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METHODS TO DETECT DEVELOPMENTAL TOXICANTS SESSION

METHODS FOR DETECTING LONG-TERM CNS DYSFUNCTION AFTER PRENATAL EXPOSURE TO NEUROTOXINS

Charles V. Vorhees

Division of Developmental Biology
Children's Hospital Research Foundation
and
Department of Pediatrics
University of Cincinnati College of Medicine
3333 Burnet Avenue
Cincinnati, OH 45229-3039

ABSTRACT

Current U.S. Environmental Protection Agency regulatory guidelines for developmental neurotoxicity emphasize functional categories such as motor activity, auditory startle, and learning and memory. A single test of some simple form of learning and memory is accepted to meet the latter category. The rationale for this emphasis has been that sensitive and reliable methods for assessing complex learning and memory are either not available or are too burdensome, and that insufficient data exist to endorse one approach over another. There has been little discussion of the fact that learning and memory is not a single identifiable functional category and no single test can assess all types of learning and memory. Three methods for assessing complex learning and memory are presented that assess two different types of learning and memory, are relatively efficient to conduct, and are sensitive to several known neurobehavioral teratogens. The tests are a 9-unit multiple-T swimming maze, and the Morris and Barnes mazes. The first of these assesses sequential learning, while the latter two assess spatial learning. A description of each test is provided, along with procedures for their use, and data exemplifying effects obtained using developmental exposure to phenytoin, methamphetamine, and MDMA. It is argued that multiple tests of learning and memory are required to ascertain cognitive deficits; something no single method can accomplish. Methods for acoustic startle are also presented.

Address correspondence to: Charles V. Vorhees, Ph.D., Div. of Developmental Biology, Children's Hospital Research Foundation, 3333 Burnet Ave., Cincinnati, OH 45229-3039.
Phone: 513-636-8622, Fax: 513-636-3912
E-mail: charles.vorhees@chmcc.org

Driscoll (1) has summarized the EPA developmental neurotoxicity guidelines. There are several tiers of testing that the EPA requires for such assessments. In all three there are functional evaluations required. In the first tier neurobehavioral screening tests are indicated. In the second tier, the same neurobehavioral methods may be used as in the first. When one gets to tier-3 evaluation, the experimental design becomes focused on more specific hypotheses and discovery of mechanisms of action, and more detailed behavioral methods may be required. The measures of functional development specified in the guidelines include assessments of motor activity, auditory startle, and learning and memory. These assessments are to be performed at various ages under the assumption that during different stages of development one may see different manifestations of treatment-related effects. With this regulatory context in mind, the central problem for the investigator becomes how to determine the utility and theoretical rationale for selecting among the universe of possible tasks that are available. This is the subject of the present discussion. In addition, I will argue that the category described in the guidelines as "learning and memory," is not a single category at all, but is actually a whole collection of categories and that no single test of learning and memory is therefore adequate for meeting this aspect of the guidelines.

To begin, I provide a framework for thinking about neurobehavioral teratogenesis. Wilson (2) showed the relationship among malformations and embryolethality as two parallel sigmoidal dose-response curves. Butcher and I later expanded this to include three curves (3) and later still to four curves (4), each parallel to the previous ones, but shifted to the left, i.e., the new curves were lower on the dose axis (the abscissa). The most severe manifestation of developmental insult is embryolethality as described by Wilson. The second curve represents the dose-response profile for malformations, and lies to the left of that for lethality. The next curve, as we proposed it, is for growth abnormalities, and lies to the left of the curve for malformations. The curve furthest to the left is for functional effects. Functional effects may be of the central nervous system, as for neurobehavioral teratogenesis, or they may represent effects on other organ systems, such as immunological or cardiovascular dysfunctions. Some disagree with the exact order and the spacing of the curves relative to one another, but the overall relationships have been documented numerous times. Effects on body weight and dysfunction are probably very close together for some compounds and there are cases where the effects on body weight are to the left of the curve for functional effects. Hence, functional effects of the central nervous system cannot be said to always be more sensitive than other measures, but what is clear is that more often than not, the functional effects curve is the furthest to the left.

Wilson (2) also conceptualized the period of maximum vulnerability to malformations. He portrayed this relationship as a slightly skewed bell-shaped curve spanning the period of organogenesis. This depiction is accurate for malformations, especially major malformations, in which the period of susceptibility begins with the onset of organogenesis and the period of maximum vulnerability peaks during the early-to-middle of this phase of development, then declines to the end of embryogenesis, which is usually defined as the closure of the hard palate in experimental animals. One of the things that Wilson's curve fails to capture very well, however, is development of the central nervous system (CNS). The development of the CNS does not fit this scheme because, except for the neural tube, large portions of brain and spinal cord development occur outside the period of organogenesis. Rodier (5) has presented a set of curves, in which tritiated thymidine incorporation into various brain regions during periods of maximum neurogenesis are plotted. What can be seen in her review is that for different parts of the brain, cell division occurs in each region during different stages of development. Most importantly for this discussion is that by the end of organogenesis as traditionally defined in teratology, much of central nervous system neurogenesis remains incomplete. The incomplete portions extend through fetal development and even extend into the postnatal period. This pattern for neurogenesis is, in fact, an underestimate of the various kinds of later developmental events that go on in CNS ontogeny. That is, neurogenesis represents only the tip of the iceberg of brain development. Figure 1 shows general development as adapted from Wilson on the top half, and CNS development on the bottom half. As can be seen, neurogenesis is but one stage of brain ontogeny and there is also migration of the cells to their final destination, and proper settling of neuroblasts within each nucleus or cortical layer. These events are followed by terminal differentiation (not shown) and synaptogenesis where neuronal processes come into apposition to one another to form synapses. Gliogenesis occurs simultaneously and is followed by myelination . In rodents, fetal developmental stages and even the postnatal period are analogous to human second and third trimester intrauterine CNS development (6). Therefore, different ways of thinking about developmental exposures are needed when contemplating possible effects on the central nervous system. When considering critical or sensitive

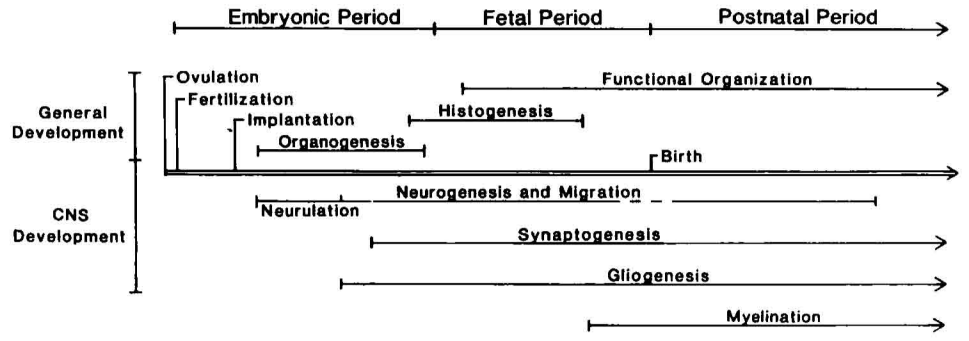

FIGURE 1

Time line for early rodent development. Top: General development from ovulation through early postnatal life. Bottom: Overlapping processes of CNS development to illustrate the prolonged ontogeny of the brain and spinal cord compared to that for most other organ systems.

periods of development, the windows of vulnerability are a function of whether the drug or chemical in question is affecting events of synaptogenesis or of neurogenesis, or whether it is affecting myelination, or receptor development. There are many events that have to be considered but we do not yet possess specific behavioral tools which can assess dysfunctions associated with specific disruptions of each of these processes. For this reason apical assessments of CNS function are usually necessary. However, virtually all behavioral methods are apical, therefore, selecting an apical behavioral test provides little guidance to the investigators. A further degree of specification is required and this is derived from what are called functional domains, i.e., the major functions that the central nervous system possesses (7). The EPA guidelines described above perform this function for the investigator by indicating that the functional domains of locomotor assessment and auditory startle are required. The guidelines appear to provide this for the category of learning and memory, but as already mentioned, this one is actually too broad to be very useful and further specification must be imposed in order to make this meaningful. Once tasks are selected, one ends up with a series of tests that measure a myriad of component processes acting as integrated systems, the output of which is locomotion, an acoustic startle response, etc.

The first test of learning and memory to be discussed is the Cincinnati maze. It was named the Cincinnati maze because it is a derivation of a simpler maze call the Biel maze (8). Both mazes are depicted in Fig. 2 (9). When animals are put in at point A and swim to point B, both the length of time it takes to traverse the maze and the number of errors of commission they make are recorded. An error of commission is defined as passing an imaginary line to a dead end alley. The dead-end alleys are all dual-ended cul-de-sacs shaped similar to the letter "T," and hence the maze is called a multiple T-maze. It is one of many kinds of multiple-T mazes. Swimming is selected for a variety of reasons. There are many developmental toxins that affect growth and body weight and when one uses food deprivation as the motivation and has animals of different body mass, then one gets into very complicated issues when trying to equate motivation across treatment conditions. For instance, are two animals of different weight both reduced to 90% of their free feeding weight equally motivated to swim to an escape ramp? There are some elaborate procedures for equating motivation, but by the time one gets done doing these procedures in a screening context, one would not have time left to find out whether the animals have cognitive impairments. Water mazes avoid many of these problems because within a wide range of body masses it has been demonstrated to have virtually no effect on swimming performance (10).

Some teratogens and most developmental neurotoxins do not produce substantial body weight changes at the doses used, therefore, within the range of typical body weight changes, water mazes effectively equalize

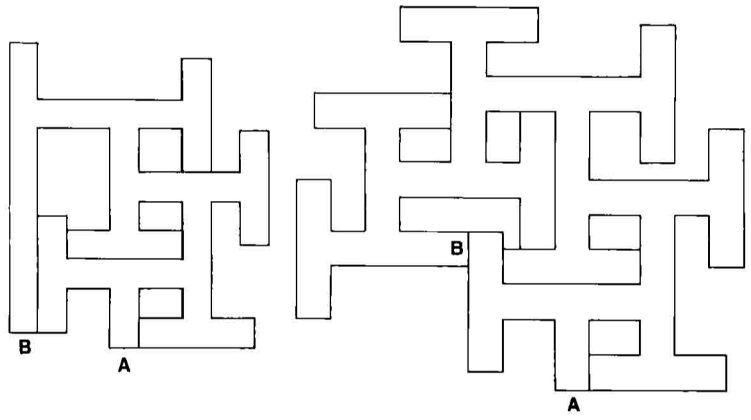

FIGURE 2

Schematic representation of two multiple-T mazes. Left: Biel water maze. Right: Cincinnati water maze. Drawn to scale, i.e., Cincinnati maze is both more complex than the Biel maze (it has more choice points and alleys), and is larger (wider channels and longer wall segments).

motivation and prevent the experimenter from having to conduct elaborate control procedures to equalize motivation across trials.

When an animal swims from point A to point B in the Cincinnati maze, it is called path A. After they have mastered that, the escape ramp is moved to point A and the animal then enters the maze at point B. Now the animal must swim from B to A and this is called path B. This will become important in looking at some of the data used to illustrate what can be obtained using such a test. The Biel maze is a six unit multiple-T maze, whereas the Cincinnati maze is a nine unit multiple-T maze, therefore, the Cincinnati maze is 50% more complex. Channel widths are also wider to accommodate larger animals.

Figure 3 shows data from the Biel maze after prenatal exposure to the anticonvulsant phenytoin. What can be seen in terms of errors and total time to escape from the maze for paths A and B combined, is that one obtains a dose-response impairment in performance in the offspring as a function of prenatal phenytoin dose. Since phenytoin is a teratogen at higher doses, let me stress that these maze effects occur at doses that are not teratogenic, i.e., they do not produce birth defects. This illustrates that behavioral teratogenic effects are dose-dependent, in contrast to what has sometimes been said about behavioral teratogenic effects, and that the dose-response curve for learning impairments is to the left of that for malformations.

Fig. 4 shows a subset of animals from the same experiment shown in Fig. 3. In Fig. 4 the dose-dependent effects of phenytoin are not quite as clear, but that is because the group sizes are much smaller. In this subset of animals some of the animals went through the Biel maze and some through the Cincinnati maze. One would expect even control animals to make more errors in the more complicated maze, and that is exactly what happens. Similarly, one would expect the treated animals to make more errors as well, and they do. What is important to note, however, is that the treated animals make proportionately more errors than the controls. This you would expect only if the impairment seen in the treated animals is a true learning deficit and only if the less complex maze in effect underestimates the true magnitude of the impairment in the treated animals. The data confirm that both statements are correct, the Cincinnati maze increases group differences disproportionately to the increase in errors seen among controls. This is most clearly seen in the low dose (100 mg/kg) group, which in the Biel maze shows an increase in errors that is not large enough to be significantly different from controls, whereas in the Cincinnati maze, this same group shows an increase in errors which is significantly increased compared to controls. The high dose

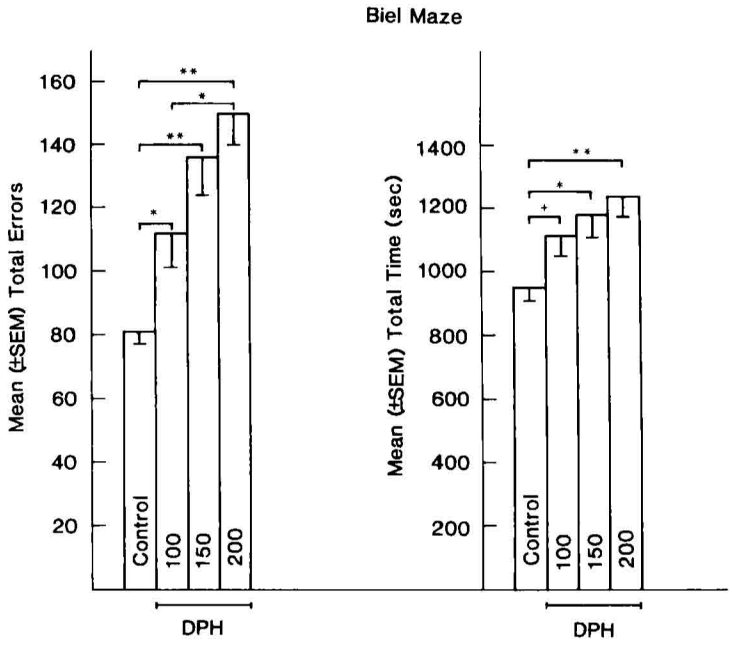

FIGURE 3

Effects of prenatal exposure to phenytoin on offspring performance in the Biel water maze (data analyzed using litter averages). Left: Average number of errors of commission across 12 maze trials (6 trials in path A and 6 trials in path B). Right: Average time to solve the maze across trials. Doses of phenytoin are expressed in mg/kg body weight administered by gavage once per day on E7-18. *$p < 0.05$, **$p < 0.01$ compared to controls or other doses.

(200 mg/kg) also shows the effect, in that the increase in errors compared to controls in the Biel maze is less than double (<100%), whereas in the Cincinnati maze the increase is more than double (>100%.). This is the principal advantage of the Cincinnati maze over the Biel maze.

In another experiment with prenatal phenytoin using two different doses administered on embryonic (E) day 7-18, the results of testing the offspring in the Cincinnati maze (full maze) in path B versus only a portion of it (Biel maze), are shown in Fig. 5. In this figure, animals were excluded if they had overt neurological impairments. Note that the curves for treated animals are fairly similar in shape regardless of whether they were tested in the full or partial maze, and both treated groups differ significantly from controls. However, the animals tested in the more complex (full or Cincinnati) maze, show a much clearer differentiation of the dose-dependent nature of the deficit. Better differentiation of effects is, I suggest, a goal of most assay methods.

This same experiment was used to demonstrate another point about the Cincinnati maze, namely, that path order did not make a lot of difference. In this case, we took different animals out of each litter, and we gave some of them path A followed by path B and some path B followed by path A. What one can see in Fig. 6 is that path order made little difference in terms of the numbers of errors made, but path type (A or B) made a very large difference in error rates.

We have obtained similar findings using the Cincinnati maze after prenatal treatment with valproic acid (VPA) and its major metabolite trans-2-ene-valproic acid (11). An interesting thing happens when one

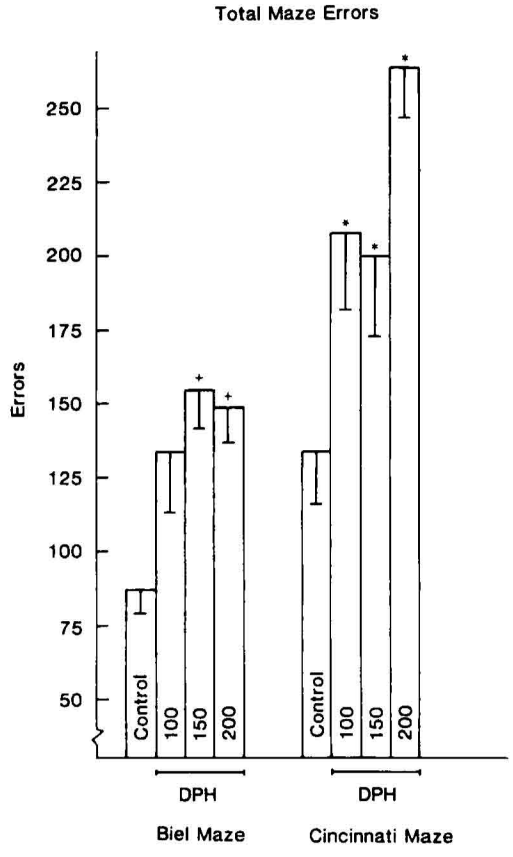

FIGURE 4

Effects of prenatal exposure to phenytoin on maze errors in a subset of animals shown in Fig. 3. Biel maze (left) and Cincinnati maze (right). Significance indicators and dosing are as in Fig. 3.

looks at Cincinnati maze errors for these two compounds. The doses used here in rats are ones which do not produce malformations. VPA at 300 mg/kg to the dams produces an increase in errors, whereas when one looks at the trans-2-ene-VPA at the same dose as valproic acid, one sees a non-significant trend toward increased errors. However, if one gives a higher dose of trans-2-ene-VPA (400 mg/kg), these offspring show increased errors. The increase in errors at this higher dose is virtually the same as that for VPA at a 25% lower dose. We concluded that trans-2-ene-VPA's effects are different from its teratogenic profile. For CNS effects, trans-2-ene-VPA is a neurobehavioral teratogen but the curve for such effects is shifted to the right compared to VPA. This illustrates another point, which is that if one thinks that one knows everything about the developmental toxicity of a compound by looking at its teratogenesis, fetal resorptions, and fetal growth, one can be misled unless tests appropriate for CNS dysfunction are included. We have recently completed a new experiment using 3,4-methylenedeoxymethamphetamine (MDMA), which is sold on the illegal market as 'Ecstasy.' It is an amphetamine stimulant, but is abused because of its hallucinogenic, rather than stimulatory, effects. We administered it to rats neonatally and the animals were evaluated on a Cincinnati maze (path B) at doses of 5, 10 and 20 mg/kg given twice per day on days

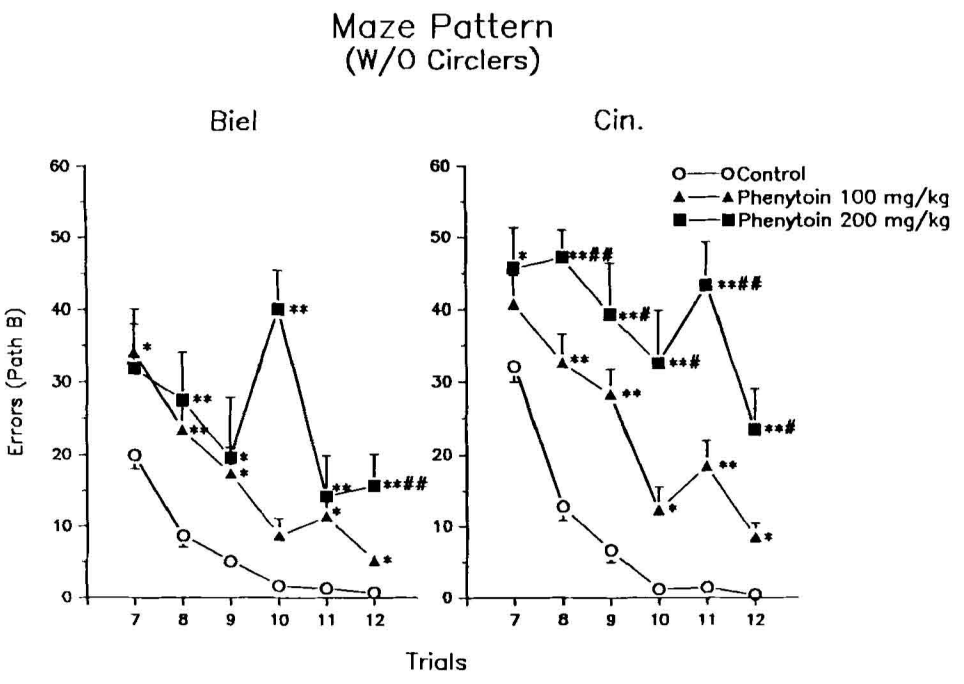

FIGURE 5

Comparison of errors in the Cincinnati maze (right) or in the Cincinnati maze with the last three cul-de-sacs blocked to make it comparable to the Biel maze (left) in offspring prenatally exposed to phenytoin on E7-18. Data were analyzed using litter averages. Rats with overt neurological impairments were excluded. *$p < 0.05$, **$p < 0.01$ compared to control. #<0.05, ##$p<0.01$ compared to the 100 mg/kg phenytoin group.

1-10 or 11-20. We found a dose-dependent increase in Cincinnati maze errors. We then separated the data by exposure period and found that the effect occurred exclusively in those exposed on days 11-20 (12). These data illustrate that even later stages of brain development are vulnerable to certain types of developmental neurotoxic insults and that in order to see such effects, one must consider postnatal exposure in rodents in order to make comparisons to human fetal brain development.

The Cincinnati maze measures sequential learning. The next test of CNS function for discussion measures a completely different aspect of cognition than does the Cincinnati. This is spatial learning and memory. Spatial learning is often assessed in a test called the Morris maze (13,14). The Morris maze is a large open tank of water. The animal must swim to find a hidden platform which is submerged beneath the surface of the water. Furthermore, the platform is camouflaged by making the water opaque or by making a black tank with a black platform so that there is no contrast and the animal cannot see the platform. There are control procedures one can run to make sure the animal cannot see the platform (15). The task is to find the hidden platform efficiently over successive trials. It is a spatial task because the inside of the maze is featureless, so there are no local or proximal cues for the animal to use to learn to navigate to the platform. The only cues that are available in the room are those on the ceiling and the walls that surround the tank. These are so called distal cues. What the animal has to do is navigate to get to the target platform. Also, one has random start points, such that on trial 1 they may start at North, on trial 2 they may start at South,

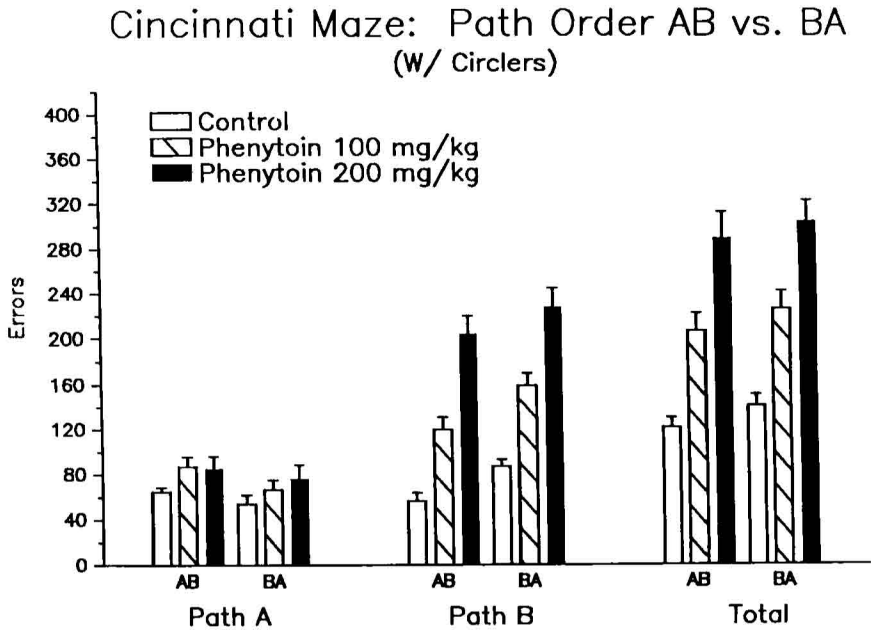

FIGURE 6

Comparison of path order on errors in the Cincinnati maze after prenatal exposure to phenytoin. Data are from the same experiment as in Fig. 5. Effects in both phenytoin groups for path B and total errors are significantly increased compared to controls. Note that path order had little effect on errors, but that path B was more difficult for treated animals.

trial 3 may be West, etc. This ensures that the animal never knows where they are going to start and cannot learn a route to the goal. The orientation to the goal requires the rat to truly navigate using distant or distal reference points in order to be able to find the platform's place in an undifferentiated space. Of course, rats will virtually always find the platform eventually, the question for the experimenter is whether they are doing so using a spatial strategy or some other way of solving the problem. There is a control procedure called the visible platform procedure in which one puts a flag or elevates the platform above the water and then give the subject additional trials. What one can determine is whether the animal has a general motor impairment, has a general learning impairment, or if it simply cannot see well enough to locate the target. There is a substantial literature that shows that if one disrupts hippocampal function with lesions, NMDA glutamate receptor antagonists (16), or by knocking out molecules which are important in the pathway of the NMDA receptor, such as CaMKII (17), that animals can solve the visible platform condition normally but when they are put in the hidden platform condition they cannot solve it. If one oblates the hippocampus, then animals cannot find the hidden platform (18), whereas if one uses a more subtle pharmacological (19) or gene targeting approach (17), the animals learn but at a much slower rate. There are variations of this task that can be used. One arranges the Morris maze as a working memory task. To understand this, recall the normal or so-called reference memory procedure described above. Animals enter the maze at different points around the perimeter and they swim to the platform which is in a fixed location. Because the platform is in a fixed location, the problem is an example of reference or long-term memory, i.e., the information to be remembered is invariant from one trial to the next. To test or probe the animal for its memory of the platform's location, the platform is removed and the animal is

scored for its ability to go to where the platform used to be. Performance on the probe trial is quantified by measuring the time in the target quadrant, distance traveled in the target quadrant, number of platform site crossings, and proximity to the target location (termed average distance from platform).

In a working memory version what one does is to give animals sets of trials, usually one set of trials per day. For the first trial or run of each set, the platform and start are randomized. The animal solves the first run by trial and error. The second or test trial is identical to the first or example trial. What the animal has to do is remember from run 1 to run 2 where the platform is to be found. When a new set of runs begins, what was learned previously is of no use. The animal has to find the new position of the platform, once again, by trial and error. What one sees is savings between run 1 and run 2 of a given set of trials. In an animal that has a working memory deficit, savings is reduced and there is little or no decrease in the time that it takes the animal on the second run as opposed to the first. This arrangement requires the animal to use trial-dependent or short-term memory to solve the task efficiently, since no permanent memory of the platform's location is possible.

The Morris maze can also be arranged with two platforms as either a visible or hidden platform discrimination task. In this arrangement, one platform is rigid and fixed, and if the animal reaches it they can climb on it and escape the water. The other one is mounted by tethers and floats. If the animal tries to climb on it, it sinks. The animal has to learn to look at these two platforms and based on either a cue difference between them, or differences in their locations, associate one with escape and the other with failure to escape.

Another example that illustrates the use of these tasks comes from our work on methamphetamine (MA) treatment to rats on postnatal days 1-10 or 11-20. I want to show that just because the Cincinnati maze is a good test of sequential learning, does not mean that it is a good test of all types of learning. For example, MA has essentially no effect on Cincinnati maze performance (20). The same animals that are unaffected in the Cincinnati maze, however, when placed in the Morris maze, show a marked increase in latency to find the hidden platform. Then when the platform is moved to the opposite quadrant as a test of reversal learning, one again sees that the MA animals have difficulty finding the hidden platform in its new location. Interestingly, the MA rats treated on days 11-20 show this abnormality clearly, while those given the same dose on days 1-10 are spared. The acquisition portion of this is illustrated in Fig. 7 (left). We have replicated this experiment a number of different times over the last five years. We have replicated the effect in different strains of rats and at different doses of MA. The latency data shown are for animals administered a dose of 30 mg/kg of d-MA twice per day. This is a high dose but it is not outside the boundaries of what some heavy drug users take.

Another experiment (21) compared the effects of MA exposure in Sprague-Dawley rats to that seen in ACIs (Fig. 7, right). The ACI is an inbred strain of rats that we used to search for differences in MA's effects that might be a function of differences in P450 metabolism. ACI females are reported to be deficient in the debrisoquine-type of P450 metabolism. The goal of this experiment was to determine whether the neurotoxic effect of MA was partly attributable to the parent compound or a metabolite. The reason the data are separated by sex is that, as mentioned, only the ACI females are reported to be deficient in P450-db1 metabolism. We tested only females in this experiment and one can see that both strains are affected, however, when expressed as a percentage of their respective control groups, as in Fig. 7, the deficit in the ACI's was not as large as that seen in the Sprague-Dawley's. Despite this, an analysis of variance of treatment group by strain, did not reveal a significant interaction between MA treatment and strain. This suggests that the deficit in the ACI strain was not sufficiently different from that found in Sprague-Dawley's to conclude that MA metabolism was a significant determinant of maze outcome. Hence the goal of this experiment was not realized.

In the next experiment, we used a so-called dark agouti or DA strain to again test the role of P450 metabolism in the developmental neurotoxicity of MA. In this strain, only half the dose of MA could be given compared to that in earlier experiments (15 mg/kg b.i.d.). Only half the dose was administered because higher doses proved lethal to DA rats. The Morris maze results showed that both the DA's and Sprague-Dawley's had deficits in Morris maze hidden platform acquisition (not shown; unpublished observation). While these data are still being analyzed, the acquisition findings do not support the idea of differential P450 metabolism as accounting for a major portion of MA's developmental effects on later Morris maze acquisition.

In our most recent experiment we looked at two different dosing patterns. In this case we gave MA either 4 times a day at 10 mg/kg or 2 times a day at 20 mg/kg. For hidden platform acquisition, no differences

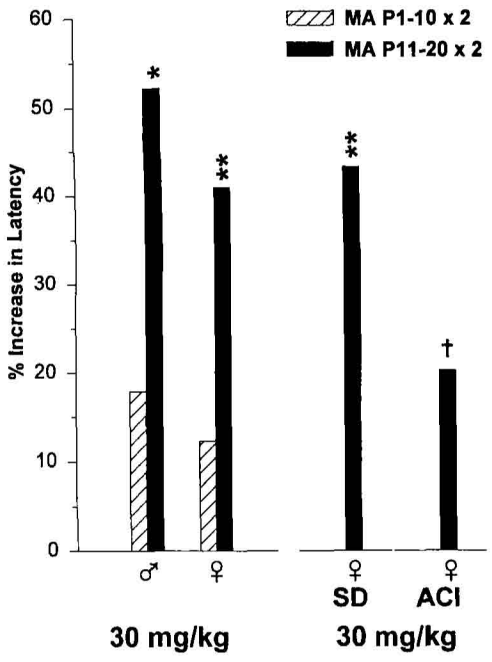

FIGURE 7

Effects of neonatal methamphetamine (MA) exposure on Morris hidden platform maze latency on acquisition trials. Dose was 30 mg/kg d-MA b.i.d. † < 0.10, *p < 0.05, **p < 0.01 compared to controls. SD = Sprague-Dawley.

between dosing regimens was found, however, as seen in the previous experiments, the MA groups performed significantly worse than controls. In this experiment we also changed the procedure for the Morris maze in two ways: (1) We ran the animals in the visible platform procedure prior to trials in the hidden platform condition. This meant that all animals had extensive maze experience prior to being given hidden platform trials; and (2) we administered the same number of acquisition trials, 24, as before, but we gave them as 4 trial per day sessions rather than as 8 trial per day sessions. It is known that distributed practice improves performance and we found that because of these two changes, both MA groups and controls performed the hidden platform much better than in our previous experiments. In fact, latencies were on average about half of those seen in our previous experiments. Despite this, the differences between the MA and control groups persisted, demonstrating that MA's effects are robust and do not change as a function of changes in specific procedural details.

We have recently completed a similar experiment to those described above using MDMA (12). We saw an overall effect of treatment group that sorted out as the high dose group exposed on days 11-20 was impaired in Morris maze hidden platform performance compared to controls (unpublished observations). Groups exposed on days 1-10 showed a similar but smaller effect.

The last test that I will discuss is the acoustic startle response. In the laboratory, this reflex is evaluated in an apparatus in which a rat or mouse is placed in an acrylic cylinder, which is enclosed in a sound attenuating chamber. There is a piezoelectric force-transducer mounted on the underside of the platform to which the cylinder is affixed. When the animal reacts to the startle signal, the animal's movement produces a deflection of the platform which induces a voltage change in the piezoelectric transducer. This signal is

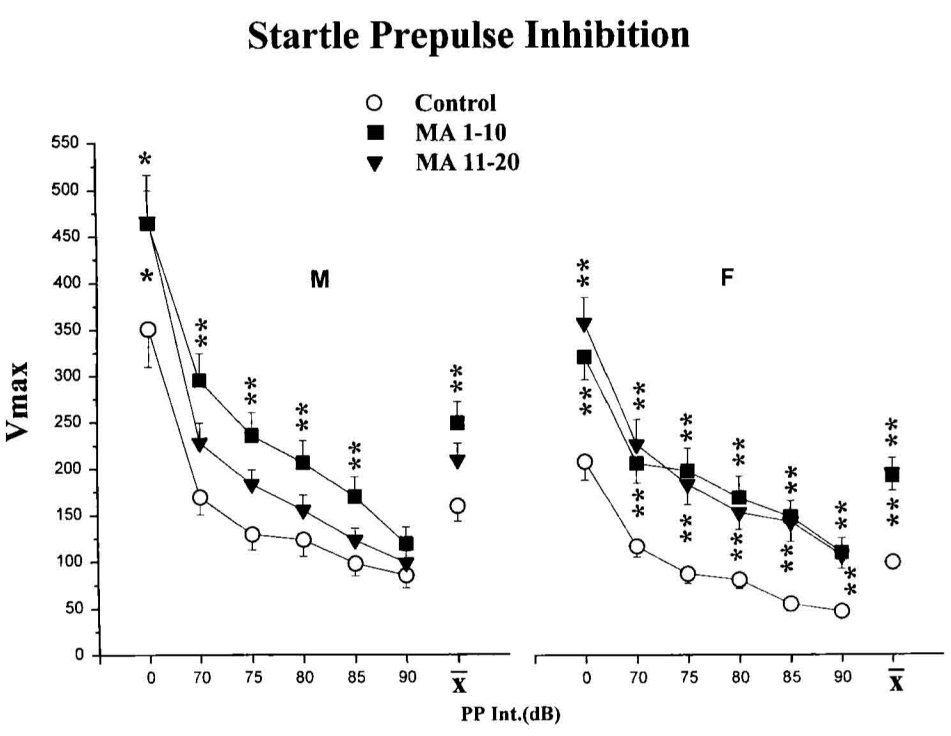

FIGURE 8

Effects of neonatal methamphetamine (MA) exposure on acoustic startle amplitude in a prepulse inhibition paradigm. V_{max} is the maximum voltage change in response to the startle stimulus averaged across 6 trials at each prepulse intensity (expressed in decibels on the A scale). *p < 0.05, **p < 0.01 compared to control. $\bar{x}$ is the mean response across all prepulse intensities. M = males, F = females.

amplified and converted to a digital signal for analysis. The data one obtains is of the amplitude of the response and the latency to maximum response. In rats prenatally exposed to MA, we see a significant augmentation of the startle reflex. The startle signal is typically 110 to 120 dB, and it can be a pure tone or a mixed frequency signal (white noise). The response is recorded for 100 milliseconds after signal onset. Because there is variation in the animals response from trial to trial, it is standard practice to administer multiple trials and average blocks of trials together to smooth out the data. As can be seen in Fig. 8, the MA-treated animals are hyperactive in terms of their maximum startle response amplitude; there was no appreciable change in response latency. This is typical as most neurotoxins produce their effects on the amplitude of the response, not on latency.

Another example of effects of MA on startle occurred in animals following neonatal exposure to the drug. Unlike the Morris maze findings, for acoustic startle both groups exposed on days 1-10 and 11-20 showed enhanced reactivity compared to controls (Fig. 8) (20).

Last I will mention what is called the prepulse inhibition paradigm. Prepulse inhibition is where one presents a weaker stimulus before the startle stimulus. This additional signal is typically presented about 40-50 milliseconds prior to the startle stimulus. What happens when this is done is that the prepulse

inhibits the strength of the startle response. If the amplitude of the prepulse stimulus is varied, one can obtain an inhibition curve where the degree of suppression of the startle amplitude increases as the magnitude of the prepulse signal is increased (Fig. 8). Prepulse modification has been discussed as a measure of sensory gating or filtering. Furthermore, humans with certain disorders have alterations in their prepulse inhibitory responses. It has been found, for example, that schizophrenics show an impairment in prepulse inhibition. People chronically exposed to amphetamines also show a deficit in prepulse inhibition. For rats exposed neonatally to MA, there was no interaction between prepulse amplitude and the magnitude of response inhibition, although there was a male-female difference as can been seen in Fig. 8.

In closing, I mention another test called the Barnes maze of spatial learning (22,23). The apparatus is a large circular plate or disk that is mounted above the floor and into which 40 holes around the perimeter have been cut. Under one of the holes is an escape box. We use a light, a tone, and a fan mounted above, which are activated on when the animal is put in the center of the arena. The animal's task is to explore the 40 holes until they find the one that provides escape. When the animal reaches the goal box then the stimuli are terminated and that provides relief from the aversive stimuli, which is reinforcing. The nice thing about the task is that one can assess spatial learning without using swimming and there are some advantages to this. Swimming is physically demanding, and it has been reported that mice tire quicker than rats in swimming tasks (23). However, the real power of the Barnes maze comes from the fact that one can do something that cannot be done in the Morris maze. One can actually analyze problem solving strategies in the Barnes maze. It turns out that animals go through a series of phases in learning a spatial solution using distal cues. The first strategy animals use is a random one in which they visit non-adjacent holes until they find the goal by trial and error. After they have used this strategy several times, they shift to a more efficient strategy which employs serial searching. In this second phase of learning, the animals move from the center to the perimeter, then travel sequentially from one adjacent hole to the next until they arrive at the goal. This strategy is more efficient than random searching, but still does not demonstrate spatial learning. As animals use the serial strategy over several trials, their accuracy for the direction of the goal gradually increases until they eventually go straight to the goal or miss it by no more than one hole. Once this step is mastered, the animals are relying on spatial reference points and regardless of their orientation at the start, will traverse the arena directly to the goal. One can map the acquisition curves for each of the three types of learning and can find out whether the animals are learning spatially or are remaining stuck in the phase of serial or random problem solving. One can also determine if treated animals are learning a spatial strategy, but at a slower rate than controls, but they ultimately are capable of spatial navigation.

The task clearly has some advantages, but it has disadvantages as well. One is that it is a more time consuming task to run because one has to run the animals more trials and trials are limited to one or just a few per day. Rats learn this maze more rapidly than mice, therefore, how time consuming it is depends on the species used. One also gets animals that will not perform in the Barnes maze that present problems for data analysis; this problem is non-existent in water mazes.

This concludes my overview of how one might go about assessing acoustic startle and cognitive function, which are both mandated under the EPA Developmental Neurotoxicity Guidelines. The take home message for cognitive assessment is that there is not one kind of learning, and therefore, not one kind of learning task that will provide blanket coverage for this category of function. One really must decide which types of learning one wants to assess and then ascertain which task is best suited for that specific type of learning.

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