Prenatal methylazoxymethanol exposure alters evoked responses in fetal rats

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Abstract

Although there is considerable interest in identifying methods to detect central nervous system impairment early in development, few behavioral assessment tools are available for detecting CNS deficits in the fetus. In the present study, methylazoxymethanol [MAM; Midwest Research Institute, (MRI)] was used to induce deficits in CNS development in fetal rats to assess effects on coordinated fetal behavior. Fetuses were exposed by administering MAM to pregnant rats on E17 of gestation via intraperitoneal injection and then were prepared for behavioral testing 3 days later on E20. After externalization from the uterus into a warm saline bath, fetal subjects received either an intraoral infusion of lemon extract to evoke a facial wiping response or were presented with an artificial nipple to evoke an oral grasping response. Intatural coordination and paw–face contact during facial wiping were disrupted in MAM-exposed fetuses. Similarly, MAM exposure diminished the ability of fetuses to grasp or maintain oral contact with the artificial nipple. Although clear disruptions of movement coordination were seen in the MAM-treated subjects, there were no significant differences from saline controls in weight or anatomical measures. Together, these findings suggest that behavioral assessments of fetal motor coordination may be useful in identifying neural insult during prenatal development.

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1. Introduction

Scientists have suspected, since the mid-1960s, that neurological disorders (in particular, amyotrophic lateral sclerosis or ALS, some forms of cerebral palsy, Parkinson’s Disease, and autism) may be the result of early neonatal or prenatal exposure to toxins [14,36,49,50,53]. Consequently, methods either to screen chemicals for toxicity or to assess impairments to early nervous system functioning are of considerable interest to both researchers and clinicians. Despite this interest, the exact mechanisms by which toxins damage the developing organism, particularly damage leading to late onset neurological disorders, have yet to be discovered. Methods for determining an association between early exposure and neurological outcome have been used for years, but methods for determining the immediate developmental effects of a toxin on the organism have, until recently, been unavailable. Studying these direct developmental effects is most important if the early causal mechanisms of these neurological disorders are to be discovered.

Direct observation of fetal behavioral development may prove useful in exploring the early causal mechanisms of prenatal neurotoxin exposure. Researchers have found that changes in observed prenatal behavior are directly related to underlying changes in the central nervous system [2,8,27]. This relationship suggests that, as in adult organisms, the observation of behavior in the fetus may be useful as an indicator of the integrated output of the developing nervous system. Moreover, overt behavior may be a particularly sensitive measure of neural function during early development, revealing patterns that are not readily apparent from anatomy or physiology alone. This sensitivity suggests that observation of fetal behavior may lead to the development of useful measures for the early diagnosis of neurological disorders [24].
Two methods in particular have been used extensively for the direct measurement of behavior in fetal rats. The first is a facial wiping response to lemon infusion, which is a complex chemosensory stimulus. During facial wiping, older rat fetuses exhibit a bout of organized forelimb activity that is similar to adult grooming. Studies have shown clear developmental patterns in the emergence of this species-typical response [28–31,42–45], suggesting that it may be useful in measuring sensory–motor disruption caused by toxin exposure.

A second method of direct measurement in the rat fetus utilizes an oral grasping response elicited by presentation of an artificial nipple. As with the facial wiping assessment, the oral grasping response emerges prenatally and shows a clear developmental pattern [32–34,47]. Disruption of this important response prenatally might greatly compromise a pup’s ability to receive sustenance from its mother after birth. This deprivation, in turn, could lead to other developmental delays, or even death. Consequently, assessment of this prenatal behavioral response may provide one way to differentiate between direct and indirect effects of neurotoxins on the fetal nervous system.

Previous work with normal development has shown these tests sensitive enough to assess not only developmental differences over the prenatal period, but also consistencies and differences in developmental patterns that exist between species [28,31], suggesting the applicability of the results of these measures to humans. Also of interest are the findings that these evoked behavioral measures are sensitive enough to reveal developmental patterns in the fetus exposed to drugs of abuse, such as cocaine [38–40], demonstrating their sensitivity to chemical perturbations.

In the present study, these measures of fetal behavioral development were used in the assessment of direct effects of the neurotoxin methylazoxymethanol acetate [MAM; Midwest Research Institute (MRI)] over a range of doses. Several important properties have led MAM to be used extensively in the study of postnatal behavioral effects of prenatal administration. These unique characteristics are the following: (a) antimitotic/antiproliferative effects, methylating guanine in cellular DNA [3,11,15,16,20]; (b) specificity for actively dividing neurons, leaving other cell types and differentiated neurons relatively unaffected [35,48]; (c) the ability to readily cross the placental barrier [20,48]; and (d) activity that remains in fetal tissue for a limited period of 2 to 24 h, after intraperitoneal administration to the pregnant dam, with peak activity at 12 h [16].

Exposure to MAM (which is found naturally in certain Pacific cycads) also has been implicated in the development of both ALS and Parkinson’s disease on Guam [3,11,14,49,50,53], suggesting that this neurotoxin has both basic and applied research relevance. Despite this chemical’s utility, no studies have looked at the direct effects of MAM on CNS function and behavior in the fetus to date. The use of direct fetal observation of behavior in this study, after prenatal administration of MAM, allows detailed analysis of the early behavioral deficits of a prototypical neurotoxin.

2. Methods

2.1. Data collection

2.1.1. Subjects

Subject fetuses (n = 48) were the offspring of 34 Sprague–Dawley Norway rats (Rattus norvegicus). During a 16-h breeding period, adults were housed together (two estrus females with one male) in polycarbonate cages with hardwood bedding and provided with food and water ad libitum. Cages were kept in colony rooms where temperature and humidity were controlled, and a 12:12-h light/dark regime was maintained. Date of conception (E0) was determined by positive vaginal smears, which were collected at the end of the 16-h breeding period. At this time, the male rat was removed, and the time-mated females remained housed together until experimental treatment on Day 17 of gestation (E17). At all times, both adult and fetal subjects were cared for in accordance with animal care guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals [21]. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Iowa.

2.1.2. Preparation of subjects

Pregnant females were assigned to one of four treatment groups on E17. The four groups were as follows: (a) 0.9% buffered saline, (b) 10 mg/kg MAM, (c) 20 mg/kg MAM, and (d) 30 mg/kg MAM. Solutions of MAM were prepared with a 0.9% saline vehicle. Because collection of data for both behavioral observations was not practical in all pregnancies, 34 dams were necessary to provide n = 8 fetal subjects for each observation in each treatment group. Subsequently, pregnant females were assigned to treatment groups in the following numbers: (a) saline, n = 10; (b) 10 mg/kg MAM, n = 10; (c) 20 mg/kg MAM, n = 11; and (d) 30 mg/kg MAM, n = 3. Treatment consisted of one intraperitoneal injection to the pregnant dam, at a volume of 1.0 ml/kg. The MAM acetate used in this study was obtained from MRI. E17 was chosen for the age of administration because it is the beginning of the period of fetal behavior that has been well described in the rat [28,41].

After treatment, the dams were housed individually in filter top cages until the time of testing. E20 was chosen as the day of testing because many fetal behaviors, including those used in this study, are fully expressed at this time of gestation. Additionally, because MAM is readily metabolized from the rat’s body within about 24 h after administration [16], no direct effects of the neurotoxin should be present on E20 (72 h after administration). Consequently, any behavioral changes observed should be due to the
neurotoxic effects of MAM and not the immediate presence of this chemical in fetal nervous system tissue.

On the day of testing, the pregnant females were prepared under brief ether anesthesia by injecting 0.1 ml of 100% ethanol into the spinal canal between the first and second lumbar vertebras. This procedure produces irreversible spinal anesthesia, eliminating sensation in the lower body of the female. After spinal preparation, the female was secured in a Plexiglas holding device and immersed to chest level in a buffered isotonic saline bath (Locke’s solution) maintained at 37.5 °C (see Fig. 1). The uterus then was externalized into the bath through a midline laparotomy, and the female was left undisturbed for 20 min to ensure that both mother and fetus had fully recovered from the ether anesthesia.

After acclimation, two individual fetuses were externalized from the uterus into the saline bath and their embryonic membranes, both chorion and amnion, were removed to facilitate visibility. The first subject was fitted with an intraoral cannula [28,31], which consists of a 2-cm length of PE-10 polyethylene tubing. The tip of the cannula is flanged and rests on the tongue of the fetus. After insertion of the cannula, a micrometer syringe was attached to the cannula to allow delivery of a lemon infusion (1:1 dilution of Schilling brand lemon extract with a 0.9% saline solution). The fetus then was placed supine on a small, submerged platform. After a 1-min baseline period, a 2-s 20-μl pulse of lemon solution was administered through a rotary infusion pump that permits precise delivery of the infusion. Both baseline and response to the lemon infusion was videotaped in S-VHS format with simultaneous time code generation (30 frames/s), which permits identification and precise synchronization of individual video frames during playback.

After testing of the first subject was complete, a second subject was prepared for placement in a holding device. Briefly, a drop of cyanoacrylate adhesive was applied to an area of the back caudal to the forelimbs. A small steel post was then gently pressed to the area. This post fit into a small hole in a magnetic holding device (1.9 × 2.5 × 0.5 cm) upon which the fetus was placed in a supine position. Behavioral assessment of this fetus consisted of a 1-min baseline period, followed by presentation of an artificial nipple to the subject’s oral area for approximately 3 min. The artificial nipple was fashioned from a block of soft vinyl dissection pad material, and was 25 mm long, 5 mm wide at the base and tapered to 1 mm wide at the rounded tip. To facilitate presentation to the fetus, the base of the nipple was attached to a handle. Presentation consisted of holding the tip of the nipple in contact with the oral area of the fetal subject. As with the subject tested with the lemon infusion, this observation was videotaped in S-VHS format with time code generation.

For all subjects, fetal attachment to the placenta and uterus by the umbilical cord was maintained, and the preparation was monitored throughout the testing period for signs of deterioration or distress. This experimental procedure for externalization and subsequent observation of fetal rodents has been extensively employed in previous research on fetal behavior [46].

2.2. Scoring and analysis

2.2.1. Anatomical measures

After behavioral testing, fetal subjects were euthanized with cold anesthesia, weighed, and photographed on a platform with a Sony digital camera (MVC-FD91) mounted above the platform. Subjects from both the facial wiping and nipple attachment experiments were pooled for anatomical measures, providing n = 16 subjects for each dose. Two whole body views of each subject were taken: (a) a dorsal view with the fetus in a prone position, and (b) a lateral view (Fig. 2). Care was taken in placing each subject to provide a consistent positioning and camera angle. Measuring scales were placed along the side of the subject in each photograph. The digital images then were imported into Scion Image for Windows, Beta 4.0.2 version (a PC program based on NIH Image, Scion), where measures were calibrated to the photographed scales. From the dorsal view, the following measures were taken: (a) cranial width—between the ears at the fullest part of the head, forming a line
perpendicular with the nose; (b) forelimb length—from the tip of the longest forelimb digit, along a line traversing the forelimb to the proximal end of the ulna; and (c) pedal length—measured from the longest hindlimb digit, along a line parallel to the bottom of the foot to the calcaneous (Fig. 2, left). The following two measures were taken from the lateral view: (a) cranial length—from the tip of the nose to the back of the head, through the midline of the ear, and (b) crown–rump length—in a line bisecting the body from the apex of the head to the posterior of the rump, excluding the tail (Fig. 2, right).

From these basic measures, ratios of relative proportion were created (Table 1). These measures are useful when developmental perturbants change relative, but not overall growth patterns. This type of phenomenon often is seen in malnourishment, where the cranial size often is very large in proportion to the body size [5,19,22,37]. Another possible relative change occurs with nervous system damage that results in cranial–facial abnormalities, most notably seen in Fetal Alcohol Syndrome and Effects (FAS/FAE) [9,51]. Finally, toxins or compromise of the fetal environment (e.g., oligohydramnios) may result in arrested movement, leading to fetal akinesia deformation sequence. Among the many outcomes of this process is a stunting of limb growth, caused by even short periods of movement decrements [17,18]. The use of these proportional indices may provide more detailed information about relative changes in growth between the treatment groups, revealing deformations that may not be evident upon casual examination or with absolute measures of anatomical size. Prior to statistical analysis, the individual measures of subjects from the same pregnancy were combined to provide litter means. However, in some pregnancies, data from only one of the behavioral observations were collected. These additional measures resulted in sample sizes of \( n = 10 \) Saline, \( n = 10 \) MAM 10, and \( n = 11 \) MAM 20. All measures and indices were analyzed in a series of one-way analyses of variance (ANOVAs) using an \( \alpha \) of .05. Because the indices are proportions, they first were normalized with an arcsine transformation.

![Fig. 2. Depiction of the anatomical measures collected in E20 rat fetuses. (Left) Cranial width (A), forelimb length (B), and pedal length (C). (Right) Cranial length (A) and crown–rump length (B).](image)

crown–rump length—in a line bisecting the body from the apex of the head to the posterior of the rump, excluding the tail (Fig. 2, right).

### Table 1

<table>
<thead>
<tr>
<th>Measure</th>
<th>Saline</th>
<th>MAM 10</th>
<th>MAM 20</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td>Absolute measure</td>
<td>Mean</td>
<td>S.E.M.</td>
<td>Mean</td>
<td>S.E.M.</td>
</tr>
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<td>Weight (g)</td>
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<td>0.107</td>
<td>3.953</td>
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<td>Cranial width (mm)</td>
<td>10.214</td>
<td>0.077</td>
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<tr>
<td>Cranial length (mm)</td>
<td>16.196</td>
<td>0.123</td>
<td>15.951</td>
<td>0.179</td>
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<tr>
<td>Crown–rump length (mm)</td>
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<td>0.368</td>
<td>36.888</td>
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<tr>
<td>Forelimb length (mm)*</td>
<td>10.631</td>
<td>0.101</td>
<td>10.119</td>
<td>0.120</td>
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<tr>
<td>Pedal length (mm)*</td>
<td>6.963</td>
<td>0.088</td>
<td>6.643</td>
<td>0.094</td>
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<td>0.008</td>
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<td>0.521</td>
<td>0.006</td>
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<tr>
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<td>Forelimb length/crown–rump length</td>
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<td>0.005</td>
<td>0.718</td>
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<td>Pedal length/crown–rump length</td>
<td>0.551</td>
<td>0.003</td>
<td>0.552</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* Forelimb length and pedal length are averages of both right and left limb measures.

b Each proportional index is the ratio of two anatomical measures. These ratios were normalized by an arcsine transformation before statistical analyses were performed.
2.2.2. Facial wiping response

Videotape was played back frame-by-frame to determine the exact timing and number of forelimb movements during the wiping response. Timing of the stimulus onset was determined by when a cloud of lemon was observed leaving the mouth of the fetus. The latency to first wipe was calculated from the time of stimulus onset until the first observable forelimb movement, and the length of the wiping bout was measured from the first forelimb movement until the end of the last forelimb movement. Frequency of wiping responses was scored in the following manner: (a) flails—movement of each limb that did not result in contact with the head of the fetus, (b) single wipes—movement of single limbs that resulted in a wiping motion of the paw across the subject’s face, and (c) conjugate wipes—simultaneous movement of both forelimbs, with both paws making contact with the face during the wiping motion.

Because not all subjects exhibited conjugate wiping, these frequencies were analyzed with a Chi-square Test of Independence in Microsoft Excel. Latency to first wipe and the total wiping bout length were analyzed in separate one-way ANOVAs. A two-way ANOVA was used to analyze the two wiping responses exhibited by all treatment groups (flails and single wipes). Post hoc tests of pairwise comparisons for significant ANOVAs were run using Fisher’s Protected Least Significant Difference. All ANOVAs and post hoc tests were run using StatView software version 5 (SAS Institute, Cary, NC).

2.2.3. Response to an artificial nipple

Latencies and frequencies for the subjects exposed to an artificial nipple also were calculated from frame-by-frame playback of video. Quantification of response to the nipple presentation was characterized as follows: (a) latency to first grasp or grasp attempt—the time from the first contact of the nipple on the subject’s face until the first grasp or attempt at grasping was observed, (b) grasp—a firm clenching of the jaw while the nipple is in the subject’s mouth, (c) grasp attempt—a closing of the mouth around the nipple that does not result in a grasp, (d) grasp length—the average length of time in seconds that the nipple was grasped by each subject, (e) grasp percent of time—the percentage of nipple presentation time that was spent attached to the nipple, (f) general movements—movements of the head (away from the nipple) and body (trunk curls), (g) oral events—licking and mouthing behaviors, and (h) forelimb activity—treading, which consisted of alternated paddling movements, and wiping motions made with the forelimbs. These last three sets of behavior were further characterized as to whether they occurred during a grasp or nongrasp period during nipple presentation. Because there was a variable presentation time for each fetus, grasps and grasp attempts were expressed as a rate per minute of nipple presentation. General movements, oral events, and forelimb activity were standardized as rates per minute of time spent either attached to or unattached from the artificial nipple. Resulting quantifications were analyzed in a series of one-way ANOVAs, and post hoc pairwise comparisons were analyzed for significant results (α=0.05) in the same manner as was used for the facial wiping data.

3. Results

3.1. MAM 30 mg/kg dose

The first three pregnant dams that were administered the 30-mg/kg dose of MAM were severely compromised, with two dying on E20. The third dam was euthanized on E19, after observing lassitude and adipsia, characteristic signs observed in the first two pregnant females. Postmortem inspection of all three dams revealed that the fetuses had died earlier than E19, as was evidenced by extensive reabsorption of all fetuses. Consequently, no additional pregnant females were assigned to this treatment group, and the 30-mg/kg dose was removed from the study.

3.2. Anatomical measures

Table 1 shows the results of the various anatomical measures and indices. No significant differences were found, despite the wide range of doses used and low variability in the measures for each dose (evidenced by low standard errors, S.E.M.).

3.3. Facial wiping response

No significant difference was found for Latency to first wipe, \( F(2,21)=1.88, P=0.178 \). The means were 1.6 ± 0.3 s for the Saline group, 2.3 ± 0.6 s for MAM 10 subjects, and 2.1 ± 0.5 s for the MAM 20 group. Likewise, total wiping bout length, \( F(2,21)=1.23, P=0.314 \) also showed no difference. The means for bout length were 9.9 ± 1.4 s for the Saline subjects, 11.1 ± 0.9 s for the MAM 10 group, and 12.8 ± 1.5 s for MAM 20 subjects. Observation of the wiping responses, however, revealed clear differences among the three treatment groups. Lemon infusion to Saline controls elicited a typical E20 facial wiping response. Subjects performed a number of alternated single wipes to the face, often culminating in one or more conjugate wipes. In contrast, MAM subjects often responded with flails (single limb wipes with no facial contact), that were replaced with alternating wipes. MAM 20 mg/kg subjects could be further differentiated in that, typically, only one limb was involved in the forelimb actions, with the other limb held rigid at a point in the wiping stroke.

The two-way ANOVA for frequencies of single limb responses (flails and single wipes) confirmed a significant main effect of wiping responses, \( F(1,21)=16.07, P=0.0006 \), and an interaction of wiping response and dose, \( F(2,21)=11.91, P=0.0003 \). Analyses of simple main effects of dose for each type of single limb
response also were significant. Follow-up pairwise comparisons showed the MAM 20 group significantly lower than both the MAM 10 group and the Saline controls for single wiping. Analysis of flailing movements, however, revealed both MAM-treated groups were significantly elevated from Saline controls, but not different from each other. Chi-square analysis of conjugate wipes also showed a significant difference, \( \chi^2(2, n=24) = 12.63, P = 0.002 \), with 63% of control subjects exhibiting at least one conjugate wipe and no subjects from either MAM-exposed group showing any conjugate wipes. Fig. 3 shows these effects, with overall differences between the three types of wiping observed and interactions with the three doses.

3.4. Response to an artificial nipple

There were no significant differences between MAM-treated subjects and Saline controls in the following: (a) latency to first grasp or first grasp attempt, \( F(2,21) = 1.55, P = 0.235 \); (b) forelimb activity, \( F(2,21) = 2.06, P = 0.153 \); (c) general movements, \( F(2,21) = 0.07, P = 0.935 \); or (d) grasps or grasp attempts, \( F(2,21) = 0.53, P = 0.601 \) (Fig. 4, left). Once attached to the artificial nipple, however, the MAM-treated subjects showed significant decrements in length of grasp, \( F(2,21) = 5.10, P = 0.016 \) (Fig. 4, middle), grasp percent time, \( F(2,21) = 4.76, P = 0.019 \) (which showed a similar relationship to grasp length), and oral events, \( F(2,21) = 4.66, P = 0.021 \) (Fig. 4, right). Only oral events occurring during a grasp or grasp attempt, expressed as a rate per minute of grasp length, were significantly lower in the MAM groups. Oral event rates during nongrasp times were not different among the three groups, \( F(2,21) = 2.53, P = 0.104 \).

4. Discussion

The results of this study reveal the utility in direct investigation of fetal behavior after exposure to a known neurotoxin. It is of particular interest that behavioral deficits were seen, despite otherwise normal appearance of exposed fetuses. Even indices of potential changes in the relationship between various body and limb measures, between MAM-exposed subjects and controls, showed no differences. That there also was no significant difference in weight across the groups is surprising, in light of studies reporting postnatal weight differences [1,4,7,10]. However, because the weight measures were collected in close proximity to the toxin administration (72 h later), there may not have been adequate time for a reduction in weight to accumulate. Prior work has documented the rapid growth of the rat fetus with mean weights of approximately a gram on E17 that quadruple by E20 [28]. Together, these findings suggest that the weight loss seen at the time of birth is a secondary effect of MAM exposure, and not a primary effect of this neurotoxin.
Another unique finding in this study was the mortality of pregnant females in the 30-mg/kg treatment group. Numerous other studies have used this dose with no reported loss [7,23,52]. However, no other studies have administered a dose of 30 mg/kg on E17. Because the fetuses were in an advanced state of reabsorption, the timing of the dose, and not the dose itself, may have been catastrophic to fetal development. It is possible that 30 mg/kg administered on E17 creates such an extensive insult that movement, which has normally just commenced at this time, is effectively arrested. Unlike fetal akinesia deformation sequence [17,18], which typically occurs after movement is well established, this early cessation of movement might be fatal. Experiments involving behavioral observations within 24 to 48 h after low-dose MAM administration are currently underway in order to explore the question of early reduction in movement as a contributor to later behavioral deficits seen in this study.

An alternative explanation for the catastrophic fetal mortality, however, could be that by E17–E18, the fetal liver may be capable of metabolizing MAM, in addition to the maternal biotransformations. Metabolism of such a high dose as 30 mg/kg may exceed fetal hepatic capacity, causing acute toxicity. That other researchers have used this higher dose without fetal mortality may be due to the timing of the administration, which in other studies was 2–3 days earlier. Because little is known about fetal metabolism of MAM, studies of biotransformation of MAM in the fetus, along with the timing of maturation of metabolic systems responsible for its biotransformation, may help explain the results found at the 30-mg/kg dose in this study.

The specific types of deficits seen in the behavioral observations indicate that these deficits from prenatal MAM exposure were not confined to the period of neurotoxin activity (2–24 h after administration to the pregnant female). In both the facial wiping and nipple attachment experiments, MAM fetuses exhibited dose-dependent reductions in the ability to execute coordinated movement. Fetuses were responsive to the stimuli presented, as was evidenced by no difference in latencies to respond or frequencies of responding. However, in the facial wiping experiment, none of the MAM-exposed fetuses were able to progress from single alternated wiping to the more coordinated conjugate wiping seen in control subjects. Similarly, MAM-exposed subjects attached to the artificial nipple with the same latency and frequency as saline fetuses, and showed the same rates of activity prior to attachment, but did not evidence the typical rates of rhythmic mouthing and licking shown by control subjects once the nipple was grasped.

The sensitivity of these behavioral measures is evidenced in that the observed behavioral deficits were seen at even the very low dose of 10 mg/kg. Because few postnatal studies have reported significant findings at this dose [35], the findings of this study stand in contrast. There are several possible explanations for this difference. One is that the deficits seen in the fetuses are transient, and that the subjects would eventually recover from the toxic insult with little or no deficit in behavior. The second is that exposed animals never recover, but compensate for the nervous system damage. Third, it is possible that some postnatal behavioral tests are not sensitive enough to assess subtle damage that may persist. Finally, findings of prenatal deficits with only occasional reports of postnatal outcome deficit are suggestive of a phenomenon called prenatal programming, where early insults create a vulnerability, or silent damage [26], that is only evidenced later under specific circumstances or testing [6]. In such cases, the timing of the behavioral assessment, rather than its sensitivity per se, may be critical in determining whether the vulnerability is detected. In order to determine which of these possible developmental trajectories occur after this particular nervous system insult, systematic studies across early development that bridge the time period from these prenatal observations to existing postnatal outcome studies will be necessary.

The results from this study clearly demonstrate that these early measures are sensitive to changes in fetal behavior brought about by perturbations to the developing nervous system. Consequently, there may be utility in using such measures of fetal behavior in screening other chemicals or prenatal insults for their potential to cause early behavioral deficits. This type of early testing should be particularly useful for evaluating teratogens that disproportionately impact nervous system development. In addition to screening, these measures may have basic research utility for assessing prenatal nervous system functioning to identify underlying mechanisms of behavioral development.

Finally, assessment of behavior in fetuses may be useful in the development of specific animal models of neural insult during prenatal development (e.g., cerebral palsy or autism). As connections are discovered between early toxin insults and later predisposition to develop neurological disease, such as Parkinson’s disease or ALS, (referred to as silent toxicity [26], or prenatal programming [6]), sensitive fetal measures of nervous system functioning may become increasingly important in gaining an understanding of the mechanisms underlying these etiologies as well.

Acknowledgements

Preliminary findings of the results reported herein have been presented at a meeting of the International Society for Developmental Psychobiology [12], the annual meeting of the Society for Neuroscience [25], and the annual meeting of the Neurobehavioral Teratology Society [13].

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