Developing Brain as a Target of Toxicity

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The human brain forms over an unusually long period compared to other organs. While most of the basic structure is laid down before birth, neuron proliferation and migration continue in the postnatal period. The blood–brain barrier is not fully developed until the middle of the first year of life. The number of synaptic connections between neurons reaches a peak around age two and is then trimmed back by about half. Similarly, there is great postnatal activity in the development of receptors and transmitter systems as well as in the production of myelin. Many of the toxic agents known to damage the developing brain interfere with one or more of these developmental processes. Those with antimitotic action, such as X-ray and methyl mercury, have distinctly different effects on structure depending on which neurons are forming at the time of exposure. Vulnerability to agents that interfere with cell production decreases rapidly over the early postnatal period. Other toxic substances, such as psychoactive drugs and agents that alter hormone levels, are expecially hazardous during synaptogenesis and the development of transmitter systems, and thus continue to be damaging for years after birth. Still other toxic substances such as lead, seem to have their greatest effects during even later stages of brain development, perhaps by interfering with the trimming back of connections. Guidelines designed to protect human populations from developmental neurotoxicity need to take into account the changing sensitivity of the brain as it passes through different developmental stages, as well as the fundamental differences in the effects of toxicants on the mature and the developing brain. — Environ Health Perspect 103(Suppl 6):73–76 (1995)

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The nervous system in the adult is often described as one of the best-protected systems of the body. It is physically protected by being encased in bone and chemically protected by the blood–brain barrier, which prevents the random passage of many toxic agents into the brain. It is common for adults to experience toxic effects in the body systems most exposed to the outside world: the gastrointestinal system, respiratory system, and skin; and in the internal organs most exposed to blood-born toxic agents: the liver and kidneys. Brain effects are relatively rare.

Unfortunately, the developing central nervous system (CNS) is much more vulnerable to injury from toxic agents than the adult CNS. In fact, if we consider congenital defects, we see the brain as the major target of toxicity. If you try to list all the causes of birth defects that you know, you will find that virtually all damage the nervous system, while most do relatively little damage to other organs. For example, consider rubella (*1*), metals like lead (*2*) and methylmercury (*3*), alcohol (*4*), retinoids (*5*), and thalidomide (*6*). Among these, rubella can cause heart defects, alcohol causes changes in facial features, retinoids cause abnormalities of the ears and face, and thalidomide causes limb defects; but CNS effects are seen with each of these agents, and often at lower doses than those required to affect other parts of the body.

To understand why the CNS is so subject to developmental injury, it is necessary to have some basic idea of how the CNS develops and how its development compares to that in other organs. The nervous system arises from the ectodermal layer of the germ disc, the flat surface that faces the amnion. A groove appears along the midline of the disc and the folds on either side touch and join, creating a tube that is wide open at the head and tail ends of the embryo. The process of tube formation begins in what will be the cervical region and proceeds toward the head and then the tail. The closure of the tube is usually described as occurring between day 21 and 26 after conception. Failures of the process are common. About 1 in 1000 births in the United States exhibits a neural tube defect. Some of these, like anencephaly, are rapidly fatal. Others, like spina bifida occulta, are compatible with life, but lead to varying degrees of abnormal innervation of the lower extremities. Fortunately, a major cause of neural tube defects has been discovered recently, and it is hoped that folic acid supplementation of the diet will prevent many of these defects in the future (*7*).

More common still are failures of the development of the internal structure of the CNS. Many of the agents we know to be toxic to the developing CNS act by interfering with specific developmental processes. Some important processes are represented in Figure 1.

Neuron Proliferation

Even before the neural tube closes, neurons are being formed. The generation of these critical cells continues throughout gestation and well into the first year of life. Unlike cells in many organs, where only a few cell types are repeated thousands of times, the CNS is made of dozens of different types of neurons. Typically, a set of neurons destined to be similar in morphology and function is generated in a short period, sometimes as little as a few weeks or even a few days of gestation. Large motor neurons, like those that stimulate muscles, are produced first, followed by sensory neurons. Nuclear groups in the brain stem and diencephalon tend to form early, while complex layered structures like the cerebral cortex, hippocampus, and cerebellum add cells over a long period (*8*). In many structures of the CNS, cell production creates numbers of neurons in excess of the number actually needed, and the proliferation period is followed by a wave of cell death that establishes the proper final number of neurons (see below).

The first panel of Figure 1 depicts the neural tube with cells on its inner surface dividing. Some divisions add more cells to the generative population, while others

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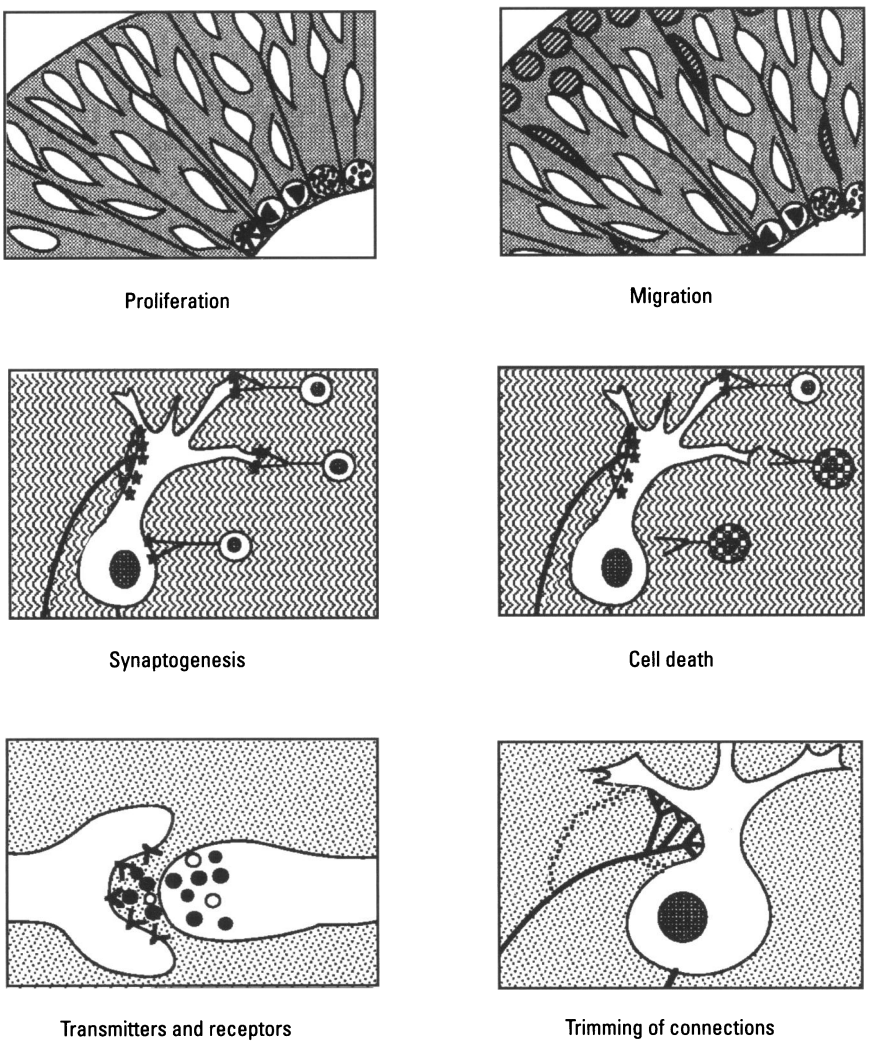

Figure 1. Developmental processes.

produce cells that leave the mitotic cycle and become definitive neurons. An insult to cell proliferation affects only the particular types forming in its presence. Thus, agents with this action have very different effects on brain development when they are delivered at different stages. They can reduce the number of a few cell types and leave many other groups, formed before or after the injury, apparently normal (*9*). Environmental agents with the property of killing neurons as they are born include ionizing radiation (*10*) and methylmercury (*11,12*).

Obviously, cell proliferation occurs in all parts of the body. Yet, even agents that interfere with all cell production seem to injure the CNS more than other organs. This may be because the production of unique units is a constant feature of CNS development, while other organ systems tend to acquire all their basic cell types early, with subsequent growth consisting of increasing the numbers of these cells, rather than adding new cell types. In addition, we know that the neuron is a cell type that proliferates only during development. Whereas many tissues retain the capacity to add cells throughout life, any loss of neurons is permanent.

Cell Migration

The second panel shows that neurons may travel from their place of origin to their final positions in the nervous system. In structures like the cerebral cortex, where cells are born on the inner surface of the neural tube and then move to the outer surface, the distances traveled can be substantial. Cell migration does not proceed at a constant rate throughout development. Like cell proliferation, it occurs in waves associated with different cell types. Most migration occurs early in gestation, when distances within the brain are small. The long migrations of small cells of the cerebral cortex, hippocampus, and cerebellum continue for several months after birth.

Occasional neurons in the wrong location occur in normal brains, but patches of misplaced neurons are characteristic only of brains with serious developmental injuries. Because toxic agents that lead to migration failure are often known to interfere with proliferation as well, it has been difficult to determine whether there are agents that affect migration exclusively, or whether migration failures result when cells are lost, changing the environment of the cells that form later. Ionizing radiation (*13*) and methylmercury (*14,15*) provide examples of the dual effect on cell survival and migration. In any case, neurons that are out of position cannot make the proper connections with the neurons that should be their neighbors, so they cannot develop normal function.

Synaptogenesis

To achieve mature function as transmitters of signals, neurons must form connections. This requires the development of specialized structures on the surfaces of the sending and receiving neurons. The point of contact is a synapse. A typical large neuron is covered with thousands of these. Research indicates that receptors appear on large groups of like cells simultaneously, and release of appropriate transmitters from cells projecting into the region follows soon after. Classic causes of deficient synaptogenesis are malnutrition (*16*) and hypothyroidism (*17*). Lead also interferes with the process of synaptogenesis (*18*).

While neurons retain the ability to make new synapses throughout life, the developmental period is critical for the formation of the basic circuitry of the nervous system. In addition, many lines of evidence suggest that some of the early communication of neurotransmitters to their receptors has a developmental purpose, signaling information for further development, rather than signaling to control body functions, as in the adult (*19*). Because of this, messengers that have transient effects in the adult may have permanent effects in the developing organism. The result is that agents that block signals or increase them can interfere with CNS development.

Our society uses countless numbers of products designed to affect neural transmission. These include drugs for human consumption and drugs directed at pests. As a class, substances that act as transmitters or hormones, or which can mimic the actions of these messengers, are suspect for altering synaptogenesis. Such agents are likely to disturb CNS development. An example is the herbicide TOK (2,4-dichlorophenyl-*p*-nitrophenyl ether), which lowers thyroxin levels. Mice exposed to TOK prenatally have abnormalities in many systems, including the CNS (*20*).

Cell Death

Substantial progress has been made in understanding how cells die in the normal course of CNS development. What determines which cells die has been more difficult to ascertain. It is likely that one factor is the number or kind of connections a neuron has made before the cell death period. The period of cell death typically coincides with the period when connections are actively forming. Whatever the determining factor, it is clear that naturally occurring cell death is not a passive process. Rather, the cells are removed in an aggressive and efficient manner. Presumably, such action serves to leave the CNS with an optimal number of well-connected neurons. Indications of failure of the necessary cell loss have been reported in some brain regions of individuals with autism (*21*). On the other hand, the active removal of cells could go beyond what is needed.

Transmitters and Receptors

The story of the differentiation of neurons as message carriers goes beyond the process of making connections. For example, the level of activity of a neuron seems to influence the development of receptors in the cells to which it projects (*22*). Further, the nature of the transmitting chemicals a cell produces appears to be controlled by the stimulation the cell receives at particular stages of development (*23*).

When the immature brain is compared to that of the adult, there are striking differences in the location and number of receptors (*24*). Release of excitatory amino acids is a response to ischemia at all ages, and contributes substantially to subsequent cell loss by overstimulating neurons with appropriate receptors. McDonald and Johnston have proposed that the remarkable effects of hypoxia–ischemia on the developing brain may be due in part to the rich supply of excitatory amino acid receptors in the immature tissue (*25*).

Trimming of Connections

Just as cells form in numbers greater than required by the mature nervous system, connections form in great excess during development. The winnowing of these connections to the needed number is a longer process than cell death. Thus, we see very high numbers of connections in the cerebral cortices of weanling-age rats and cats (*16,26*) and in 2-year-old children (*27*). As with cell death, we assume that the synapses that disappear are less useful in some way than those that are retained. However, we know little about the process by which some connections are eliminated and others are preserved. This process is represented in the last panel of Figure 1. Recent data on the interaction of low doses of lead with several processes involved in transmission at the synapse have suggested the hypothesis that this metal may interfere with the process of synapse trimming by reducing the difference in level of activity between active and inactive synapses (*28*).

Myelination

The coating of axons by sheets of glial tissue provides insulation and makes transmission along the axon more rapid. We know from many demyelinating diseases in the adult how important myelin is for CNS function. A number of agents are thought to interfere with the deposition of myelin in the developing brain, and since new waves of myelin formation continue into adulthood, such agents could be hazardous over an extremely long period (*29*).

Development of the Blood–Brain Barrier

In addition to the sensitive processes just described, the developing brain is distinguished by the absence of a blood–brain barrier. The development of this barrier is a gradual process beginning *in utero* and complete around 6 months after birth in the human (*30*). Thus, some toxic agents that never enter the mature brain enter the developing brain freely. Examples include cadmium (*31*) and monosodium glutamate (*32*).

In summary, the brain is vulnerable to agents that interfere with any of the processes involved in its development. Because of the complexity of a tissue with thousands of interconnecting circuits, the establishment of the mature systems of neural tissue involves more developmental processes than those of other tissues. Because of this, there are more opportunities for injury. Probably the most important feature of the CNS with regard to developmental accidents, however, is the sheer length of time over which development of the CNS proceeds.

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