Arch Toxicol (1981) 48: 69–88

Archives of
TOXICOLOGY

Review Article

Experimental Methods in Behavioral Teratology

G. Zbinden

Institute of Toxicology, Swiss Federal Institute of Technology and University of Zürich, Schorenstrasse 16, CH-8603 Schwerzenbach, Switzerland

Abstract. The possibility that the exposure of the embryo to certain chemical substances can lead to behavioral disturbances is known from human epidemiological studies, e.g., in chronic poisoning with mercury and ethanol. Therefore, efforts are made to develop toxicological techniques with which new behavioral teratogens can be recognized.

The review describes the most important experimental methods which are presently explored, and which are based on a rich body of knowledge accumulated by experimental psychologists. Most of the tests were developed with small animals, mostly with rats. They range from a rather straightforward determination of various reflexes to complex behavioral situations involving mechanical devices, operant conditioning techniques and procedures evaluating social behavior.

In applying these methods in routine toxicology, it is important to remember, that many behavioral effects determined in newborn and adult animals are subtle. Moreover, they are influenced by a large variety of environmental factors affecting the health and the behavior of the mothers and of the offspring in the early and later phases of development. Therefore, the experiments must be conducted under highly standardized conditions and must be controlled rigorously. It is concluded that the best experimental strategy for the evaluation of potential behavioral teratogens is not yet established. Therefore, it would be premature to decide on a fixed protocol to be included in routine animal safety experiments for drugs and other chemical substances.

Key words: Behavioral teratogens – Reflex development – Operant conditioning

1. Basic Concepts

Few toxicological concepts have been endorsed and promoted more readily and with less experimental and epidemiological evidence than that behind behavioral

0340-5761/81/0048/0069/$ 04.00

teratology. Greatly aided by ancient folklore, we are more than willing to believe that environmental influences to which a pregnant mother is exposed, also affect the developing brain of the embryo, and will thus shape the offspring's personality and behavior. As a consequence, classical investigations on somatic ontogeny are now being extended into the areas of behavioral and sensory development. Moreover, with the rapidly growing knowledge on biochemical processes in brain function, a new discipline, biochemical ontogeny, has assumed an equally important role (Sobrian 1977a, Sobotka et al. 1974).

Experimental evidence is rapidly accumulating showing that the emergence of autonomic, neuromuscular, sensory, emotional and memory functions, and the maturation of the biochemical infrastructure of the brain are as well programmed as the processes of cell proliferation, differentiation and migration, occurring during the early development of the central nervous system. However, those who want to study the behavioral aspects of ontogeny, find themselves confronted with difficult problems. For, there are no direct and unambiguous measures of behavior, as there are for cell proliferation and migration, and for the emergence of biochemical characteristics, such as for example the assemblage of catecholamine depots in the developing brain. Even the simplest behavioral response results from an interplay of many parts of the whole nervous system, and it is always modulated by an unique set of information stored in the memory of the experimental subject. No one should be upset, therefore, that behavioral scientists and toxicologists have established new trains of thought, and have created their own lingo, to come to grips with phenomena that cannot be assessed and measured by conventional biological techniques.

Behavioral teratology is a new and rapidly developing discipline. Fortunately, it can lean upon the experience of many experimental psychologists who have for many years studied the behavioral ontogeny of newborn experimental animals. Faced with the enormous problem of assessing unmeasurable phenomena, such as emotionality, intelligence, anxiety, and memory, psychologists have learned to record and to quantify a large number of visible responses of the animals under defined experimental conditions. And based on a large body of experimental evidence they have attempted to make inferences from such responses to the subject's personality and its actual state of mind. The main purpose of this review is to survey the techniques developed by experimental psychologists and to discuss whether they could be used within the framework of teratological studies of chemical substances. The review will also point to some serious precautions that should be observed when the methods of experimental behavioral psychology are incorporated into the routine teratological test procedures.

2. Test Methods for the Assessment of Behavioral Development

From a review of the pertinent literature it is evident that the overwhelming majority of the studies deal with behavioral development of the laboratory rat. This is fortunate, since the rat is also one of the two animal species which is used for conventional teratological experiments. For the study of gross malformations the pups are usually secured by caesarian section a few days prior to the expected delivery. For the evaluation of behavioral and developmental abnormalities some of the pregnant mothers must be allowed to litter normally, and the offspring must then be followed for at least 8–10 weeks.

2.1. Physical Signs

Behavioral evaluation of developing animals traditionally begins with a careful listing of physical signs, and the age of the animals at which they become evident. An inventory of the most commonly recorded physical signs in rat pups is shown in Table 1. Such observations have, of course, nothing to do with behavior. But they serve as indicators for pre- and postnatal adversities. One of the most important consequences of a developmental disturbance is a reduction in weight gain. Since this could also be due to maternal neglect or a reduced flow of milk it may be necessary to have some of the pups raised by foster-mothers.

2.2. Behavioral Tests

The repertory of behavioral responses of the rat that can be measured is limited. It consists exclusively of movements. For this reason the test procedures used in behavioral teratology will be subdivided into a few groups, depending on the experimental interventions necessary to elicit the rat's movements.

2.2.1. Development of Reflexes. The most straightforward situation exists when we deal with movements that are enforced by acute manipulations. The information we are looking for in this group of tests is the time of appearance of the various reflexes (Table 2). Take the free fall righting, for example. The rat is dropped from about 35 cm, back downward. Either it lands on all fours or it doesn't, so there is no argument about the presence or absence of this reflex. The same is true for the startle response to an auditory stimulus. It is usually elicited by the snap of a mouse trap, 15 cm over the head of the rat (Smart and Dobbing 1971, Sobrian 1977a). The rat's startle, i.e., the jerk of the head and the extensions of the hind limbs, is a clear-cut response, and its presence or absence can be readily recognized.

Table 1. Physical signs and their approximate time of appearance in rats[1]

Pinna detachment (unfolding of external ear)	Day 2
Primary coat of downy hair	Day 5
Incisor eruption	Day 8
Development of fur	Day 9
Ear opening	Day 11
Eye opening	Day 14
Testes descent	Day 25
Vaginal opening	Day 30

[1] From Alder and Zbinden (1977)

Table 2. Movements enforced by acute manipulations. Information: Time of appearance

Test	Time of appearance in rats[1]
Righting reflex[2]	Day 1.8
Negative geotaxis[3]	Day 4.8
Cliff avoidance[4]	Day 4.8
Palmar grasp[5]	Day 6.3 (waned)
Auditory startle	Day 11.4
Vibrissae placing[6]	Day 12.5
Free fall righting	Day 17.5
Visual placing[7]	Day 17.6
Convulsive behavior[8]	

[1] From Smart and Dobbing 1971. For reflex ontogeny in mice, see Fox (1965)
[2] Rat placed on back, turns over on ventral surface
[3] Placed head down on 20° C slope, turns to face up
[4] Moves away from edge of bench
[5] Grasps a paper clip with forepaws if stroked
[6] Held by tail, vibrissae touching vertical surface, lifts head and extends forelegs in direction of surface
[7] Same as [6] without vibrissae touching surface, same response
[8] Measures response latency and seizure type, e.g., following flurothyl inhalation (Golub and Kornetsky 1974; Su and Okita 1976) or audiogenic seizures (Davis and Lin 1972)

Doing these experiments in one's own laboratory one may find that the various reflexes appear considerably earlier or later than indicated in Table 2, or that the variability of the findings is more marked. The major reasons for such discrepancies are strain differences, perhaps also lack of experience and skill of the experimenters. Moreover, the criteria of successful performance may be different from those of other investigators. Such discrepancies are of no concern as long as the results from one's own laboratory remain stable and reproducible.

A special case in this group are tests that measure convulsive responses. Here the latency of convulsive response and the type of the induced convulsions are recorded. For example, a significant decrease in latency of flurothyl-induced convulsive seizures was observed in mice prenatally exposed to methylmercury (Su and Okita 1976).

2.2.2. Spontaneous Movements. This next group of tests includes experiments which measure spontaneous movements in familiar surroundings. The animals are left in places that do not frighten them. From these experiments one gets two kinds of information: the time of appearance of various movements, and the rate. Table 3 shows the types of movements of rat pups that can be recorded.

For the measurement of the spontaneous activity of small animals various home-made or commercially available activity cages have been proposed (Lasky et al. 1977). Although they provide rather crude measurements, they are

Table 3. Spontaneous movements in familiar surroundings[1]. Information: Time of appearance and rate[2]

Crawling	Pivoting (circular movement)
Head lifting	Standing on hindlegs
Jumping	Climbing
Huddling behavior	Emergence from home cage
Hyperactivity induced by L-dopa[3]	

[1] From Altman et al 1971, Martin et al. 1978, and others
[2] Time of appearance and rate depend on experimental set-up and must be established for each procedure
[3] s.c. injection of 100 mg/kg L-dopa induces vigorous crawling (Fechter and Annau 1977)

occasionally helpful as a preliminary screening device. For example, transient hyperactivity could be detected in rat pups that were exposed to morphin sulfate in utero (Sobrian 1977b). Altered habituation to the activity cage environment as demonstrated by a lesser decrease in motor activity with time was found in 10 day old rat pups prenatally exposed to dichloromethane (Bornschein et al. 1980). This effect may be due to elevated maternal carboxyhemoglobin concentrations which are known to be associated with behavioral effects in newborn rats (Fechter and Annau 1977). A simple test procedure that measures spontaneous behavior of rats and that can give information about a variety of neuromuscular and emotional factors, is called emergence from home-cage (Ader and Conklin 1963). All one has to do, is to take the cover off the home-cage and to measure the time until the animal gets out. The best time to do this experiment is when the rats are 60–100 days old.

An activity that is very characteristic for rat pups is huddling. Looking at the nestling in a heap of young rats, one would never surmise that there is system even in this disordered scramble. But if one identifies each pup with a readily visible marking, and records the huddle from above with a TV camera, one can reconstruct the motion of the individual animals through the huddle. And what comes out is a graceful floating of the pups, up from the bottom of the pile to the top, and back down again (Alberts 1978). The huddling behavior could probably be used for the detection of animals with brain damage. Alberts (1978) has introduced an anesthetized pup into a huddle of healthy crawlers and has observed that the handicapped animal remained at the bottom of the pile, the least desirable spot, when the ambient temperature was high (36° C), but was lifted up to the top in a cold environment (24° C), when the healthy competitors preferred the cosyness at the depth of the heap.

2.2.3. Performance Tests Requiring Special Equipment. With these procedures movements enforced by mechanical apparatus are measured. In this area of research human inventiveness has been particularly productive. It would take many pages to describe all the machines that have been built and published, many pages more to tell about those that were abandoned. The fascination with these tests lays in the fact that they do not only record an all-or-none effect (the

Table 4. Movements enforced by mechanical apparatus. Information: Time of appearance and achievement score[1]

Hanging – grip strength[2,3]
Clinging to inclined screen[4,5]
Clinging to and descending vertical rope[3]
Climbing vertical rod[3]
Climbing inclined screen[6]
Locomotion on parallel rods[2,7]
Homing (walk over from box to home-cage)[3]
Rotarod performance – rim escape test[12]
Activity wheel[10,11]
Swimming ability[8,13]

[1] Time of appearance and achievement score depend on experimental set-up and must be established for each procedure
[2] Werboff et al. 1961
[3] Altman et al. 1971
[4] Cabe et al. 1978
[5] Sobotka et al. 1974
[6] Werboff et al. 1962
[7] Furchtgott and Echols 1958
[8] Spyker et al. 1972
[9] Butcher et al. 1972
[10] Lasky et al. 1977
[11] Bornschein et al. 1980
[12] Balancing ability on the rim of a glass beaker with possibility to move towards refuge box (Smart and Dobbing 1971)
[13] Klaus and Hacker 1978

enforced movement is present or absent), but can also obtain a quantitative measurement of achievement. In Table 4 a small selection of these tests is listed. It is clear that these procedures should be able to detect loss of muscular strength and impaired neuromuscular functions resulting in a falling-off of the animals from the apparatus. Young rats are quick to learn that such mishaps do not hurt, and from then on their performance becomes erratic. A bucket of cold water placed below the apparatus acts as a more persuasive deterrent than a padded table top, and thus improves reproducibility of the test results.

Sometimes a paradoxical effect is observed in that brain-damaged animals do not fall off the devices but cling to them tenaciously. This was observed with offspring of malnurished rats (Altman et al. 1971) and with rat pups that were exposed to methylmercury, 0.1–2.5 mg/kg p.o. on days 6–15 of gestation (Sobotka et al. 1974). In the rotarod test offspring of rats treated with 200 mg/kg diazepam daily from day 7–20 of gestation performed significantly better than the controls, a finding that is, in the opinion of the authors, difficult to interpret (Butcher and Vorhees 1979).

2.2.4. Behavior in Unfamiliar Environment. In this group of behavioral tests spontaneous movements of the animals in unfamiliar surroundings are observed. A selection of these procedures is listed in Table 5. Note that the information obtained from these tests is quite varied: first we can measure latency, that is the

Table 5. Spontaneous movements in unfamiliar surroundings. Information: Latency, rate, organization of behavior

Open field procedure	Residential maze[1]
Exploratory maze	Spontaneous alternations[2]
Elevated platform[3]	

[1] Elsner et al. 1979
[2] Rats, age 17 days or more, are placed repeatedly in *T* maze. *T* maze is cleaned after each trial. Normally rats alternate between the two goal arms (Sobrian 1977a)
[3] Rat is placed on 7 × 7 × 3 cm platform and the time until it steps down is measured (Lasky et al. 1977)

time necessary for the animal to decide to do anything. Next, we can record rate, e.g., by observation of motor activity, and finally we can score various qualities of movements.

The most widely used test in this series is the open field procedure of which there are innumerable modifications. The equipment consists of a wooden board with squares drawn on it. Rats are put in the open field, and ambulation, i.e., number of center squares and peripheral squares crossed per unit time, rearing, grooming, backings, head liftings, etc., are scored (Spyker et al. 1972; Su and Okita 1976; Lasky et al. 1977; Winneke et al. 1977).

In the open field situation another behavioral response, i.e., deposition of fecal pellets and water puddles can be measured. It is believed that with these activities the animal reveals its emotions. The results observed with rat pups damaged by pre- and postnatal exposure to lead may be cited as an example. These animals exhibited increased ambulation, rearing and grooming, but no emotional changes (Winneke et al. 1977). In contrast, methylmercury administered to pregnant mice caused increased latency, lower number of groomings, and decreased frequency of urination of the offspring. This mental state was interpreted as "indifference regarding a new environment" (Su and Okita 1976).

The open field procedure has a much greater information content than that which can be gathered with simple observational methods. Moreover, it would be most desirable to extend the observational period over a much longer period of time than that which can realistically be demanded even from the most dedicated scientist. It is in this area of biological research that the computer must take over a large share of the work. A good example of how a computerized procedure can extract data from complex behavioral pattern over a prolonged period of time was described by Elsner et al. (1979). The movements of a rat in a relatively complex residential maze were registered with the help of 18 infrared optical gates. In 24 h subdivided in a 12 h dark and 12 h light cycle an animal signalled approximately 25,000 movements that were either due to locomotion, defined as consecutive crossings of different gates, or to local activity, i.e., consecutive crossings of the same gate. Computer programs were established that recorded and evaluated locomotor and local activity as a function of time and maze location. In addition, the time spent per visit in each location could be evaluated. The maze has five types of bifurcations where the animal has to make a decision where it wants to go. For each type of bifurcation the computer can calculate the probability for each possibility the animal can choose. It was found that the behavior at the different decision points was, by no means, random, but very characteristic and highly reproducible. The behavioral pattern of the rats varied characteristically over the 24 h observational period, was highly influenced by the light-dark cycle and clearly demonstrated significant differences between various maze locations. From these results it is evident that there is more to spontaneous animal movements than can be recorded by simple observation.

2.2.5. Operant Conditioning. The next stage in complexity is represented by test procedures that measure movements enforced by reward and punishment. From the list shown in Table 6 it is evident that these tests are ambitious undertakings that attempt to assess such things as learning, discrimination, e.g., between various tone frequencies (Walker and Furchtgott 1970), light intensities or visual patterns (Winneke et al. 1977), and memory. Despite the complexity of these messages, all we can observe and record are the animal's movements. But in this case, the movements are not enforced by simple manipulations or crude devices,

Table 6. Movements enforced by reward and punishment. Information: Responsiveness, learning (acquisition, retention, and extinction) and discrimination

Scheduled controlled behavior[1]
Conditioned avoidance learning (e.g., shuttle box learning)[2,3,4]
Rewarded swimming maze behavior (escape learning)[5,6,7]
Rewarded maze behavior[6,8]
Appetitive learning[9,10,11]
Discriminative learning[12]
Taste aversion learning[13,14]
Odor aversion learning[15]

[1] Müsch et al. 1978
[2] Walker and Furchtgott 1970
[3] Bush and Leathwood 1975
[4] Bornschein et al. 1980
[5] Butcher et al. 1972
[6] Coyle and Singer 1975
[7] Smart et al. 1977
[8] Werboff et al. 1962
[9] Amsel et al. 1976
[10] Amsel et al. 1977
[11] Kenny and Blass 1977
[12] E.g., learn the right way to take to reach home cage (Altman and Bulut 1976), or to obtain food reward (Lamptey and Walker 1978), or learn to jump at door marked with proper pattern to obtain food (Winneke et al. 1977)
[13] Gregg et al. 1978
[14] Baker et al. 1977
[15] Rudy and Cheatle 1977

and they cannot be scored by the look-and-count technique. Highly sophisticated equipment is necessary to force the animals to collaborate.

The most frequently used method in this group of tests is based on operant conditioning procedures. Around this behavioral concept an immense edifice of scientific speculations and experimental facts has been built in recent years. It is based on the experimental situation in which the animals's behavior is determined by its consequences. Let us, to understand this concept, look at a simple example. A rat, after being placed in a cunningly rigged box, more or less by accident stumbles over a lever, an act which is automatically rewarded with food or drink by the machine. The rat will usually try this trick once more, and if the reward comes on again, it will go on pressing, demonstrating its ability to learn by experience. We now can alter the rules of the game and give the reward only when the lever is pressed at a high rate. And when this task is mastered, we can switch again and reward only if the subject ceases to operate the machine for a certain length of time. We can introduce sound or light cues to signal which one of the rules of the game is on, and with such interventions we rapidly push the animals to the limits of their learning ability. We can also see how long the rat remembers what it has learned (memory) and how long it takes to forget the original lesson if the expected rewards are not forthcoming any more (extinction).

Operant conditioning is, as was mentioned above, a vast scientific discipline of its own. It has obvious applications in behavioral teratology. For example, rats that were exposed to methylmercury prenatally (0.01–2 mg/kg p.o. on gestation days 6–9) performed poorly in a situation which required learning that high rate bar pressing was the thing to do to be rewarded with food (Müsch et al. 1978; Bornhausen et al. 1980).

To perform adequately in tests of this kind rats have to have a certain age to be able to press the levers or run through the various obstacle courses. Could tests be devised in which learning is assessed in very young pups? Behavioral scientists have long wondered at what age learning begins. They have tests ready with which it is possible to demonstrate learning abilities in rat pups as young as 2 days. A series of these tests is listed in Table 6. One procedure is called appetitive learning. In this test the pup must crawl towards its mothers's nipple and must learn whether the right or the left alley of a simple Y-maze leads to the desired goal (Kenny and Blass 1977). In a variation of this procedure the time for crawling to the mother is recorded when reward is given (faster) and when it is withheld (slower) (Amsel et al. 1976). Recently Johanson and Hall (1979) described a set-up in which appetitive learning ability of one day old rats can be tested. The pups must only lift their head to be revarded with a small amount of milk.

In another test rat pups learn to associate a novel taste with an unpleasant experience. They receive a little saccharine solution by direct injection into the mouth and are made ill immediately thereafter by an injection of Li Cl (2% of body weight of a 0.12–0.15 M solution). A few days later they remember this unpleasant experience and refuse to swallow saccharine solution (Gregg et al. 1978) or, if they are a little older, prefer to drink tap water when offered tap water on one day and saccharine solution on the following day (Gregg et al.

1978). This technique is called the taste aversion test. It is very difficult to use and often gives erratic results, mostly because pups tend to forget their bad experiences quite rapidly (Baker et al. 1977). A similar procedure is the odor aversion test. Here the rat pup is exposed to a novel odor, e.g., lemon or garlic. After this novel experience comes again the punishment in the form of a LiCl injection. A few days later the pup is placed in a cage with a wire mesh bottom under which wood shavings with two distinct odors are placed, one of the odors being that which the rat associates with ill effects. The animal shows us that it remembers the traumatic experience by avoiding the part of the cage where it must smell the material it has learned to hate (Rudy and Cheatle 1977).

2.2.6. Social Behavior. Another group of tests is concerned with measuring movements that are induced by placing the test animals in contact with other animals. A small sample of these procedures is listed in Table 7. They are designed to give information about social behavior, and they open up a whole new field of behavioral research.

It is quite conceivable that the ability of a rat to interact socially with another rat can be altered by a prenatally acquired brain lesion. But it is also readily understandable that social behavior will greatly depend on experimental circumstances.

2.2.7. Development of Sensory Functions. To end this series of tests it should be mentioned that the ontogeny of sensory function must also be considered. Many behavioral tests could be greatly upset if there were a disturbance of hearing, smelling or taste or if the vision of the test animal were impaired (Winneke et al. 1977). Test procedures in which sensory functions can be assessed, have therefore become part of the routine in behavioral teratology. Table 8 lists the most important mile stones of sensory development in rats.

Table 7. Movements enforced by placing the test animals in contact with other animals (social behavior)

Preference behavior[1]
Interaction with animal of same age[2]
Approach and contact behavior to sibblings and (anesthetized) dam
Pup retrieving[3]
Nest reconstruction[3]
Nursing behavior[3]
Water-spout competition[4]
Adult sexual behavior[5]

[1] Subject must choose between two companions, sitting in the two arms of a *T* maze (Peters 1978)
[2] Treated identically or control. Aggression, walk over, allogroom, mount (Peters 1978, Whatson and Smart 1978)
[3] Broitman and Donoso 1978
[4] Two thirsty rats (23 h water deprived) are given access to water for 3 min. (Whatson et al. 1975)
[5] Herrenkohl and Whitney 1976

Table 8. Sensory development of the rat and some tests for its assessment[1]

Hearing (auditory startle, day 11–13)
Vision (visual placing, day 14–18)
Taste (taste preference)
Odor[2 3] (odor preference, day 3–12, aversion day 3–8)
Heat perception (e.g., hot plate)

[1] Adapted from Alder and Zbinden 1977
[2] Sobrian and Cornwell-Jones 1977
[3] Cornwell-Jones and Sobrian 1977

In the beginning of this chapter it was mentioned that the major behavioral responses of rats consisted of various movements. Another response that may be recorded is ultrasonic vocalization. Rats emit ultrasonic signals of 20–50 kHz particularly under two conditions: when they are very young, and when they copulate. Ultrasound can be recorded with special microphons and, when played back at low speed, becomes audible to us. Rat and mouse pups emit ultrasonic calls when they are taken away from their nest, most probably in response to low ambient temperature (Okon 1972). At the age of 17–20 days when they have reached homoiothermy (Sales and Skinner 1979) these calls are not anymore elicited.

It was found that the ultrasonic calls by rats that are isolated and cold will attract their mothers. In contrast, the calls that follow tactile stimulation stops the adult and could thus be considered of a defensive nature (Noirot 1972). From this information it can be concluded that ultrasonic vocalization of rat pups contains some interesting information that is not adequately exploited by simply counting the number of calls and measuring their intensity. Up to now ultrasonic vocalization has not been used to assess prenatal brain damage. But the response is so predictable and reproducible that it could certainly be considered as one of the parameters to be scored in a toxicological test battery.

3. Behavioral Tests in Teratology

In the introduction to this paper it was stated that toxicologists never seriously questioned the soundness of the behavioral approach to teratology. Reading the many excellent papers on behavioral ontogeny they felt that all they needed to do was to pilfer a number of the elegant animal tests that worked so well in the hands of the behavioral scientists. This feeling is perhaps best expressed in a statement by Joan M. Spyker (1975) written in an excellent review on behavioral teratology. It reads as follows: "Behavior is at least as susceptibel to teratogenic influence as other developing systems. However, unlike structural birth defects, subtle behavioral abnormalities are not readily evident and may be revealed only by special tests during postnatal life. Particularly at low levels, teratogens may cause behavioral changes in the absence of gross functional or structural defects."

In 1974 the British Department of Health and Social Security issued revised guidelines on reproduction studies. In keeping with the general enthusiasm, the Department requested for the first time behavioral studies with the progeny, as documenteed by the following statement:

"5. Examination of the progeny

(b) Late effects of the drug on the progeny in terms of auditory, visual and behavioural function should be assessed . . .

(c) Under certain circumstances some of the progeny may be allowed to live and reach maturity so that their reproductive capacity could be assessed, and other late effects of the drug on the progeny in terms of behavioral, visual and auditory function determined."

Medicines Act. 1968, Notes for Guidance on Reproductive Studies. June 1974.

The shock waves that were created by this edict, were soon felt in all toxicology laboratories of the world. Although everybody was in favor of looking at behavioral effects in teratology, nobody really knew how to go about it. And those who tried the seemingly simple test procedures of the behavioral scientists saw their high expectations largely unfulfilled. Perhaps their expectations were too high. Most probably toxicologists did not read carefully enough the behavioral literature. If they had done so, they would have noticed that one word appears with conspicuously high frequency in papers on behavioral testing. It is the word "subtle". For example Spyker (1975) uses it 16 times in her 9 page review on behavioral teratology referred to above. "Subtle", according to Webster's New World Dictionary, means "hard to solve, detect, or understand"; it also stands fos "misterious", "abstruse" and "sly". It would be detrimental to the further development of behavioral teratology if this fact would not be clearly recognized. The behavioral changes that can be observed in such experiments, are often not dramatic. Only those studies that are carefully controlled and are conducted under highly standardized conditions and by well trained and experienced persons can be expected to yield useful and reproducible results.

In order to illustrate the subtleties of the effects observed in teratological experiments, a few examples gathered from the literature will be presented. As pre- and postnatal insults various forms of nutritional deficiency were selected. These represent well recognized teratogenic insults that greatly affect growth and development of the brain.

The first two experiments were done with mice on low protein diet from day 7 of gestation through the end of the lactation period (Bush and Leathwood 1975), and with rats whose food intake was severely restricted from day 7 of gestation through lactation (Smart and Dobbing 1971). To give an idea how severely the pups were affected, it should be noted that 37% of the protein-deficient mice pups died (none of the controls). Their forebrain was 16%, the cerebellum 20% smaller than that of the controls, and the DNA content in these brain regions was reduced by 15% and 23% respectively. At weaning the deficient mice weighed 7 g, the controls more than double (16 g). In the rat experiment the undernurished pups weighed about 50% less than the properly fed controls. It is thus no surprise that there was a delay in physical development in the deficient pups. But the magnitude of these effects was not impressive: in mice ears unfolded with a delay of 1 day, eyes opened 2 days later than in the controls, and fur appeared with a 3 days' delay. In rats incisor eruption occurred after 8.7 days and in controls after 8.1 days. Eye opening was delayed an average of 1.2 days. These differences were statistically significant, but they might have been easily missed if the experiments had not been conducted with great care.

In the deficient mice pups the various reflexes appeared with a 2–3 days' delay. In rats only four of eight reflexes tested had a significantly delayed onset. And here again, the differences were less than impressive: palmar grasp weaned with a 1.5 days' delay, auditory startle occurred 1 day later than in the controls, the response to visual placing was delayed by 2.2 days and free fall righting by 3 days.

Learning was tested in the mice at the age of 8–11 weeks. Acquisition of avoidance behavior was used, and the performance in the 14th session was considered. The well fed controls successfully avoided the shock by responding to the conditioned stimulus (light and sound) in 74% of the trials. This was only slightly better than the 52% of the undernourished animals.

A similar effect on learning was also observed in rats whose mothers had received a diet deficient in essential free fatty acids from day 14 of gestation through lactation. The pups that continued to receive the deficient diet showed poor performance in a Y-maze discrimination test with food reward. Over a 7 day testing period these animals made only 40% correct responses. The well fed controls did not perform spectacularly either, 63% correct responses was their score (Lamptey and Walker 1978).

It is often suggested that a variety of tests be used to assess the effects of potential behavioral teratogens. Although this appears to be a reasonable proposition, it is possible that the many results that are obtained with such a test battery are sometimes difficult to interpret.As an example some of the data of Werboff et al. (1962) who used prenatal X-irradiation as the teratogenic insult will be presented. Pregnant rats received this treatment either on day 5, 10, 15, or 20 of gestation. To illustrate the results I limit myself to the observations made in pups that had received the highest dose of 100 r.

In Table 9 statistically significant increases in motor and emotional activities compared to controls are indicated with ↑, significant decreases with ↓. In the maze learning situation ↑ means improved performance, ↓ means poorer performance than controls. Differences that were not statistically significant are listed as "NS".

The information provided in this experiment is very difficult to assess. Motor activity varies with time of testing and experimental procedures. In the maze learning procedure there were even differences between male and female animals.

To end this discussion of the quality of behavioral effects that can be observed in teratological experiments a study using the open field will be mentioned. The experiment was reported by Spyker et al. (1972) and was conducted with 30 day old mice. Their mothers received single i.p. injections of 8 mg/kg methylmercury diamide on day 7 or 9 of gestation. This is a hefty dose,

Table 9. Results of a teratologic experiment with rats (Werboff et al. 1962)

Test	Irradiated on			
	day 5	day 10	day 15	day 20
Motor activity on inclined plane at age of 25 days	↑	NS	↓	↓
Open field test at age of 55 days; motor activity	↑	↓	↑	NS
Open field test; emotional activities	NS	↓	NS	↓
Maze learning at age of 75 days, females	↑	NS	↓	NS
Maze learning at age of 75 days, males	NS	NS	↓	NS

corresponding to one fourth or one third of the LD_{50}. There were a few statistically significant differences between treated pups and controls: the controls waited an average of 5.1 s until they decided to move, methylmercury-treated animals had a center latency of 7.1 s. They deposited an average of 1.7 fecal pellets and left 0.52 urine puddles during the 2 min observation period. The controls produced an average of 2.9 fecal pellets and 0.89 urine puddles. In the same experiment a very striking effect was observed: one half of the methylmercury-treated pups made 3 or more steps backwards, whereas only 1 of 19 controls produced this movement. This observation shows that behavioral tests may indeed be able to detect marked differences between brain-damaged animals and untreated controls. However, there are not enough experiments available to decide which of the many possible tests is the most likely to detect such differences, nor do we know for sure whether different kinds of brain lesions are likely to manifest themselves in the same types of test procedures.

4. Interfering Factors in Behavioral Experiments

The goal that counts most in a young rat's life is survival in an extremely hostile world. For this purpose nature has endowed these animals with an ability to adjust to their environment, a gift that is so mysterious that it is totally beyond our comprehension. Recent experimental studies have only given us fleeting glances at the enormous plasticity of a rat's responses to extraneous influences. Many subtle changes of behavior were discovered to occur as a consequence of environmental conditions that the pup encounters when it is born. Whether it finds its cage full of toys (this is called an enriched environment), or cold and bare (a deprived environment) may affect various responses including learning and open field behavior (Coyle and Singer 1975; Ardila et al. 1977). Quite important for the pup's emotional and behavioral development are also unpleasant experiences, such as temporary solitary confinement, heat and cold, electric shock and painful injections (Salama and Hunt 1964; Hutchings 1967).

Moreover, we must not forget that we, the experimenters, are also part of the young animals' environment. With our squeeking shoes, loud voices and strange body odors, we are, most probably, as revolting to them as the noise of the telephone, the scratch of the stomach tube and a pinch with tweezers.

The number of sibblings that share the nest and compete for mother's nipples, as well as the abundance of the milk flow decisively shape the pup's future behavior. Young rats that had to fight for their fair share turned out to be more lively and less excitable later in life (Sadile et al. 1977). We also must keep an eye on the duration of gestation since it is often altered in drug-treated animals and may upset the developmental time-table of the offspring.

A very decisive factor appears to be early handling of the pups. A large body of experimental evidence indicates that rats that were gentled regularly in early life will later show many positive character traits: they explore more and defecate less in the open field situation, they learn faster and make fewer mistakes, show less vocalization and are easier to pick (Ader and Conklin 1963; Weinberg and Levine 1977). This reduction in emotionality could even be proven by chemical means: rat pups that were handled early in life showed a lower rise in plasma corticosterols than the controls after open field exposure, heat or electric shock treatment (Pfeifer et al. 1976).

The most important influence in a young rat's life is, of course, its mother. It is no wonder, therefore, that the dam's behavior is of critical significance for the future of the offspring. Even the slightest disturbance of the dam's health, a minor hormonal imbalance, drug-induced reduction of milk-flow or alterations of social behavior (Golub and Kornetsky 1974) may leave distinct imprints on the personalities of the offspring that may never be erased (Martin et al. 1978). And something more, there is little doubt that various environmental stresses to which the pregnant females are subjected, can also affect the future behavior of the offspring. Among such stresses we must include crowding, tilting, swimming, conditioned avoidance learning, open field exposure, sounds and repeated foot shocks (Archer and Blackman 1970; Sobrian 1977a). The finding is, of course, of importance for behavioral teratology, since administration of teratogens to pregnant animals be they chemical, physical, or nutritional, often entails stressful procedures.

It would be convenient if we could distinguish between these stress-induced behavioral alterations, mediated perhaps through hormonal, nutritional or circulatory disturbances in the pregnant female, from direct damage to the offspring. But from the enormous literature on this subject there is little hope that such an easy solution will ever be possible. Archer and Blackman (1970) call the present literature on prenatal stress "an untidy jumble of findings, many of which are not readily interpretable". Nevertheless, we must expect to see variations in offspring behavior occurring in various test procedures that are not a consequence of a teratogenic effect on the pup's brain, but merely due to stressful interventions to which the pregnant mothers were subjected. Even gentle handling of pregnant rats significantly affects the behavior of the offspring.

5. Consequences for Behavioral Teratology Testing

If one looks at the complexities of behavioral responses, it is readily apparent that one is faced with a difficult situation: on the one hand we have the subtleness of the biological effects, on the other the great variety of extraneous influences on dams and pups that can deeply affect offspring behavior. It is easy to understand, therefore, that the literature on behavioral teratology is replete with controversial, often absurdly contradictroy findings. Here is one example:

In 1963 Werboff and Kesner conducted an ambitious experiment: They treated pregnant rats on gestation days 5–8, 11–14, or 17–20 at eight hourly intervals with reserpine, chlorpromazine, or meprobamate s.c., the daily doses being 0.1 mg/kg, 6 mg/kg, and 60 mg/kg respectively. When the pups had reached the age of 82 days they underwent a 7-day training period in a straight alleyway with food reward. After this they were tested in a Lashley type III maze to obtain information on learning ability. The criterion of this test was 2/3 errorless consecutive runs in 20 s or less. The results obtained were the same for all three treatment periods, an observation which is by itself rather suprising. Of the three groups treated with psychotropic drugs the meprobamate rats were the only ones that differed significantly from the water controls and the two other treatment groups. Meprobamate, according to the authors, was detrimental to the learning ability. This is a very strong word considering that these rats needed an average of 12.7 runs to reach the criterion which was not that much worse than the 9.5 runs scored by the controls. The author's commented the result of the study as follows: "The finding that only meprobamate of the three tranquillizing drugs administered to gravid rats detrimentally altered maze learning ability of the offspring was anticipated." What the authors did not anticipate, however, was the outcome of an exact copy of their experiment conducted two years later by Hoffeld and Webster (1965). First of all, these authors found clearcut differences between the various time periods of treatment. Of the pups exposed early in gestation those receiving chlorpromazine showed a significant impairment of learning ability when compared to the controls and the meprobamate-treated rats, but not in comparison with the reserpine group. Of the animals exposed in mid-gestation the controls showed the greatest behavioral changes. Their performance was significantly worse than that of the meprobamate rats. Treatment in late gestation had no effect on learning ability in either group.

From this example, one must not conclude that the observed behavioral results were not real. On the contrary, the data are certainly valid under the conditions that prevailed in the two laboratories. The discrepancies, however, clearly demonstrate, that extraneous influences that are often impossible to control may upset or even subvert experimental results. Similar observations have been reported with other drugs. For example, Robertson et al. (1980) found increased activity and decreased latency in the open field in offsprings of rats treated with chlorpromazine, and this effect was present at various time periods. Earlier workers either saw no effect of chlorpromazine in open field behavior of the offspring (Werboff and Havlena 1962), or reported increased or decreased activity depending on the time of testing (Clark et al. 1970). From such observation we must conclude that great care must be exercised when results of behavioral studies in teratology are evaluated. And it appears important that more research on non-specific effects on offspring behavior be conducted.

For the evaluation of behavioral changes in teratological experiments it is important to observe the overall development of the offspring which is best reflected in their growth curves. In most cases reported in the literature some evidence of maternal toxicity or developmental retardation of the offspring is associated with the behavioral abnormalities. For example, in an experiment reported by Vorhees et al. (1979) in which pregnant rats received 25 mg/kg prochlorperazine edisylate, 20 mg/kg fenfluramine hydrochloride or 40,000 IU/kg vitamin A palmitate p.o. from day 7–20 of gestation there were significant effects on body weights of dams and offspring, drug-related deaths of dams and reduction in numbers with viable litters. A variety of behavioral changes demonstrated in the pups were therefore associated with reproductive and growth effects. It is thus not easy to separate the consequences of maternal and fetal toxicity from true behavioral effects. It would be important to repeat these experiments with lower, non-toxic doses. With propoxyphene hydrochloride (75 mg/kg), on the other hand, no maternal and fetal toxicity was observed. The fact that behavioral changes were observed in these animals is remarkable and justifies perhaps the author's suggestion that propoxyphen can be considered a "pure" behavioral teratogen. This conclusion would be more convincing, though, if the pups had been raised by foster-mothers, since an effect of the drug treatment on maternal behavior cannot be excluded with certainty (Spyker and Spyker 1977).

6. Conclusions

From the data reported in this review we must conclude that the behavioral sciences provide a large number of exciting test procedures, many of which will certainly find an application in behavioral teratology. We must also be aware, however, that the probability to discover a subtle behavioral alteration caused by a teratogen is reduced by postnatal test procedures that also affect behavioral responses. The current practice to subject pups to a large battery of different behavioral tests (Grant 1976; Butcher and Vorhees 1979) is likely to introduce so many new variables that a subtle behavioral effect that is related to the teratogen may not be recognized with certainty (Archer and Blackman 1970). Behavioral teratologists should, therefore, curb their enthusiasm and limit themselves to a small number of carefully selected procedures (Grant 1976). Unfortunately, it is not yet possible to pinpoint the tests that should be done. But what we see clearly is the direction our research must take. We need procedures that can quantify a broad spectrum of behavioral characteristics, tests that involve a minimum of manipulations and training of the animals. We must also seriously study the question whether our behavioral experiments should be done with very young animals, or whether it might not be better to wait until the pups have reached maturity. Until these very crucial problems are solved, it serves no useful purpose to legislate the general adoption of behavioral test batteries whose usefulness has not been validated and which may become obsolete in a few years.

Since results of behavioral teratology studies are often fickle, it would be most desirable, if all experiments were done with several doses, and not with just one as it is mostly the case. In all instances, when the observed effects are so subtle that complicated statistics are necessary to make them look respectable, it should be mandatory to repeat the experiment. There is no doubt that many irrelevant observations would remain unreported if behavioral toxicologists abide by these rules.

References

Ader R, Conklin PM (1963) Handling of pregnant rats: effects on emotionality of their offspring. Science 142: 411–412

Alberts JR (1978) Huddling by rat pups: Group behavioral mechanisms of temperature regulation and energy conservation. J Comp Physiol Psychol 92: 231–245

Alder S, Zbinden G (1977) Methods for the evaluation of physical, neuromuscular and behavioral development of rats in early postnatal life. In: Neubert D, Merker HJ, Kwasigroch TE (eds) Methods in prenatal toxicology. Thieme, Stuttgart, pp 175–185

Altman J, Bulut FG (1976) Organic maturation and the development of learning capacity. In: Rosenzweig MR, Bennett EL (eds) Neural mechanisms of learning and memory. MIT Press, Cambridge MA, London, pp 236–240

Altman J, Sudarshan K, Das GD, McCormick N, Barnes D (1971) The influence of nutrition on neural and behavioral development: III. Development of some motor, particularly locomotor patterns during infance. Dev Psychobiol 4: 97–114

Amsel A, Burdette DR, Letz R (1976) Appetitive learning, patterned alternation, and extinction in 10-d-old rats with non-lactating suckling as reward. Nature 262: 816–818

Amsel A, Radek CC, Graham M, Letz R (1977) Ultrasound emission in infant rats as an indicator of arousal during appetitive learning and extinction. Science 197: 786–788

Archer JE, Blackman DE (1970) Prenatal psychological stress and offspring behavior in rats and mice. Dev Psychobiol 4: 193–248

Ardila R, Rezk M, Polanco R, Pereira F (1977) Early handling, electric shock, and environmental complexity: effects on exploratory behavior, "emotionality", and body weight. Psychol Rev 2: 219–224

Baker JJ, Baker TB, Kesner RP (1977). Taste aversion learning in young and adult rats. J Comp Physiol Psychol 91: 1168–1178

Bornhausen M, Müsch HR, Greim H (1980) Operant behavior performance changes in rats after prenatal methylmercury exposure. Toxicol Appl Pharmacol 56: 305–310

Bornschein RL, Hastings L, Manson JM (1980) Behavioral toxicity in the offspring of rats following maternal exposure to dichlormethane. Toxicol Appl Pharmacol 52: 29–37

Broitman ST, Donoso AO (1978) Effects of chronic imipramine and clomipramine oral administration on maternal behavior and litter development. Psychopharmacology 56: 93–101

Bush M, Leathwood PD (1975) Effect of different regimens of early malnutrition on behavioral development and adult avoidance learning in Swiss white mice. Br J Nutr 33: 373–385

Butcher RE, Vorhees CV (1979) A preliminary test battery for the investigation of the behavioral teratology of selected psychotropic drugs. Neurobehav Toxicol [Suppl 1] 1: 207–212

Butcher RE, Brunner RL, Roth T, Kimmel CA (1972) A learning impairment associated with maternal hypervitaminosis-A in rats. Life Sci 11: 141–145

Cabe PA, Tilson HA, Mitchell CL, Dennis R (1978) A simple recording grip strength device. Pharmacol Biochem Behav 8: 101–102

Clark CVH, Gorman D, Vernadakis A (1970) Effects of prenatal administration of psychotropic drugs on behavior of developing rats. Dev Psychobiol 3: 225–235

Cornwell-Jones C, Sobrian SK (1977) Development of odor-guided behavior in Wistar and Sprague-Dawley rat pups. Physiol Behav 19: 685–688

Coyle IR, Singer G (1975) The interaction of post-weaning housing conditions and prenatal drug effects on behavior. Psychopharmacologie 41: 237–244

Davis WM, Lin CH (1972) Prenatal morphine effects on survival and behavior of rat offspring. Res Commun Chem Pathol Pharmacol 3: 205–214

Elsner J, Looser R, Zbinden G (1979) Quantitative analysis of rat behavior patterns in a residential maze. Neurobehav Toxicol [Suppl 1] 1: 163–174

Fechter LD, Annau Z (1977) Toxicity of mild prenatal carbon monoxide exposure. Science 197: 680–682

Fox WM (1965) Reflex ontogeny and behavioral development of the mouse. Anim Behav 13: 234–241

Furchtgott E, Echols M (1958) Locomotor coordination following pre- and neonatal X irradiation. J Comp Physiol Psychol 51: 292–294

Golub M, Kornetsky C (1974) Seizure susceptibility and avoidance conditioning in adult rats treated prenatally with chlorpromazine. Dev Psychobiol 7: 79–88

Grant LD (1976) Research strategies for behavioral teratology studies. Environ Health Perspect 18: 85–94

Gregg B, Kittrell EMW, Domjan M, Amsel A (1978) Ingestional aversion learning in pre-weanling rats. J Comp Physiol Psychol 92: 785–795

Herrenkohl LR, Whitney JB (1976) Effects of prepartal stress on postpartal nursing behavior, litter development and adult sexual behavior. Physiol Behav 17: 1019–1021

Hoffeld DR, Webster RL (1965) Effect of injection of tranquillizing drugs during pregnancy on offspring. Nature 205: 1070–1072

Hutchings DE (1967) Infantile body temperature loss in rats: effects of duration of cold exposure, rate of heat loss, level of hypothermia, and rewarming on later emotionality. Psychol Rep 21: 985–1002

Johanson IB, Hall WG (1979) Appetitive learning in 1-day-old rat pups. Science 205: 419–421

Kenny JT, Blass EM (1977) Suckling as incentive to instrumental learning in pre-weanling rats. Science 196: 898–899

Klaus S, Hacker E (1978) Die Entwicklung des Schwimmverhaltens bei Mäusen als Verhaltenstest in der Toxikologie. Z Versuchstierk 20: 81–87

Lamptey MS, Walker BL (1978) Learning behavior and brain lipid composition in rats subjected to essential fatty acid deficiency during gestation, lactation, and growth. J Nutr 108: 358–367

Lasky DI, Zagon IS, McLaughlin PJ (1977) Effect of maternally administered heroin on the motor activity of rat offspring. Pharmacol Biochem Behav 7: 281–284

Martin JC, Martin DC, Sigman G, Radow B (1978) Maternal ethanol consumption and hyperactivity in cross-fostered offspring. Physiol Psychol 6: 362–365

Müsch HR, Bornhausen M, Kriegel H, Greim H (1978) Methylmercury chloride induces learning deficits in prenatally treated rats. Arch Toxicol 40: 103–108

Noirot E (1972) Ultrasounds and maternal behavior in small rodents. Dev Psychobiol 5: 371–387

Okon EE (1972) Factors affecting ultrasound production in infant rodents. J Zool (Lond) 168: 139–148

Peters DP (1978) Effects of prenatal nutritional deficiency on affiliation and aggression in rats. Physiol Behav 20: 359–362

Pfeifer WD, Rotundo R, Myers M, Denenberg VH (1976) Stimulation in infancy: unique effect of handling. Physiol Behav 17: 781–784

Robertson RT, Majka JA, Peter CP, Bokelman DL (1980) Effects of prenatal exposure to chlorpromazine on postnatal development and behavior of rats. Toxicol Appl Pharmacol 53: 541–549

Rudy JW, Cheatle MD (1977) Odor aversion learning in neonatal rats. Science 198: 845–846

Sadile AG, Lat J, Cioffi LA (1977) Late effects of early postnatal malnutrition on habituation dynamics of spontaneous exploratory activity in adult rats. Activ Nerv Sup 19: 135–137

Salama AA, Hunt JMcV (1964) "Fixation" in the rat as a function of infantile shocking, handling, and gentling. J Gen Psychol 105: 131–162

Sales GD, Skinner NC (1979) The effect of ambient temperature on body temperature and on ultrasonic behavior in litters of albino laboratory mice deprived of their mothers. J Zool (Lond) 187: 165–281

Smart JL, Dobbing J (1971) Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behaviour in the rat. Brain Res 28: 85–95

Smart JL, Whatson TS, Dobbing J (1977) Perseverative behavior by adult offspring of underfed mother rats. Activ Nerv Sup (Praha) 19: 128–129

Sobotka TJ, Cook MP, Brodie RE (1974) Effects of perinatal exposure to methylmercury on functional brain development and neurochemistry. Biol Psychiat 8: 307–320

Sobrian SK (1977a) Aversive prenatal stimulation: effects on behavioral, biochemical, and somatic ontogeny in the rat. Dev Psychobiol 10: 41–51

Sobrian SK (1977b) Prenatal morphine administration alters behavioral development in the rat. Pharmacol Biochem Behav 7: 285–288

Sobrian SK, Cornwell-Jones C (1977) Neonatal 6-hydroxytryptamine alters olfactory development. Behav Biol 21: 329–340

Spyker DA, Spyker JM (1977) Response model analysis for cross-fostering studies: prenatal versus postnatal effects on offspring exposed to methylmercury dicyanodiamide. Toxicol Appl Pharmacol 40: 511–527

Spyker JM (1975) Assessing the impact of low level chemicals on development: behavioral and latent effects. Fed Proc 34: 1835–1844

Spyker JM, Sparber SB, Goldberg AM (1972) Subtle consequences of methylmercury exposure: Behavioral deviations in offspring of treated mothers. Science 177: 621–623

Su MQ, Okita GT (1976) Behavioral effects on the progeny of mice treated with methylmercury. Toxicol Appl Pharmacol 38: 195–205

Vorhees CV, Brunner RL, Butcher RE (1979) Psychotropic drugs as behavioral teratogens. Science 205: 1220–1225

Walker S, Furchtgott E (1970) Effect of prenatal X-irradiation on the acquisition, extinction, and discrimination of a classically conditioned response. Radiat Res 42: 120–128

Weinberg J, Levine S (1977) Early handling influences on behavioral and physiological responses during active avoidance. Dev Psychobiol 10: 161–169

Werboff J, Havlena J (1962) Postnatal behavioral effects of tranquillizers administered to the gravid rat. Exp Neurol 6: 263–269

Werboff J, Kesner R (1963) Learning deficits of offspring after administration of tranquillizing drugs to the mothers. Nature 197: 106–107

Werboff J, Goodman I, Havlena J, Sikov MR (1961) Effects of prenatal X-irradiation on motor performance in the rat. Am J Physiol 201: 703–706

Werboff J, Havlena J, Sikov MR (1962) Effects of prenatal X-irradiation on activity, emotionality and maze-learning ability in the rat. Radiat Res 16: 441–452

Whatson TS, Smart JL (1978) Social behavior of rats following pre- and early postnatal undernutrition. Physiol Behav 20: 749–753

Whatson TS, Smart JL, Dobbing J (1975) Dominance relationships among previously undernourished and well fed male rats. Physiol Behav 14: 425–429

Winneke G, Brockhaus A, Baltissen R (1977) Neurobehavioral and systemic effects of long term blood-lead elevation in rats. I. Discrimination learning and open field-behavior. Arch Toxicol 37: 247–263

Received July 10, 1981