates were examined morphologically and functionally during aging, decreases in synaptic densities and deficits in spatial learning became apparent, providing empirical evidence for accelerated aging as a result of developmental exposure (73).

There is both epidemiological and experimental evidence that exposure to the environmental contaminant methyl mercury can produce delayed neurotoxicity (353). Methyl mercury toxicity is largely characterized by abnormalities in motor function and impairment in the visual, auditory, and somatosensory systems. As early as 1975 it was recognized that manifestations of Minamata disease (MD) could worsen over time even after cessation of exposure to methyl mercury (354,355). A study in Japan including > 90% of persons diagnosed with MD and age-matched controls identified deficits in the ability of persons with MD to independently eat, bathe, use the toilet, dress, and wash their faces (356). The relative deficit between controls and people with MD increased with age in a statistically significant manner even though exposure to methyl mercury had ceased in all individuals 20–30 years before assessment.

Delayed neurotoxicity has also been observed in monkeys exposed developmentally to methyl mercury. Monkeys exposed to methyl mercury from birth to early adulthood developed clumsiness beginning in middle age (357), which was probably a consequence of somatosensory dysfunction (358). This cohort of monkeys, as well as a cohort exposed to methyl mercury in utero

through puberty, developed deficits in fine motor control during middle age (353). The group of monkeys exposed in utero through puberty displayed exacerbation of auditory impairment as they aged (359). Individuals in both cohorts developed constriction of visual fields during old age that had not been present when they were younger (360).

An interaction between aging and neurological disease during childhood has also been observed. It has become apparent over the last decade that up to 25% of people who had contracted polio as children had apparently recovered and were functioning normally, then suffered a recurrence of clinical symptoms as they moved into middle age. Evidence of degeneration in peripheral nerves of these individuals was detected; this may reflect premature senescence of nerves compromised during the acute phase of the disease, or accelerated aging produced by increased metabolic demands on spared normal nerves. Exposure to the polio virus during childhood may also result in the development of motor neuron disease decades later (361).

The potential consequences of an interaction of developmental exposure to a neurotoxic agent and acceleration of the aging process are profound. As the large cohort of baby boomers ages, the costs of neurological or psychological dysfunction will be significant, and any added increase in dysfunction as a consequence of exposure to neurotoxic agents may be expected to place additional burdens on the cost and infrastructure of the health care system.

Antisocial Behavior Associated with Early Neurotoxic Exposure

Another form of latent consequences after developmental exposure to neurotoxic agents is exemplified by magnification of early deficits with maturation to adulthood. There is increasing evidence linking aggressive and criminal behavior with decreased IQ (362,363) as well as with other deficits typical of exposure to neurotoxicants, such as impulsivity and poor executive control. In a study of aggressive and nonaggressive adolescent boys, the best predictor of aggressive behavior was deficits in executive function (364). Children diagnosed with hyperactivity disorders have increased incidence of depression and aggression at 17–18 years of age (365), whereas individuals with various psychiatric disorders are at increased risk for criminal behavior (366). There is also increasingly compelling evidence for an association between aggressive behavior during childhood and later increased criminality in males, but not females, as a result of developmental exposure to neurotoxicants. Determination of such long-term effects requires long-term follow-up of cohorts in which exposure during development was

determined. Three toxicants have been studied long enough to perform such assessments: lead, alcohol, and cigarettes. Exposure resulting in an increased body burden of lead was largely postnatal, whereas maternal alcohol consumption or cigarette smoking resulted in prenatal exposure to the fetus. All three agents produce deficits in IQ, attention problems, increased impulsivity, and deficits of executive function. All three are also associated with increased criminality during adulthood as a result of developmental exposure.

Consequences of Developmental Lead Exposure

An association between lead exposure and antisocial and other problem behaviors has been identified in children as young as 2–5 years of age (367,368). In a study in children 8 years of age, tooth dentine lead levels were associated with total scores of problem behavior on the Teachers Report Form of the Child Behavior Profile (369). Tooth lead levels were also associated with internalizing (consisting of scores for anxiety or withdrawal) and externalizing (consisting of inattentive, nervous-overactive, and aggressive scores). There was also a weak association between tooth lead levels and prevalence of extreme problem behavior scores.

The consequences in older children of this poor social adjustment were explored in a retrospective cohort study of the association between bone lead levels and measures of social adjustment in boys at 7 and again at 11 years of age (370). At 7 years of age, borderline associations after adjusting for covariates were observed between teachers' ratings and lead levels for aggression, delinquency, social problems, and externalizing behaviors on the Child Behavior Checklist (CBCL). When these children were 11 years of age, parents of subjects with higher lead levels reported significantly more somatic complaints, more delinquent and aggressive behavior, and higher internalizing and externalizing scores. Teachers' ratings of children at 11 years of age were associated with bone lead levels for a number of categories, including somatic complaints, anxious/depressed, social and attention problems, delinquent and aggressive behaviors, and internalizing and externalizing. Subjects with higher lead levels had higher scores in self-reports of delinquency at 11 years of age. Higher bone lead levels were associated with an increased risk of exceeding the clinical score for impaired attention, aggression, and delinquency, and these subjects were more likely to have worse scores on all items of the CBCL during the 4-year period of observation.

Consequences of Maternal Cigarette Smoking

A relatively large literature addresses the long-term consequences of maternal smoking

during pregnancy in the offspring, which have revealed impairments after control of potential confounding variables. Maternal smoking during pregnancy results in decreased birth weight (371,372), which is itself a risk factor for later behavioral and other health problems. It is therefore not surprising that maternal smoking results in deficits in IQ in the offspring (373,374). Maternal smoking was also associated with increased aggressive behavior, as well as oppositional and overactive behavior in 1,377 twin pairs 3 years of age (375). Maternal cigarette smoking was associated with increased impulsivity (commission errors) on a continuous performance vigilance task in children 6 years of age (376). Fergusson et al. (377) observed a correlation between maternal cigarette smoking and an increased incidence of conduct disorders and depression in adolescents 16–18 years of age. Other studies have also found an association between antisocial behavior and maternal cigarette smoking in middle childhood (378–380) and adolescence (381–383). Finally, two recent studies identified a dose–effect relationship between maternal cigarette smoking and persistent criminal behavior. In one study (384), an increase in persistent criminal behavior was observed by 34 years of age in a cohort of 4,169 males, as ascertained from the Danish National Criminal Register, with effects observed with as few as 1–2 cigarettes per day smoked by the mother. In a similar study in Finland (385), a cohort of 5,636 males was followed prospectively from 6 months in utero to 28 years of age. Sons of mothers who smoked had a 2-fold (adjusted) increased risk for violent or repeated crimes but not nonviolent crimes. As pointed out by Fergusson (386), these effects of maternal cigarette smoking, observed in numerous studies, are resilient to control for confounding variables associated with cigarette smoking. However, it is interesting to note that in the Räsänen et al. (385) study, maternal smoking combined with risk factors including teenage pregnancy, single-parent family, and unwanted pregnancy increased the odds ratio 9-fold for violent crime and 14-fold for repeated crime.

Consequences of *in Utero* Exposure to Ethanol

In 1973 Jones and Smith (387) provided the first description in the English literature of a distinctive pattern of abnormalities in babies born to alcoholic mothers, which they named fetal alcohol syndrome (FAS). The faces of the children with FAS have a recognizable constellation of abnormalities, including short palpebral fissures, a flat midface, indistinct philtrum and thin upper lip, a low nasal bridge, short nose, micrognathia, and minor ear abnormalities, and often also include epicanthal folds (388). These children are also small for their age and may have limb and

cardiac abnormalities. Individuals with FAS have severe dysmorphogenesis of the brain accompanied by characteristic behavioral problems, including lowered IQ and hyperactivity (389). A continuum of effects in humans is evident as a consequence of *in utero* alcohol exposure from relatively mild to severe, with individuals displaying varying degrees of the behavioral and physical anomalies associated with FAS. Children who have some but not all of the features of FAS are referred to as having fetal alcohol effects (FAE). These effects of prenatal exposure to ethanol have been replicated in animal models (390).

A longitudinal study on the effects of *in utero* exposure to alcohol has been ongoing in Seattle, Washington, since 1974–1975, at which time pregnant women were recruited into the study and questioned about their alcohol intake. Offspring of mothers consuming on average one drink per day exhibited neurological deficits as infants and deficits in memory, IQ, and school achievement, as well as attention deficits, increased impulsive behavior, and lack of cooperation (391–393). At 14 years of age, children in this cohort whose mothers drank an average of one drink per day during pregnancy still exhibited increased behavior/learning deficits (394). Individuals aged 16–27 with IQ scores in the normal range also exhibited impairment in tests of attention, memory, and executive function (395). Binge drinking (fewer than two drinks/occasion) by the mothers resulted in a profile of adolescent antisocial behavior (394).

The consequences of *in utero* exposure to alcohol are associated with other behaviors that make it difficult for an individual to integrate successfully into society. Further studies with persons with FAS/FAE identified a 50–60% incidence of mental health problems, trouble with the law, and inappropriate sexual behavior (389,396). In this group of adolescents, prenatal alcohol exposure was predictive of alcohol use, whereas living in an alcoholic family was not (397). In the Seattle cohort, 30 women with FAS/FAE had given birth to a child by 1997. Of these, 57% no longer had the child in their care, 40% drank during pregnancy, 17% had children with FAS/FAE, and an additional 13% had children with suspected FAS/FAE. These long-term effects of FAE represent a human tragedy of epic proportions, particularly because the estimate of full-blown FAS is 2.7–4.6 per 1,000 live births in the United States (388). The financial and social costs are enormous.

Monetary Consequences of a "Small" Effect on Cognition

"Small" functional effects often stimulate intense debate in the risk assessment community. A decrement in function that is still considered to be in the normal range may

nonetheless have important consequences for a society, particularly if a large segment of the society is exposed. It is becoming increasingly well established that a common effect exposure to developmental neurotoxicants is a small decrement in IQ. The consequences of a shift in the IQ distribution have been discussed (398,399). Even a small shift to the left in the normal distribution has a large effect on the tails of the distribution. For example, a shift in the IQ of the population by 5 points results in a doubling of the number of individuals with IQs < 70 who would require greater educational resources in addition to other social supports. There would be a concomitant decrease in individuals with IQs > 130 by a factor of 2.5. There is also considerable evidence that there is a high correlation among IQ, education, and lifetime earnings. One of the most powerful and extensive databases is the government-funded National Longitudinal Survey of Youth (NLSY) (400), which has followed more than 12,000 young people from 3,000 households, now in their 30s, since 1979. Murray (401) used the NLSY database to calculate the consequences of childhood IQ to later earnings. He identified 710 sibling pairs in which one had a typical IQ (normal, 90–110) and one had an IQ either > 110 (bright) or < 90 (dull). He found that higher IQ was associated with higher incomes (Figure 17), more education, fewer total offspring, and

Figure 17. Earnings of paired siblings in which one sibling was labeled "normal" (IQ 90–110) and the other was either "bright" (IQ > 110) or "dull" (IQ < 90), based on the National Longitudinal Study of Youth (400). Adapted from Murray (401).

fewer offspring outside marriage. Whatever the cause of the IQ decrement (genetic and/or environmental, with exposure to a neurotoxic agent potentially contributing to both categories), the consequences have important implications for society.

The monetary cost associated with the ubiquitous exposure of fetuses and children to lead in industrialized societies has been calculated by Schwartz (402) in an estimation of the benefits of a 1-μg/dL reduction in the population mean blood lead concentration. The analysis was based on the monetary savings of reducing lead levels in children with blood lead concentrations between 10 and 20 μg/dL. Note that a 1-μg/dL reduction in blood lead concentrations would result in a shift in the population of < 1 IQ point based on meta-analyses (403,404). Schwartz (402) estimated medical costs associated with treatment of children with undue lead exposure, the increase in remedial education, and the costs associated with reduced birth weight and reduced gestational age, among other factors. The largest single cost was lost earnings as a result of decreased intellectual capability: $5.06 billion in 1994 dollars. The total cost, including increased medical care, compensatory education, and neonatal mortality and morbidity, was $6.94 billion 1994 dollars. In a later similar analysis using the NLSY database (400) to monetize the effect of decreased cognitive ability on earning capacity, the estimated gain in earnings was $7.5 billion U.S. per year for a decrease in blood lead levels of 1 μg/dL in the U.S. population (405). It is obvious that the small effect of lead on IQ is reflected in an enormous cost to society in lost potential and increased need for medical care and special education.

A similar analysis has been performed with respect to the societal cost of *in utero* exposure to cocaine (406). It is estimated that between 45,000 and 875,000 cocaine-exposed children are born in the United States each year. Lester et al. (406) performed a meta-analysis of studies of school-age children and calculated the effects of cocaine exposure on IQ and receptive and expressive language abilities

(Table 2). The meta-analysis estimated a 3.26-point drop in IQ as a result of prenatal exposure, which would increase by 1.6 times the number of children with IQs < 70 (2 SDs below the mean IQ) for a cost of between $4 and 35 million dollars/year for special education, whereas children with an IQ of < 78 (1.5 SD below the mean) would cost between $10 and 80 million/year. The effect on language is even greater than that on IQ (as measured by effect size) and would result in additional total costs of $33–272 million/year. So, although the early concerns that cocaine-exposed babies would suffer from overt brain damage and intellectual impairment never materialized, the monetary cost of the more subtle impairment identified in these children is still enormous based on increased needs for educational intervention alone. Lester et al. (406) did not estimate costs of attentional, behavioral, or emotional problems, the cost of decreased earnings, or the possibility of antisocial behavior during adulthood.

Summary

It is important to reiterate that the nervous system develops over a very long period of time extending from the embryonic period through puberty, with both synaptogenesis and myelination continuing through puberty in both animals and humans. Even during adulthood there is continued recapitulation of synaptogenesis in the form of synaptic plasticity. This plasticity is modified by experience and environmental stimuli and continues to play a role in synaptic remodeling in both the developing and adult nervous system and is believed to underlie the efficacy and strength of synaptic transmission. Many agents can cause developmental neurotoxicity as a result of alterations in the developmental processes we have reviewed in this article. The actual mechanisms by which these processes are perturbed by vastly different chemical classes is an enigma and a continued research question. However, identification of perturbations of these developmental processes by xenobiotics and clinical disorders has led us to the recognition that a developmental insult can initiate
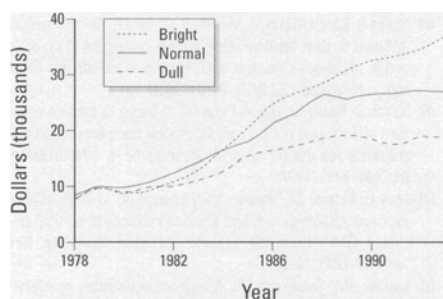
Table 2. Societal burden of prenatal cocaine exposure.

Measure	Effect (SE)	Percent < 2 SD (normal = 2.28%)	Increase	Additional affected children per year (n)	Additional cost for services (millions)	Percent < 1.5 SD (normal = 6.68%)	Increase	Additional affected children per year (n)	Additional cost for services (millions)
IQ difference	3.26 (2.01) IQ points	3.75	1.6 ×	1,688–14,062	$4–35	10.03	1.5 ×	4,514–37,612	$10–80
IQ effect size	0.33 (0.13) SD units	4.75	2.0 ×	2,138–17,812	$7–59	12.10	1.8 ×	5,445–45,375	$15–129
Receptive language effect size	0.71 (0.26) SD units	9.85	4.3 ×	4,432–36,938	$22–180	21.48	3.2 ×	9,666–80,550	$42–352
Expressive language effect size	0.60 (0.29) SD units	8.08	3.5 ×	3,636–30,300	$17–138	18.14	2.7 ×	8,163–68,025	$33–272

The calculations for children with IQs < 2 SD are included in the calculations for < 1.5 SD. Both calculations are provided because in some cases special education services are provided to children beginning at 1.5 SD below the mean (IQ < 78) and sometimes at 2 SD (IQ < 70). Adapted from Lester et al. (406).

a cascade of alterations which may not be detected structurally or functionally until much later in life. Thus, these effects may be manifested at a time much removed from the critical developmental window when the exposure occurred. The developmental effects may be manifested as persistent deficits, developmental delays, or transient deficits. Developmental delays and transient deficits in these developmental processes and ontogeny of function may have more insidious consequences later in life. These two types of effects may result from the continued growth and differentiation of the nervous system resulting in apparent compensation. Such apparent recovery may be reversed during aging, when the compensatory ability of the nervous system may be significantly reduced. The critical nature of time of assessment for adverse effects after developmental perturbations may extend into aging because many structures of the brain (e.g., temporal lobe of the cerebral cortex) undergo age-related neuronal loss and concomitant loss of synaptic inputs and/or plasticity. In addition to latent neurotoxicity as a consequence of developmental exposure, there are tremendous impacts on society of small effects on function that may impair either cognitive or social performance (i.e., impulsivity, emotionality, and executive function) in individuals. The societal impact of these small or transient effects on the population can be tremendous when amortized across the population. These effects can result in an increased need for remedial assistance in school, increased medical care, increased assistance later in life with basic life skills, or increased mortality and morbidity during aging. These long-term consequences after developmental exposure to environmental agents require attention to the neurobiological substrates and processes that are homologous between animals and humans and which may be perturbed by environmental agents.

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