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Critical Periods for Behavioral Anomalies in Mice

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While mice have been used less frequently than rats in behavioral research, their use has some advantages in teratological studies. The development of the mouse CNS has been investigated more extensively than that of the rat. Since time of insult has been found to be an important factor in effects on both anatomy and behavior, data on the sequence of events in CNS development are valuable in planning and interpreting behavioral assessments of potential teratogens. A comparison of studies in mice and rats suggests that behavioral effects of teratogens are similar in the two species and demonstrates that mice can be used successfully in a variety of behavioral evaluations.

The traditional use of mice in embryology has provided data that make the species desirable for teratological studies. When the teratological assessment involves behavior, however, many investigators perfer rats, mainly because there is such a wealth of behavioral data available for the second species. Rats do tend to be more vigorous, easier to train, and much easier to handle. The latter qualities can be important to the experimenter in studies where animals receive many trials, but such differences do not prohibit the use of mice, they only serve to explain why rats have been used more often. The thesis of this paper is that mice should not be considered poor subjects for behavioral studies. In fact, there are enough data available to indicate that teratogenic effects on behavior are quite similar in the two species.

For those interested in correlating behavioral deficits with alterations of the CNS, mice present one special advantage: the development of the mouse CNS has been studied more extensively than that of the rat. The idea that there are critical periods in development for the production of particular abnormalities is hardly new. Wilson (*1*) has described periods of peak sensitivity for several systems. The stages when interference with development leads to gross defects of the CNS, such as anencephaly or exencephaly, are very early in gestation. But since neuron proliferation continues even past birth, the period when the CNS is vulnerable extends into postnatal life. With teratogens leading to few gross abnormalities after the middle third of gestation, fetal and early postnatal life represent a long period during which CNS damage can occur without obvious teratologies. Even in the middle third of gestation, low doses of known teratogens may lead to functional deficits in subjects which appear morphologically normal (*2*).

Since many agents assessed for teratogenicity may interfere with cell proliferation, migration, or differentiation, some knowledge of the timing of these processes in the structures of the CNS can provide clues to the aspects of behavior most likely to be affected by a given insult. Figure 1 summarizes data from many investigations of cell origins in the CNS. The rat data are from Hicks, D'Amato, and Lowe's studies (*4*) of anatomical changes after x-irradiation at various stages of gestation. Since x-irradiation affects migrating cells, as well as those in the cell cycle, the times of peak development described might be expected to differ somewhat from the times identified by autoradiographic techniques, but, in fact, labeling studies have tended to suggest very similar time

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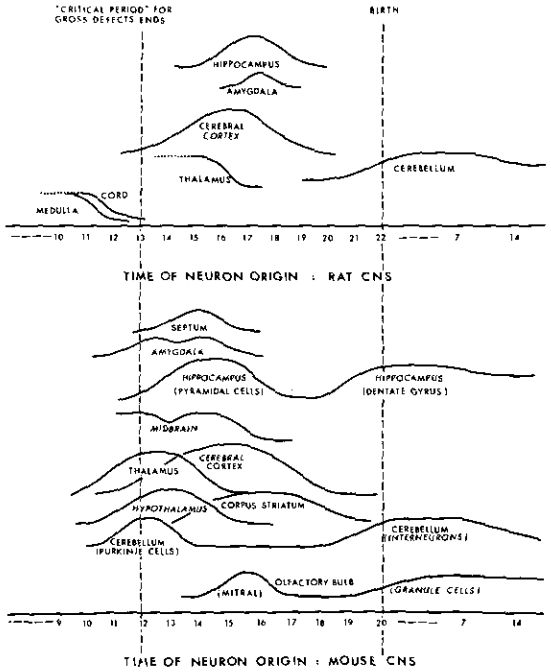

FIGURE 1. Times of neuron origins in rats are drawn from x-irradiation data by Hicks and D'Amato (*3*) and Hicks, D'Amato, and Lowe (*4*). Times in mice are based on autoradiographic studies of specific structures over time: diencephalon, Angevine (*5*); corpus striatum, Angevine and McConnell (*6*); cerebral cortex, Angevine and Sidman (*7*) hypothalamus, Shimada and Nakamura (*8*). cerebellum, Miale and Sidman (*9*) and Andreoli, Rodier, and Langman, (*10*); olfactory bulb, Hinds (*11*). Surveys across structures for a given day confirm many of these points and add others. see Langman, Webster, and Rodier (*12*) for days E 15, E 19, and PN 3; data of Rodier (*43*) for E 16 and E 18.

courses. The details of cell production periods for the cerebellum have been described by Altman (*13,14*), who has also examined a number of other cell types with postnatal origins (*15*), Nornes and Das (*16*) have charted the time of origin for neurons of the rat spinal cord.

Many more structures have been studied in the mouse CNS, and some have been analyzed in great detail. Peaks for separate cell types are shown only where long periods of inactivity intervene between the formation of the earliest type and later bursts of proliferation. The medulla and cord have not been investigated formally, but they do arise early, just as in the rat. The peaks of cell production in the midbrain, thalamus, and hypothalamus occur somewhat later. The graph is very general, but the time of formation is more specific if examined for a given nucleus or cell type within the larger structure. For example, the cells of the superior colliculus are complete before those of the inferior colliculus (*12*). The cells of some thalamic nuclei form as early as the 10th day of gestation (E 10), others as late as E 14-16 (*5*). The neurons of the hypothalamus appear in a lateral-to-medial, anterior-to-posterior gradient (*8*). During this early period, the Purkinje cells of the cerebellum originate from the rhombic lip (*9,10*), and soon afterward several other large neuron types are produced—the pyramidal cells of the hippocampus and the mitral cells of the olfactory bulb (*11,17*). The inner layers of the cerebral cortex are formed early, and each succeeding generation of neurons migrates through those previously formed to take up a position external to the older layers (*7*). The corpus striatum is another region with a long period of origin (*6*). Mitotic activity extending into postnatal life creates the dentate gyrus of the hippocampus (*17*), and the interneurons of the cerebellar cortex (*9,18,19*). Where structures have been studied in both species, the order is virtually identical. The exact time, in terms of days of gestation, is similar.

Considering the very rigid schedule on which the CNS forms, interference with basic developmental processes should damage different parts of the nervous system if delivered at different stages of development. This is evident not only in anatomy (*4,12*), but in the behavioral effects of the same treatment delivered at different stages (*20-23*). In fact, with a wide variety of teratogens, the behavioral effects are so dependent on the time of administration that many studies show very similar effects if the time variable is controlled (*24*). As one might expect, given the similarity between rat and mouse development, effects do not differ appreciably between the two species.

Table 1 compares data on rats and mice for several classes of behavior. For simplicity, the course of development has been divided into only three periods: early (the period during which many agents produce gross malformations), middle (the period of most rapid development for many CNS structures, such as the cerebral cortex, thalamus, hypothalamus, hippocampus, and corpus striatum), and late (the period when many microneurons undergo their final mitosis, including those of the cerebullum, dentate gyrus, and olfactory bulb). Behavioral abnormalities have been reported after teratological treatments in each of these periods. The early period studies cited were carried out under low-dose conditions which did not yield gross somatic defects. Thus, the studies reviewed are ones in

Table 1. Behavioral effects in rats and mice exposed to teratogenic agents at three stages of development.

Species	Early (before E 13)			Middle (E 13-E 17)			Late (E 18-PN 7)		
	Effect	Agent	Ref.	Effect	Agent	Ref.	Effect	Agent	Ref.
				I. Locomotor deficits					
Mice				"Hyper" gait	5-Azacytidine	(24)	Rotating rod	Corticosterone	(25)
				Pivoting	5-Azacytidine	(24)	"Hypo" gait	5-Azacytidine	(23)
							Pivoting	5-Azacytidine	(23)
Rats	Climbing	Hydroxyurea	(26)	Rotating rod	Methylazoxy-methanol	(28)	Rotating rod	x-Irradiation[a]	(29)
	"Hypo" gait	Hydroxyurea	(27)	"Hyper" gait	x-Irradiation	(4)	"Hypo" gait	x-Irradiation[a]	(29)
							Pivoting	x-Irradiation[a]	(14)
				II. Activity					
Mice				Hyperactivity	5-Azacytidine	(23)	Hypoactivity	5-Azacytidine	(23)
				Hypoactivity	Chlorpromazine	(30)			
Rats	Hypoactivity	x-Irradiation	(22)	Hyperactivity	x-Irradiation	(22,31)	Hypoactivity	x-Irradiation	(31,33)
				Hyperactivity	Methylazoxy-methanol	(32)	Hypoactivity	γ-Irradiation	(34)
							Hypoactivity	Corticosterone	(25)
				III. Learning					
Mice				Active avoidance ↓	5-Azacytidine	(43)	Active avoidance ↑	5-Azacytidine	(43)
				Active avoidance ↓	Chlorproma-zine	(30)	Operant	Corticosterone	(35)
Rats	Maze	Hydroxy-urea	(27)	Active avoidance ↑	x-Irradia-tion	(31)	Active avoidance ↑	x-Irradiation[a]	(39)
	Maze	Hypervita-minosis A	(36)	Maze	Methylazoxy-methanol	(28)	Maze	Barbiturates	(40)
	Maze	Acetylsa-licylic acid	(37)	Maze	x-Irradiation	(22)	Operant	Hypervita-minosis A	(41)
	Maze	x-Irradia-tion	(22)	Operant	Hypervita-minosis A	(38)			

[a] Focal irradiation. [b] Rodier, unpublished data.

which animals were found to exhibit abnormal behavior, despite the fact that they would not have been judged "abnormal" on the basis of a standard teratological screen.

The absence of differences between species is representative of all the data familiar to me, but the apparent lack of differences due to type of treatment is a function of the sample of treatments included. Most of the studies from which time or species comparisons can be drawn tend to involve agents with similar actions. Anticarcinogens and x-irradiation cause cell death in proliferative tissues, so it is not surprising that their effects are similar and dependent on time of administration. Other agents might give very different results.

Table 1 speaks for itself, but perhaps the tasks should be discussed in more detail. Rotating rod tasks present the animal with the problem of keeping its balance and footing on a moving surface. This is a challenge to sensory systems, motor systems, and systems which serve to coordinate sensory and motor functions. Middle and late period insults have been shown to affect such tasks. The "climbing" task cited is probably not as difficult, but it discriminated between hydroxyurea—treated and control animals. The "gait" data are not easily quantifiable, but they demonstrate differences between treatment times and similarity between rats and mice. The abnormalities signified by the term "hypo" gait include splaying or dragging of the hind limbs. "Hyper" gaits include hopping or stiff-legged movement with increased excursion of the hind limbs. Pivoting is a transient mode of locomotion in rodents, not unlike crawling in human infants. Persistence of pivoting after the normal time period appears to be associated with other locomotor deficits.

The "activity" data are from open field situations of several kinds. Like the locomotion data, they indicate differences dependent on treatment times and mice and rats tend to react similarly. An exception to the pattern of x-irradiation, 5-azacytidine, and corticosterone is seen in a study using chlorpromazine.

In both locomotor tasks and general activity treated animals can depart from normalcy in various ways. There is no logical problem with the idea that both hyper and hypo gaits are undesirable because they represent some sort of malfunction. For those unfamiliar with behavioral evaluations, however, it may seem surprising that "learning" scores also vary in both directions after teratogenic treatments. For example, after very early x-irradiation maze performance was improved, rather than disrupted (*22*). There are several examples of faster "learning" of active avoidance after middle-and late-period insults. Such findings must be viewed like those on other tasks. There is no such thing as a direct test of learning ability, only tests of performance on tasks which require some learning. The fact that active avoidance is enhanced by some surgical lesions of the CNS (*42*) should serve to convince the reader that observations of "improved" performance after experimental intervention should not be interpreted as a recommendation of the treatment. In all the studies cited in Table 1, animals with indications of "improved" learning were abnormal on other behavioral tests. Such data emphasize the importance of employing a battery of tests, rather than a single measure.

Even the most complete information on CNS development does not offer the possibility of complete prediction of behavioral effects. Simultaneous lesions of several brain structures have rarely been investigated by those who are interested in correlating structures and function. While single, discrete lesions have sometimes been firmly related to a set of behavioral changes, there is no "master list" of behaviors with which the effects of a given lesion can be analyzed. In the case of agents which persist in the body, or those to which exposure may be chronic, developmental data may be irrelevant. With such agents, selective destruction of CNS structures would occur only on the basis of some very specific reaction involving selective sensitivity of structures. However, since many treatments are known to have effects dependent on the stage of development of their target tissues, knowledge of the course of CNS development can be of value in planning behavioral assessments. In the studies cited here, for example, most of the deficits seen after postnatal treatments are probably related to cerebellar damage. Studies of insults at this period should include motor tasks that challenge sensory-motor integration. Earlier treatments can also lead to motor problems, but more obvious effects tend to appear on learning tasks and motivational measures.

It is reasonable to use mice because they have been the most popular subjects for studies of CNS development. There is a growing literature of behavioral evaluations in mice, and it is clear that the species can be employed successfully in behavioral studies, generally with results very similar to those observed in rats.

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