Vulnerable Periods and Processes during Central Nervous System Development

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The developing central nervous system (CNS) is the organ system most frequently observed to exhibit congenital abnormalities. While the developing CNS lacks a blood brain barrier, the characteristics of known teratogens indicate that differential doses to the developing vs mature brain are not the major factor in differential sensitivity. Instead, most agents seem to act on processes that occur only during development. Thus, it appears that the susceptibility of the developing brain compared to the mature one depends to a great extent on the presence of processes sensitive to disruption. Yet cell proliferation, migration, and differentiation characterize many other developing organs, so the difference between CNS and other organs must depend on other properties of the developing CNS. The most important of these is probably the fact that nervous system development takes much longer than development of other organs, making it subject to injury over a longer period. — Environ Health Perspect 102(Suppl 2):121-124 (1994).

Key words: teratology, CNS, development

Introduction

The nervous system is the body system most commonly disrupted by teratogenic agents. We can be sure that this is the case because of the frequency with which it is injured. Surveys of birth defects show that the most common type of gross malformation—heart defects—occurs six times in 1000 births (1), but brain damage, from frank retardation to milder learning disabilities, occurs in 10 to 20% of births (2). That is a startling discrepancy.

The mature nervous system, in contrast, is not so common a target for toxic agents. So it is not true that the CNS is an especially "weak" system. Nor is it true that young tissue is consistently more easily injured than mature tissue. In fact, toxicologists know of many agents which become toxic to particular tissues only as the body matures. Good examples are metals such as lithium (3) and inorganic Hg (4) which damage the kidney only as its filtration function becomes active.

We do know that substances in the blood pass into the brain more freely in the developing animal, because the blood brain barrier is not fully developed until well after birth (5). This is not enough to explain why developmental injuries to the brain are so common, because we have examples in which developing brain is more sensitive than mature brain even though the teratogen is transferred freely to brain at both ages (6) and even examples, such as radiation exposure (7), in which the teratogen is not bloodborne. Apparently, the combination of immaturity and CNS characteristics somehow yields a system that is "injury prone."

Research on injuries to the developing CNS has given us some explanations of why this system differs from others in its high rate of birth defects. In this manuscript some of the reasons are discussed in the context of the general course of CNS development and the processes that underlie it.

General Development

Functionally, we can observe some reflexes in utero. These are the first indication that the nervous system has some parts that are operational (Table 1) (8,9). In the neonate, we see the appearance of many new reflexes (10). Cortical dominance represents the achievement of the smoothing out of spinal reflexes as higher centers gain control. It occurs at the end of the third month in the human (11). We see rapid development of cognitive abilities from 4 to 7 years of age, when children demonstrate the capacity to categorize and order items. Puberty is another example of maturation, because it occurs when the appropriate brain systems are mature, at around 12 years of age.

Structurally, we see changes in the human CNS that are the basis for these events (Table 2). The tube from which the nervous system develops is formed from days 21 to 26 in human embryonic life, and the first neurons are born as the tube closes. Throughout gestation more and more neurons are added, and by the second trimester, the other cell type of the CNS, the glia, are increasing in number. These will finally coat many neuronal projections as insulation, allowing rapid transmission of impulses along cells. Neurons of the cerebral hemispheres begin to move away from their birthplaces at about 6 weeks in utero, and that process continues until 5 months after birth (12). The glial coating—myelination—is about half complete at 6 months in the corpus callosum (13). Visual connections are complete at around 3 to 4 years of age (14). The addition of connections and development of the transmitter and receptor chemicals which allow transmission between cells goes on for a long time. The addition of neurons and glia and the growth and elaboration of each

| Table 1. Functional development of the CNS.* |
|--------------------------------------------|
| **CNS development** | **Age** |
| Prenatal | 3 months |
| Limb reflexes | 4 months |
| Swallowing reflex | 6 months |
| Suckling reflex | 7 months |
| Startle to noise | |
| Postnatal | |
| Visual discrimination | 1-2 months |
| Cortical dominance | 4 months |
| Chewing reflex | 5-6 months |
| Walking | 1 year |
| Temperature regulation | 1-2 years |
| Ordering, classification | 4-5 years |
| Puberty | 12 years |

*Data from reviews by Sarnat and Mueller (8) and Rodier (9).
cell keeps the weight of the brain increasing up to age 20.

**Critical Processes**

In describing general development of the CNS, several processes that are important in development of many organs were mentioned. Structures are built by cell proliferation, migration and, finally, a sequence of steps called differentiation, in which the cells take on the properties that distinguish the many different cell types of the body from one another. Normal function requires a certain number of cells, in the correct location, and each of the cells must have the proper characteristics to do its job in the structure. For example, to make a cerebellum, a group of neurons is born in embryonic life; these migrate to a new location and then begin to grow and become very specialized. The cells internal and external to them proliferate, the external ones move to an internal position, making contacts with our original group as they pass by (15,16). Finally, glial cells add a myelin coating to some cell processes, increasing the speed of transmission along axons. At the end of all these events, several years after birth in the human, the cerebellum has many different types of cells, each with its own transmitter chemicals, its own receptors for messages from neighboring cells, and its own synaptic connections to other cells. In the adult, the cerebellum is important for motor coordination, balance, and skilled movements.

Even this brief outline suggests some of the things that might go wrong in CNS development. An agent could interfere with cell proliferation, so that too few cells are produced. An agent could interfere with migration, so that cells end up in the wrong place. An agent could interfere with the outgrowth of extensions from cells, with the establishment of synaptic connections, or with the development of dozens of other properties of neurons that are needed to achieve the normal function of transmitting and receiving information.

If you can picture the developing cerebellum, you can grasp this critical point: there are vulnerable periods during development only because there are vulnerable processes taking place. When cells are forming, agents which interfere with cell proliferation can cause damage. When neurons are making connections, agents which interfere with synaptogenesis can cause damage, and so forth. Because these processes occur within a rigidly controlled time frame, they create windows when the nervous system is susceptible to damage from agents which would be innocuous in mature brain.

Once all the cells have formed, all are in place, and all have differentiated, the nervous system is a much more stable organ. The mature CNS is attacked by many agents, and some of these are hazardous to the developing CNS, as well, but it must be the processes exclusive to development that make the developing CNS such a common target for toxicity, as compared to its adult counterpart.

**Cell Production**

The developmental process that we know the most about is cell proliferation. We can represent the bursts of activity as in Figure 1—here we see the some of the cells of the cerebellum, the Purkinje cells forming in mid-gestation in the rodent, the smaller cells forming mostly after birth. Now consider a few of many other other structures. The large cells of the hippocampus form in mid-gestation, and the dentate gyrus cells much later (17). Luteinizing hormone releasing hormone (LHRH) cells of the hypothalamus form a little earlier. Suppose we expose animals on day 15 in utero to an agent which disrupts cell proliferation. We might expect the injured animals to fail to produce the normal number of hippocampal pyramidal cells, while the cerebellum and the LHRH cells would be spared. That is exactly what happens (18). Animals with this kind of injury are deficient on many different tasks involving learning and memory. They are strikingly hyperactive. If we try the same experiment, but expose the animals earlier or later, we get different changes in the brain, and different behavioral effects. For example, if we go early or late enough to injure the cerebellum, we often see hypoactivity (19). This points out the importance of time of exposure. The same teratogen can have different effects, or even no effects, depending on when it is delivered.

What happens if we interfere when the LHRH cells are forming? These control the release of pituitary hormones which stimulate and maintain the ovaries and testes. When we interfere with cell division on day 12, the animals have reduced numbers of cells expressing LHRH as adults. After birth, when we stain pituitaries for LH, an important reproductive hormone, we see many cells containing the hormone in controls, but the injured animals have none. Not surprisingly, the injured animals also have immature gonads, and they go through puberty later than controls (20; CE Gavin, unpublished data).

Many agents known to cause brain damage do so by interfering with cell pro-

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**Figure 1.** Proliferation periods of several types of neurons in the rodent. Dating of cell production is based on autoradiographic studies (17).

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**Table 2.** Physical development of the CNS.

| Stage/Process                          | Age  |
|----------------------------------------|------|
| Prenatal                               |      |
| Neural tube closes                     | Days 22–26 |
| First neurons born                     | Days 22–26 |
| Cortical neurons migrate               | 6 weeks |
| Postnatal                              |      |
| Cortical migration complete            | 5 months |
| Neuron proliferation complete          | 12 months |
| Myelin 50% complete (corpus callosum) | 18 months |
| Visual system connections complete     | 3–4 years |
| Brain mature in form                   | 20 years |

*Data from reviews by Sarnat and Mueller (8) and Rodier (9).*
dution. A classic example is X-irradiation. The CNS, because of producing many cell types over an extended period, is subject to this injury at more stages than any other developing organ. Figure 2 gives the example of the liver for comparison. You can see that the period when a developmental accident could limit liver cell numbers is short, while the period when cells are forming for the CNS is extremely long. Also notice that most body systems, like the liver, need to produce only a small number of cell types. Thus, if some are lost, function may not be affected, and therefore the injury may not be expressed. In contrast, because the CNS has so many different cells with different job descriptions, each produced over a limited period, loss of a few progenitor cells could wipe out a whole category. The effect of this could be disastrous.

The CNS has another vulnerability with regard to cell proliferation. It has no ability to replace missing neurons when it is mature, as some other tissues do. In fact, in experiments in which we have interrupted proliferation for brief periods, we see that once the normal period of production of a particular cell type is over, any compensation generated by the developing tissue tends to enhance the numbers of the next cells on the production schedule, rather than the ones that are missing. Thus, the ability of the CNS to repair injuries to cell production is poor, even during development.

Cell Migration

When complex circuits are being constructed, misplaced elements can cause problems, because neuronal circuits depend on physical contact between the cells. One agent known to lead to migration failure is methyl mercury. This chemical has been responsible for mass poisonings in Japan and Iraq and is now of concern because of the high levels present in the diets of those who consume fish. Like many metals, methyl mercury can lead to neurological damage and death in adults, but the embryo and fetus are affected by much lower doses. A child fatally affected by a very high dose in Iraq exhibited misplaced neurons in the cerebellum and cerebral cortex (21). In animal experiments, we see what seem to be related failures of cell migration (22).

It is unclear whether migration failures occur because of a direct interference of methyl mercury, or other agents, with migrating cells, or whether the effects are indirect. For example, loss of supporting structures, such as glia, or changes in the surface properties of surrounding cells could affect migration. In any case, ectopic cells seem to be associated with widespread, severe brain damage, rather than occurring in isolation from other injuries.

Many structures require some cell migration in the embryonic period—the heart is one—but human neurons are still migrating long after birth. In all cases, the conditions necessary for migration are present only during development, so if migration is not accomplished on time, it remains incomplete. Again, the length of time required for its development makes the CNS subject to many injuries.

Cell Differentiation

Hypothyroidism is a classic example of a condition which leads to mental retardation. One of the things that goes wrong when neurons develop with too little thyroid hormone in their environment is that the cells fail to extend enough processes to make appropriate connections (23). Normal levels of glucocorticoids are also necessary for neuron development (24), and differentiation of some regions is dependent on sex hormones (25). These facts make it clear that neuron differentiation is dependent on the levels and balance of natural messenger substances in the local milieu of cells, as well as the genetic program of the cell. Thus, abnormal differentiation does not require exposure to agents commonly considered to be poisons—anything that shifts the composition of the milieu at a critical time may trigger abnormal differentiation.

Those who study neuroteratology are particularly concerned about exposure to psychoactive drugs because so many of them have been shown to disrupt brain development. There are probably many ways in which these agents can cause abnormalities, but one way has been described in some detail. In the adult, the release of dopamine, a neurotransmitter, can either increase or decrease as cells show high or low activity, and we have an assortment of drugs which can turn the volume in this system up or down. In the brain, as in the rest of the body, systems are constructed in such a way as to resist wild fluctuations of activity, so the receptors for dopamine adjust to different activity levels. When release is high, the number of receptors drops, making the receiving cell less sensitive to dopamine signals. When release is low, the receptor population grows to increase sensitivity. Studies of psychoactive drugs and receptors in developing animals (26) suggest that the receptors respond the same way in utero—increasing in number to low rates of stimulation and decreasing to high rates. Unfortunately, when the receptors and transmitters first appear, the adjustment of receptors seems to fail to recover. That is, after the first adjustment, the receptor numbers seem to go to a new set point permanently. There are probably many other ways in which early manipulations of neural activity influence development, but this one has been studied extensively, and is a good example of how something that is innocuous in the adult can be injurious in the developing organism.

The normal role of the CNS in communication may lead to miscues when the wrong signals are received in early life. The environment is full of substances like drugs and pesticides that are designed to affect CNS function. Thus, it is should not be uncommon for the embryo or fetus to be exposed to psychoactive agents.
Summary
We have reviewed the time course of development of the nervous system, focusing on critical processes: cell proliferation, migration, and differentiation. The fact that the nervous system has little ability to replace missing elements is probably important to its high rate of developmental injuries. The absence of the blood brain barrier is a significant difference between mature and developing CNS, which contributes to injuries of the immature brain. The great number of different cell types in the CNS doesn't make it more susceptible to damage than other organs, but it may play a role in making injuries likely to be expressed in function. We are only beginning to understand all the steps in the development of transmitter systems, but the idea that abnormal stimulation during development may cause permanent adjustments of receptors may be valuable in understanding why psychoactive agents seem teratogenic at doses which are pharmacologically effective, but not toxic, in the adult. Most importantly, the long course of CNS development is surely the key feature responsible for the frequency with which it is injured.

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