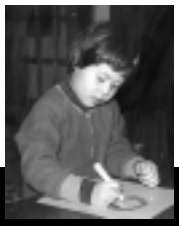
# Chapter 2



# Normal Brain Development and Developmental Toxicology

## Normal Brain Development

Brain development begins early in the child's first envir onment of the uterus and continues well beyond birth into adolescence. Normal brain development requires the intricate unfolding of a cascade of processes that do not occur during any other life stage. Consequently , developing fetuses and infants are uniquely vulnerable to disruption of these processes by environmental factors, including chemical contaminants and nutritional deficiencies. Cell proliferation, migration, differentiation, and synapse formation normally progress in a tightly programmed and orderly fashion. Subsequently , neural cir cuits ar e refined and consolidated through programmed cell death (apoptosis), a process that continues into childhood and adolescence. Interference with any stage of this cascade of events may alter normal progression of subsequent stages so that even short-term disruptions may have long-term effects later in life.

Neurons are the nerve cells in brain or peripheral nerves responsible for transmitting nerve impulses. Outgrowths from these cells, collectively called neurites, develop into long axons or shorter dendrites, each of which makes contact with neighboring neurons. Connections between neurons, called synapses, enable complex circuits to be established in the brain. Other cells, called glia, are responsible for the synthesis and maintenance of myelin, a coating around larger axons, which facilitates nerve transmission. Myelin consists largely of lipids (fats) with smaller amounts of protein. Some glial cells also provide scaffolding for the migration of neurons during development and help to maintain a normal biochemical environment.

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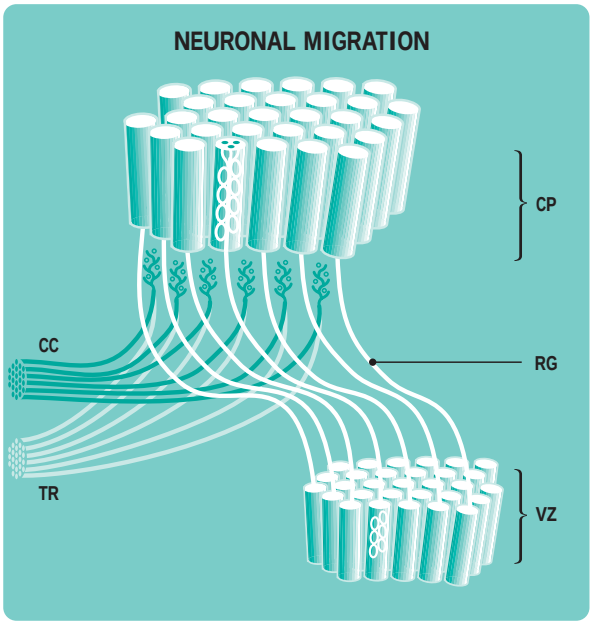
The timeline of normal brain development has been studied in detail in animals and to some degree in humans. Embryonic and early fetal development are characterized first by neuronal proliferation and migration.

Later, cellular dif fer entiation and synapse formation dominate. During normal development, neurons migrate to their final positions in a specific




During brain development neurons originate near the center of the brain (ventricular zone, VZ) and migrate along radial glial guides (RG) to their final location closer to the surface of the brain (cortical plate, CP). As the neurons migrate they intercept nerve fibers from other portions of the brain (thalamus, TR; the opposite side of the brain, CC). Later-developing neurons migrate to final positions closer to the brain surface, remaining in columns (outlined by cylinders) that correspond to columns from which they originated. (adapted from Rakic, 1988)

Rakic P. Specification of cerebral cortical areas. Science 241(4862):170-176, 1988.

sequence with those migrating to the cortex early forming the deepest layers while later arriving cells are more superficial. Proper positioning of the neurons is essential for establishing normal neural circuitry and brain function. Cell proliferation continues in the rat brain up to about 3 weeks after birth. In humans, neuron formation is largely complete at birth, and almost all neurons of the cerebral cortex have reached their final positions. Glia, however, continue to develop throughout life. Many synapses formed during the first two years of life are later eliminated as circuits are pruned. However, new synapses form throughout life, explaining how we can continue to learn and remember. Myelination continues well into the teenage years. [1]

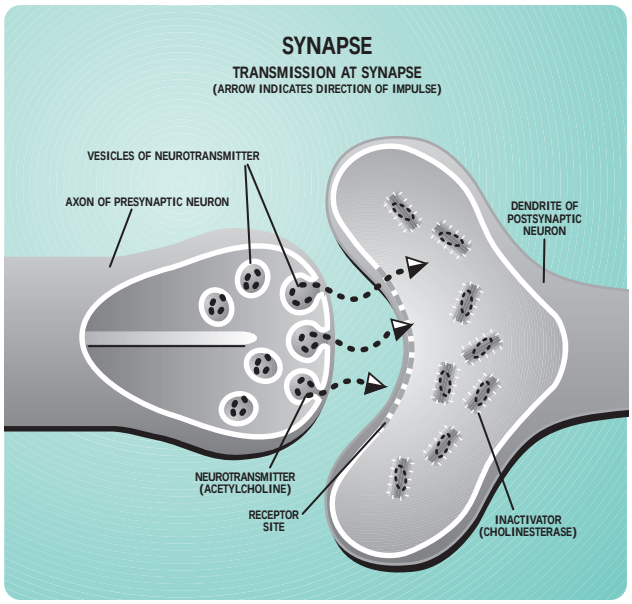
Development does not, however, progress uniformly in every area of the brain. For example, the cerebellum develops later than many other brain structures. Consequently, at any one time, some areas are undergoing cellular proliferation while others are undergoing primarily differentiation. Timing is, therefore, important when considering the potential effects of exposure to an environmental agent that disrupts specific developmental processes.

Neurotransmitters, hormones, neurotrophins, and growth factors orchestrate the intricate process of brain development. Neurotrophins are proteins that help regulate differentiation and survival of neurons. In the adult, neurotransmitters serve primarily to transmit nerve impulses from one neuron to another. In the developing brain, however, neurotransmitters serve an additional and very important role, helping to orchestrate the cascade of events necessary for normal brain development. Major neurotransmitters include acetylcholine (ACh), norepinephrine, dopamine, serotonin, gamma-amino butyric acid (GABA), glutamate, and aspartate. Growth, thyroid, steroid, and sex hormones also play important roles in brain development. Neurotransmitters, neurotrophins, and hormones exert their effects by attaching to specific cellular receptors, initiating a biological response. Receptor location and density are also determined during early brain development.

During prenatal life, neurotransmitters and their cellular receptors also develop on a specific timeline. For example, receptors for the neurotransmitter, acetylcholine, develop slowly from 16-20 weeks, followed by a lag time of about 4 weeks, and then rapid receptor formation during the last trimester of pregnancy.[2]

The class of neurotransmitters that includes dopamine and norepinephrine matures later.

The cholinergic neurotransmitter system, which utilizes acetylcholine as its chemical messenger, includes two types of receptors – muscarinic and nicotinic, so named because of their selective stimulation by muscarine (a chemical that can be extracted from certain mushrooms) and nicotine. Both types




of receptors are found in the central nervous system and their respective roles in brain development are gradually coming into focus, though considerable information is still missing. Normal development of muscarinic ACh receptors is important for later learning and cognition.[3] Initially, neurotransmitters promote DNA synthesis and cell proliferation.[4] Later, with increases in synaptic proliferation and nerve activity, the same transmitters promote differentiation of nerve cells into those with more specialized functions.

In general, cholinergic neurons frequently make contact with non-cholinergic structures, leading investigators to conclude that an important role is to modulate the activity of other types of neurons. For example, ACh released from one neuron, acting on the receptors of other neurons, modulates their release of norepinephrine, dopamine, GABA, serotonin, glutamate, and acetylcholine.[5]

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The brain undergoes rapid structural and functional changes during late pregnancy and in the neonatal period. Cognitive functions and behavior arise from multiple sources and depend on more than one neurotransmitter and more than one portion of the brain. Attention, memory, language skills, learning capacity, and behavior result from integration of multiple structural and functional factors with cultural and social forces. These complex interactions make it exceedingly difficult to study the contribution of each factor independently. These complexities also make it difficult to study and understand when, if, or to what degree environmental factors play a role. Differing professional interests also help to explain the varying approaches of investigators from separate disciplines as they attempt to understand human behavior and cognitive abilities.

## Developmental Neurotoxicology

Developmental neurotoxicants, including lead, mercury, pesticides, and others, may directly interfere with any of the processes required for normal brain development. Cell division, migration, differentiation, synapse formation, and apoptosis may be accelerated or delayed. Myelin formation may also be altered by toxic exposures or nutritional deficiencies. [6] Some neurotoxicants, like lead and alcohol, interfere with normal neurite development through a variety of mechanisms. Unique developmental processes, including myelination, synapse formation, and apoptosis continue under genetic and environmental control at least through puberty.[7] The timing, pattern, and level of toxic exposure largely determine which parts of the brain will be affected and to what degree. Various stages of development provide critical windows of vulnerability during which exposure to a chemical substance may have lasting adverse effects on brain function. Different learning or behavioral effects may result from exposure to the same agent at different times in brain development, depending on the location in the brain where susceptible neurodevelopmental events are taking place at the time of the exposure.

Some toxicants act indirectly by, for example, interfering with normal placental function, altering umbilical circulation, causing general growth retardation, or altering function or metabolism of hormones (endocrine disruptors). However, the distinction between direct and indirect toxicity is of no practical importance, since the child is still impaired. It is also critically important to keep in mind that neurotoxicants may interfere with brain development and subsequent function at exposure levels that have minimal, transient, or no effect on the adult brain.

### The Role of Thyroid Hormone

Among the various growth factors and hormones necessary for normal brain development, thyroid hormone (thyroxine), which is essential for neuronal proliferation and differentiation, plays a particularly important role. [8] It appears that any toxicant that lowers thyroxine levels, or otherwise interferes with thyroid hormone action, even to a small degree, is likely to have an adverse impact on IQ and potentially other brain functions. Even transient decreases in thyroxine in the CNS during critical developmental periods may produce alteration in neuronal branching and cellular architecture in the brain.

It has long been known that maternal and fetal hypothyroidism, as determined by distinctly subnormal thyroxine levels, produce cognitive impairment in children. However, a recent study reports that even minor reductions in maternal thyroxine levels result in reduced performance on IQ tests in children. [9] In this study, elevated levels of thyrotropin, the pituitary hormone responsible for stimulating the thyroid to release thyroxine even when slightly decreased, predicted reduced performance on the Wechsler

Intelligence Scale for Children. IQ scores were 4 points lower in children of women with elevated thyrotropin levels compared to matched controls. Fifteen percent of the children had IQ scores of 85 or less compared to 5% of control children. Only some of the women with elevated thyrotropin also had low thyroxine levels.

### Challenges to Identifying Neurotoxic Effects

One of the main problems encountered in studying the effect of chemical exposures on subsequent brain function is the possibility of a long latent period between the exposure and recognition of a functional deficit. For example, impaired language or reading skills may not become apparent until school age. Indeed, some investigators report that some chemicals administered during development have effects on brain function in subsequent generations. [10] Delays of this sort make it extremely difficult to attribute a functional brain abnormality to an earlier chemical exposure.

In addition, the symptoms of impaired brain function are not specific for each potential cause. That is, cognitive and behavioral disorders, or even mental retardation, may have multiple causes, including genetic and envir onmental factors. Mor eover , even a known neurodevelopmental toxicant, like alcohol, may cause a range of adverse ef fects including pr ematurity , cognitive disorders, mental retardation, and disturbances of sexual

differentiation of the brain. Lack of specificity of symptoms, multiple potential causes, and long latent periods between exposures and recognition of symptoms combine to ensure that establishing causal connections between symptoms and chemical exposures will be difficult.

### Neurodevelopmental Toxicity Testing

Laboratory and epidemiological research over several decades has led to considerable insight into the capacity of a few neurodevelopmental toxicants to interfere with normal brain development, often with severe and lasting consequences. Unfor tunately , extensive information is available for only a few chemicals, though more neurodevelopmental data on many others are urgently needed. As new research is contemplated, an important question focuses on the degree to which

animal testing data predict neurological consequences of exposure in humans.

A retrospective look at the evolution of understanding of the neurodevelopmental toxicity of lead, mercury, and PCBs is instructive. In an historical review of this question, Rice et al. conclude that animal studies, particularly rodent studies, are disappointing in their ability to predict "safe" exposure levels, below which no human health effects are likely to occur.[11] Rodent studies often vastly underestimate the sensitivity of the developing human brain. For example, based on comparisons of animal and human data, animal studies of lead, mercury, and PCBs predict a "safe" exposure level in humans that is 2-4 orders of magnitude (100-10,000 fold) higher than levels that actually cause effects in humans. These sobering limitations must be kept in mind as we use the results of animal testing to estimate "safe" human exposure levels. *(see Chapter 7)*

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