Neurotoxicology and Teratology, Vol. 13, pp. 13–20. Pergamon Press plc, 1991. Printed in the U.S.A. 0892-0362/91 $3.00 + .00

Behavioral and Neurochemical Effects of Prenatal Methylenedioxymethamphetamine (MDMA) Exposure in Rats[1]

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Received 30 March 1990

ST. OMER, V. E. V., S. F. ALI, R. R. HOLSON, H. M. DUHART, F. M. SCALZO AND W. SLIKKER, JR. *Behavioral and neurochemical effects of prenatal methylenedioxymethamphetamine (MDMA) exposure in rats.* NEUROTOXICOL TERATOL **13**(1) 13–20, 1991.—MDMA is a hallucinogenic drug that is used by the general public as a recreational drug of abuse. The neurobehavioral consequences of prenatal MDMA exposure are unknown. Groups of pregnant rats were gavaged with 0, 2.5, or 10 mg/kg MDMA during gestation on alternate gestational days 6–18. Gestational duration, litter size, neonatal birth weights and physical appearance at birth were unaffected by MDMA treatments. Pregnancy weight gain was significantly reduced by MDMA treatment. Progeny growth, maturational parameters (eye opening and incisor eruption times), surface righting reflex, swimming performance, forelimb grip strength, milk-induced behaviors, passive avoidance behavior, figure-8 maze activity over 48 hours, the density of brain serotonin (5-HT) uptake sites, and brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were unaffected by MDMA treatments. Olfactory discrimination on postnatal days (PND) 9–11 was enhanced in both male and female MDMA-treated progeny, while negative geotaxis (PND 7–10) was delayed in female pups. In contrast to progeny, MDMA caused dose-dependent decreases in 5-HT and 5-HIAA levels in discrete brain areas of the dam. It is concluded that prenatal exposure to MDMA at the levels used here produces only subtle behavioral alterations in developing rats. The dam is more at risk for MDMA-induced 5-HT depletion than is the conceptus.

Methylenedioxymethamphetamine (MDMA) Gestational exposure Serotonin 5-HIAA Rat brain Rat pups

THE substituted amphetamine methylenedioxymethamphetamine (MDMA) is a mild hallucinogen sometimes used recreationally by the general public (2, 22, 23) despite its listing as a Schedule I drug by the Drug Enforcement Administration (22). The possible adverse effects of MDMA in man are not well documented (7,48) and nothing is known about the drug's potential effects on fetal and child development. This is of particular societal concern since animal data indicate that in adult laboratory animals, MDMA is a selective serotonergic neurotoxin (3, 4, 10–12, 17, 40, 44, 45, 49, 50, 53). Exposure of adult rats to MDMA produces a persistent reduction of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels (3, 4, 10–13, 17, 40, 44, 45, 49, 50, 53), decreases tryptophane hydroxylase (TPH) activity (53) and causes denervation of serotonergic neurons in selected regions of the brain (4, 11, 12, 40, 49). Such MDMA exposure is also accompanied by long-term behavioral alterations in adult rats, including rate-increasing and reinforcement-decreasing effects on a differential reinforcement of low rate schedule (33).

In contrast, little is yet known about the effect of MDMA exposure on the developing organism. A variety of considerations suggest that such effects may exist, and could be severe. Serotonin is a potent teratogen/embryotoxin in early pregnancy due to direct effects on the placenta (20), and MDMA may resemble parachloroamphetamine, another selective serotonin neurotoxicant, in triggering a flood of serotonin release from CNS and periphery (27, 51, 54). Such a mechanism could explain the reported embryotoxicity of serotonin depletors in rat (38) and guinea pig (28). In addition, it has been suggested that serotonin plays a trophic, growth-promoting role in early CNS development in vivo (9, 29–31, 35) and in vitro (8,26), while pharmacological block-

[1]Presented in part at the 13th Annual Meeting of the Behavioral Teratology Society, Richmond, VA, June 4–6, 1989, and at the 19th Annual Meeting of the Society for Neuroscience, Phoenix, AZ, October 29–November 3, 1989.

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TABLE 1

MATURATIONAL AND BEHAVIORAL DEVELOPMENTAL TEST BATTERY

Procedure	Pup Number*	Postnatal Day of Testing	Number of Each Set†
Incisor eruption	1, 4, 5, 8	7 to criterion	10
Eye opening	1, 4, 5, 8	12 to criterion	10
Surface righting reflex	1, 4, 5, 8	2 to 5	10
Negative geotaxis (angle = 25°)	1, 4, 5, 8	7 to 10	10
Swimming performance	1, 4, 5, 8	7 to 20	10
Olfactory discrimination	1, 4, 5, 8	9–11	10
Forelimb grip strength	1, 5	14, 17, 22	10
Milk-induced behaviors	3, 7	6	10
Figure-8 maze activity	4, 5	21–24	5
Passive avoidance learning	4, 5	95–98	4

*Males are 1, 3, 4; females are 5, 7, 8.
†Average number/litter/group.

ade of serotonin synthesis in utero causes neurochemical and behavioral abnormalities in offspring (46). Thus, if MDMA depletes serotonin in utero as it does in adults, we would anticipate that maternal MDMA exposure would have a negative effect upon the developing brain and behavior.

To date, this possibility has not been tested. A single study has reported that MDMA exposure in rats over the first 10 days of postnatal life causes neurotoxicity not unlike that in adults (37), but we have been unable to locate any reports of prenatal MDMA exposure. This study represents a first attempt at assessing the impact of such exposure. Due to the possibility of rather severe effects on the conceptus (vide infra), MDMA was given to pregnant rat dams by gavage at relatively low doses only on alternate days over the postimplantation course of pregnancy. This relatively conservative dosing regime should serve to set lower limits to prenatal MDMA effects upon the conceptus.

METHOD

Animals

Pregnant CD albino rats from the NCTR breeding colony were individually housed under controlled environmental conditions (12:12 light:dark cycle, 23 ± 1°C and 50 ± 5% relative humidity). The rats were weighed and gavaged between 8:00 and 11:00 a.m. on even days from gestational days (GD) 6 through 18 with either 0, 2.5 or 10 mg MDMA/kg dissolved in distilled water. All dams were checked twice daily (8:30 a.m. and 4:00 p.m.) for parturition. Deliveries completed by 8:30 a.m. were designated as postnatal day (PND) 1. Pups born later that day (between 8:30 a.m. and 4:00 p.m.) were designated as PND 1 on the following morning. Offspring in each litter were counted, examined for dead pups and external malformations, sexed and weighed. Each litter was then randomly reduced to 8 pups (4 ± 1 of each sex) and foot marked by tattooing on the dorsal surface. Litters were weaned on PND 27. Gross postmortem examinations were performed on all dead pups. The experimental design called for the random selection of 10 litters/treatment group for postnatal testing and for discarding litters with less than 8 pups. However, the evaluation of maternal weight gain, length of gestation and reproductive outcome (PND 1) were based on the examination of the 12 pregnant dams per treatment group.

Postnatal Growth and Maturational Development

Offspring were weighed on PNDs 1, 4, 7, 14, 17, 22 and 27 prior to behavioral testing. Eye opening and incisor eruption were observed according to the schedule shown in Table 1 and evaluated as previously described by Mohammad and St. Omer (36).

Behavioral Development

Males and females from each litter were subjected to behavioral tests on postnatal days as described in Table 1. The spectrum of behavioral tests employed in this investigation and summarized (Table 1) are essentially standard methods described for use in mice and rats (1, 6, 14, 18, 21, 36, 41, 42, 52). Unless otherwise stated, one male and one female were tested from 10 litters in each of the three dose groups.

Surface righting reflex. Each pup was given two successive trials/day from PND 2 to 5, and timed from being placed in a supine position until it had righted itself on all four feet in ≤2 seconds. Surface righting reflects the development of labyrinthine and body righting mechanisms (21,36).

Negative geotaxis. This behavior was observed once daily on PND 7 through 10. Pups were timed for completing a 180° turn within 60 seconds when placed in a head down position on a 25° inclined surface. Negative geotaxis reflects vestibular function, motor development and activity (6, 21, 36).

Swimming performance. An aquarium filled with water (28–29°C) was used for the swimming test on PND 7, 9, 11, 15, and 20 (52). Prior to PND 7 pups floated motionless with their heads and noses under the water. Each animal was dropped from about 5 times its own height into the center of the aquarium and was observed for 5–10 seconds. Swimming performance was evaluated according to the position of the nose and head (angle) on the surface of the water (42,52). The angle of swimming was rated as follows: 0—head and nose below surface, 1—nose below surface, 2—nose and top of head at or above surface but ears still below surface, 3—same as 2 except with water line at mid-ear level, 4—same as 3 except water line at bottom of ears. After the test, pups were dried and returned to the home cages. Swimming is a measure of motor development and the integration of a coordinated series of reflex responses (42,52).

Olfactory discrimination. This test was conducted daily on PND 9–11. The apparatus consisted of a plastic container (35 × 13 × 12 cm), two small bins, a wire screen placed over the container and a clear plastic cover which was placed over the container. One end of the container contained a bin filled with clean bedding, the other end, a bin filled with home cage bedding. A

TABLE 2

PHYSICAL DATA OF DAMS AND LITTERS FROM PREGNANT RATS GAVAGE FED MDMA DURING GESTATION ON ALTERNATE DAYS 6–18

Group (MDMA) mg/kg	# Pregnancy Wt. Gain $\frac{GD\ 20-GD\ 0}{GD\ 0} \times 100$	Gestation (ave. days)	Total Pups per Litter on PND 1	Live Pups per Litter	M:F Ratio per Litter	Birth Weight (g) M	Birth Weight (g) F	Incisor Eruption (days) M	Incisor Eruption (days) F	Eye Opening (days) M	Eye Opening (days) F
0	61.5±2.0	22.7±0.1	12.8±0.9	12.3±1.1	1.4±0.2	6.9±1.1	6.6±0.2	11.1±0.2	11.1±0.4	14.5±0.2	14.3±0.2
2.5	59.9±1.9	22.6±0.2	12.1±0.5	12.0±0.5	0.8±0.1*	7.2±0.5	6.6±0.2	11.5±0.4	11.3±0.3	14.8±0.2	14.7±0.1
10	55.5±1.4*	22.8±0.1	12.5±1.0	12.3±1.0	1.2±0.2	7.0±1.0	6.6±0.3	11.6±0.3	11.9±0.4	14.3±0.3	14.4±0.2

Values are means ± SEM of 12 dams or 12 litters/treatment group (exception, values of incisor eruption and eye opening = 10).
M = male pups, F = female pups, PND = postnatal day, GD = gestational day.
MDMA = methylenedioxymethamphetamine.
*$p<0.05$. Duncan's multiple range test.
F statistic for # pregnancy wt. gain = $F(2,33) = 3.21$, $p<0.05$.

3 cm square area demarcated the center of the screen. A line was drawn on the screen above each bin. Each pup was placed on the centrally demarcated region on the screen and the latency to enter the home bedding side by crossing the designated line with the front paws and head was timed. Maximum time allowed was 2 minutes. Central placement of the pup was balanced by alternating the pup facing the experimenter or away from the experimenter. Age of home bedding was balanced across the groups and averaged 2–3 days old at testing. This test reflects a nest-seeking response mediated by the olfactory system (24,36).

Forelimb grip strength. This test was conducted on PND 14, 17 and 22. A steel wire (20 cm long by 0.2 cm thick) was supported between two poles 25 cm from the floor. Each pup was gripped at the base of its tail and suspended above the wire until it grasped the wire with both forepaws. The pup was lowered and released. The latency to fall within a maximum time of 15 seconds was recorded. Forelimb grip measures muscle strength (6,15).

Milk-induced behaviors. Prior to testing on PND 6, each pup was deprived of food and the dam in a warm (33–34°C), humidity-controlled isolette (Air-Shields Inc., C-77) for 18–20 hours. Cannulations were performed at least one hour prior to testing using the procedures described by Hall (25) for posterior tongue cannula placements. Two pups at a time were placed individually in clear plastic cups in the isolette. Each tongue cannula was attached to the milk delivery system and baseline activity of each pup was recorded for 5 minutes in the absence of milk infusions (pre-milk). Mouthing, probing forward locomotion, forelimb paddling, rolling and curling, lying still, and twitching behaviors were recorded using a time sampling procedure for 5 seconds every 20 seconds during the observation period. Immediately following this 5 minute pre-milk period, milk infusions (50% Carnation evaporated milk/water, 10 sec every minute) commenced and continued for two 5-minute observation periods (milk 1 and milk 2, respectively). Postinfusion period activity was then observed for 5 additional minutes (post-milk). The mouthing component is an index of 5-HT functional activity in early life (19).

Figure-8 maze activity. The apparatus has been described previously (27). Animals between 21 and 24 days of age were monitored for activity on 23 out of every 24 h, over two consecutive days, under conditions of total food deprivation. During the 23-hour period, housing lights were on for 11 hours and off for a total of 12 hours. Since only eight mazes were available, it was possible to test 1 male and 1 female from only 5 randomly selected litters per dose group.

Passive avoidance learning. Adult rats (approximately PND 120) were tested for 3 consecutive days, 1 day of training (acquisition latency) and 2 days of retention testing (retention latency). During testing the rat was placed on an elevated exposed platform opening onto a dark chamber with a shock grid for a floor, and the time to enter the dark chamber was timed. Entry into the dark chamber on the first day resulted in the delivery of a 5-second duration escapable foot shock (0.8 mA) through the floor of the chamber. On the next two days entry into the dark chamber was not accompanied by foot shock.

Neurochemical Analysis

On PND 27 and 29 brains from one male and one female pup per litter, and from all dams (PND 27), were rapidly removed following decapitation. PND 27 brains (rat pups Nos. 1 and 8 and all dams) were dissected into frontal cortex, caudate nucleus, hippocampus and hypothalamus (23) and PND 29 brains (rat Nos. 2 and 6) into cerebrum (prosencephalon plus mesencephalon) and the rest of the brain; tissues were placed on dry ice and stored at −70°C until processed.

5-HT and 5-HIAA concentrations of PND 27 brains were resolved by high-performance liquid chromatography (HPLC) and quantified by electrochemical detection (EC) following the method described by Ali *et al.* (5).

5-HT uptake sites were measured by a modified method of Battaglia *et al.* (11). Cerebrum tissue of PND 29 rats was homogenized in 20 volumes of 0.32 M chilled sucrose, then centrifuged (50,000 × g for 10 minutes), and the pellet resuspended in deionized distilled water (pH 7.4). Following centrifugation the pellet was suspended in 50 mM Tris-HCl (pH 7.4) buffer and recentrifuged. The final pellet was then suspended in the incubation buffer (50 mM Tris-HCl contained 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl, 0.1% ascorbate and 10 mM pargyline, pH 7.4) at a concentration of 50 mg wet tissue weight/ml. To obtain an estimate of the maximal density of ^{3}H-paroxetine-labeled 5-HT uptake sites, a saturating concentration (0.5 nM) of ^{3}H-paroxetine (23.1 Ci/mmol; New England Nuclear, Boston, MA) was incubated with 100 µl final tissue preparation in the absence and presence of 10 uM fluoxetine (Eli Lilly Co., Indianapolis, IN). The contents were incubated in triplicate for 60 minutes at 22°C in a total volume of 1 ml. After incubation, samples were diluted with 5 ml of ice cold 50 mM Tris-HCl buffer and rapidly filtered under vacuum through over presoaked Whatman GF/C glass fiber filters (Whatman Inc., Clifton, NJ) using a Brandel Cell Harvester. The filters were washed twice with 5 ml of cold Tris-HCl buffer, air dried, placed into scintillation vials containing 10 ml Scintisol scintillation cocktail (Isolab Inc., Akron, OH), mixed, and total radioactivity was quantified by liquid scintillation spectrometry (Tracor Mark III, Elk Grove Village, IL). Specific bind-

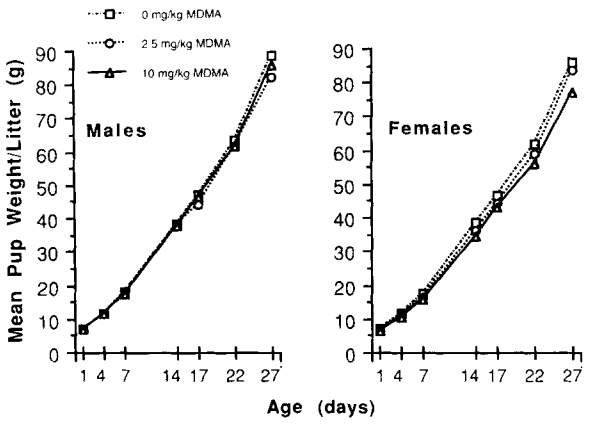

FIG. 1. Postnatal body weights of male and female rats prenatally exposed to vehicle or MDMA during gestation on alternate days 6–18. N = 10 litters/sex/group.

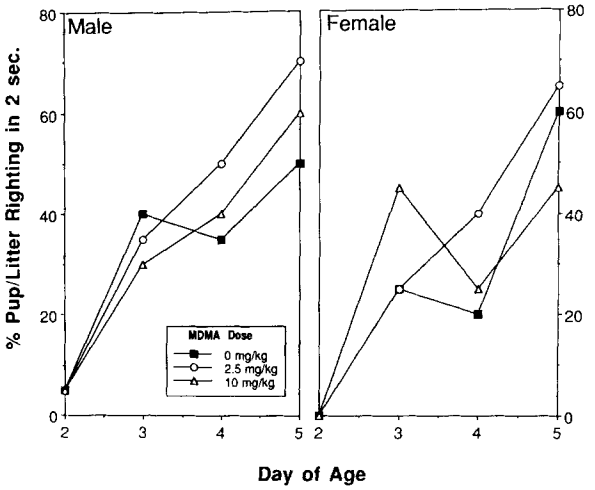

FIG. 2. Surface righting reflex response of rats prenatally exposed to vehicle or MDMA during gestation on alternate days 6–18. N = 10 litters/sex/group.

ing was calculated as the difference between the amount of ^{3}H-paroxetine alone (total binding) and that in the presence of fluoxetine (nonspecific binding). Proteins were determined according to the method of Lowry *et al.* (34).

Statistics

Maternal body weights, neurochemical data and all behavioral tests (with the exception of surface righting reflex) were analyzed by one-way ANOVA, followed by Duncan's multiple range test. Treatment effects on the surface righting reflex were analyzed by chi square tests. On all tests (except neurochemical parameters), only one pup of each sex in each litter was used as the experimental unit. The accepted level of significance was $p<0.05$.

RESULTS

Maternal Weight Gain

MDMA treatment significantly reduced maternal weight gain during gestation, $F(2,33)=3.25$, $p<0.05$ (Table 2). However, the dams' body weights during lactation were not affected by treatment (data not shown). No other physical signs of MDMA toxicity were seen in the dams.

Gestational and birth records. Length of gestation, litter size (pups/litter), and body weights of male or female offspring on PND 1 were not significantly affected by treatment (Table 2). External birth defects (malformations) were not seen in any groups. Offspring mortality was not affected by treatment and gross postmortem examinations were unremarkable and did not indicate a cause of death. Low dose MDMA treatment significantly reduced the male:female sex ratio at birth within litters, $F(2,33)=3.58$, $p<0.039$ (Table 2), but this effect was probably spurious due to a high ratio of control males.

Postnatal growth and maturation. A dose-related decrease in offspring weight gain (PND 1 to PND 27) tended to be lower in MDMA-exposed rats of both sexes (Fig. 1), although this trend

TABLE 3

MEAN HOURLY CIRCADIAN FIGURE-8 MAZE ACTIVITY OVER 48 HOURS IN PHOTOCELL COUNTS (±SEM) FOR CONTROL RATS AND RATS TREATED WITH MDMA DURING GESTATION ON ALTERNATE DAYS 6–18 AND TESTED PNDs 22–24 UNDER CONDITION OF TOTAL FOOD DEPRIVATIONS

Sex	Group (MDMA mg/kg)	1st h Activity (1000–1100 h)	Total Activity (1000 to 0900 h) / 23	Total Activity (% increase) (over control)
Male	0	223 ± 57	238 ± 36	—
	2.5	258 ± 66	251 ± 38	7%
	10	369 ± 81	314 ± 124	34%
Female	0	303 ± 53	196 ± 17	—
	2.5	294 ± 61	232 ± 25	18%
	10	258 ± 82	220 ± 52	12%

Values are means determined by first calculating a mean for each group of 5 litters (1 pup/litter) on days 1 and 2 and then calculating a mean over 48 hours for each sex using these group means.

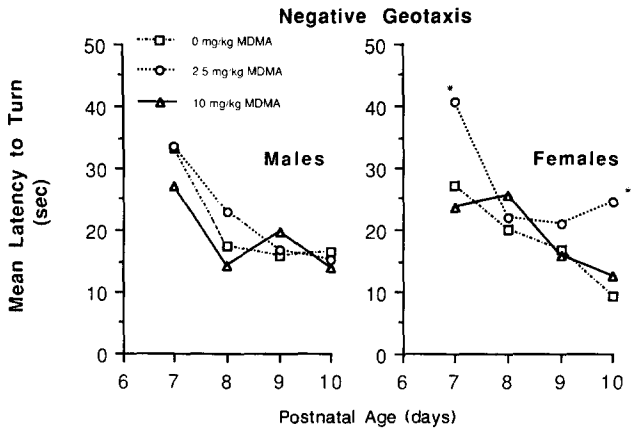

FIG. 3. Development of negative geotaxis (angle = 25°) in male and female rats prenatally exposed to vehicle or MDMA during gestation on alternate days 6–18. N = 10 litters/sex/group. *Indicates significant difference from vehicle control, $p<0.05$, Duncan's multiple range test.

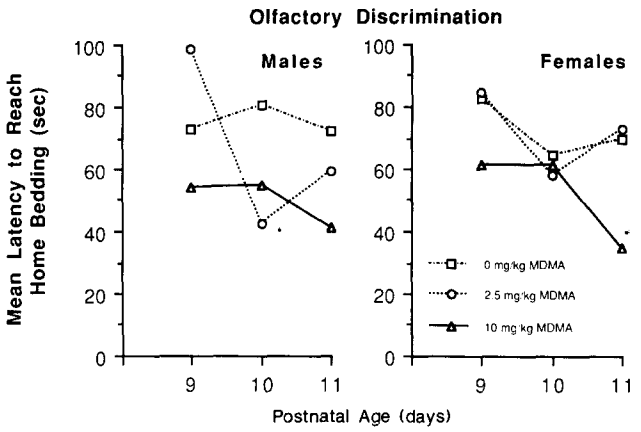

FIG. 4. Development of olfactory discrimination of male and female rats prenatally exposed to vehicle or MDMA during gestation on alternate days 6–18. N = 10 litters/sex/group. *Indicates significant difference from vehicle control, $p<0.05$, Duncan's multiple range test.

never reached statistical significance. There were no significant treatment effects on time of incisor eruption or eye opening (Table 2).

Postnatal Behavior

Surface righting reflex. The ontogeny of surface righting was not significantly affected by prenatal MDMA treatment in either sex (Fig. 2).

Negative geotaxis. As shown in Fig. 3, the ontogeny of negative geotaxis was significantly delayed only in females and only in the 2.5 mg/kg group, $F(2,27) = 4.42$, $p<0.025$. Compared to control, the turning times were significantly ($p<0.05$) slower (50% and 162%, respectively) on PND 7 and 10.

Olfactory discrimination. The overall trend of MDMA was to enhance male and female attraction to home cage bedding (Fig. 4). The ontogeny of olfactory discrimination was significantly affected by treatment in males on PND 9, $F(2,26) = 3.54$, $p<0.05$, and PND 10, $F(2,26) = 3.64$, $p<0.05$, as well as in females on PND 11, $F(2,26) = 3.71$, $p<0.05$ (Fig. 4). Relative to control values attraction to home cage bedding odor was significantly ($p<0.05$) enhanced in group 2.5 mg/kg males by 47% on PND 10 and in group 10 mg/kg females by 50% on PND 11.

Milk-induced behaviors. There was no overall apparent treatment effect on milk-induced behaviors (mouthing, probing, forward locomotion, roll/curl, forelimb paddle, lie still and twitching) in response to milk infusions on PND 6 in either male or female pups (data not shown).

Swimming behavior. Examination of swimming performance (Fig. 5) indicated no treatment effect on the ontogeny of this performance.

Forelimb grip strength. The ontogeny of forelimb grip strength in control pups as measured by latency to fall in seconds on PND 14, 17 and 22 were 11.1 ± 1.5, 13.1 ± 1.1 and 13.3 ± 1.2 for males and 12.2 ± 1.2, 13.6 ± 0.7 and 13.3 ± 0.7 for females. Forelimb grip strength was not affected by treatment (data not shown).

Passive avoidance retention. There were no significant differences in passive avoidance training or retention latencies due to treatment in adult offspring. In all groups day 2 retention latency was shorter than day 1 but since acquisition latencies were not affected by treatment, this treatment × retention day interaction was not MDMA related (data not shown).

Activity in residential maze. There were no significant differences in the first hour of diurnal or in circadian locomotor activity of male or female control rats and MDMA-treated rats recorded either singly by day (data not shown) or over 2 days in the fig-

TABLE 4

SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) CONTENT (μg/g WET TISSUE) IN THE BRAINS OF 27-DAY-OLD RATS PRENATALLY EXPOSED TO VEHICLE OR MDMA DURING GESTATION ON ALTERNATE DAYS 6–18

Sex	Group (MDMA) mg/kg	Caudate Nucleus 5-HT	Caudate Nucleus 5-HIAA	Frontal Cortex 5-HT	Frontal Cortex 5-HIAA	Hippocampus 5-HT	Hippocampus 5-HIAA
Male	0	26.0 ± 2.9	8.5 ± 0.9	21.5 ± 3.6	7.2 ± 0.8	26.4 ± 1.2	14.1 ± 0.9
	2.5	28.2 ± 3.2	11.1 ± 1.0	27.6 ± 6.1	7.6 ± 1.1	28.1 ± 2.4	12.7 ± 1.1
	10	28.4 ± 1.9	11.5 ± 1.0	21.3 ± 5.3	6.7 ± 1.3	26.1 ± 1.5	12.5 ± 0.7
Female	0	24.0 ± 3.0	10.9 ± 2.4	19.7 ± 2.4	6.9 ± 1.0	24.1 ± 1.4	13.1 ± 1.1
	2.5	27.6 ± 1.3	12.6 ± 0.6	16.6 ± 1.5	6.6 ± 0.5	24.7 ± 1.1	12.0 ± 1.3
	10	27.4 ± 2.0	10.3 ± 0.8	20.3 ± 4.6	7.2 ± 1.0	28.8 ± 1.5	14.1 ± 1.1

Values are means ± SEM for 10 litters (1 pup/litter) per group.

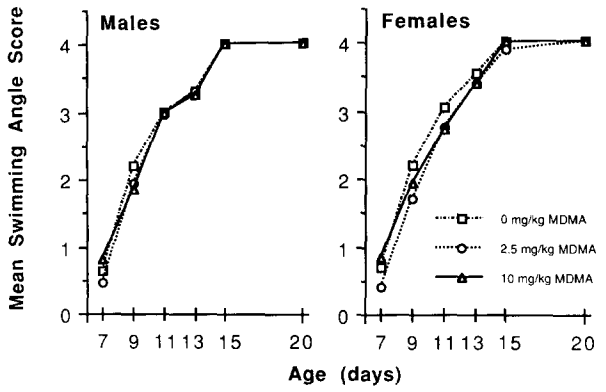

FIG. 5. Swimming performance of developing male and female rats exposed prenatally to vehicle or MDMA during gestation on alternate days 6–18. N = 10 litters/sex/group.

ure-8 maze (Table 3).

Neurochemistry

5-HT uptake sites. The density of 5-HT uptake sites in the cerebrum on PND 29 were not affected by prenatal exposure of pups to MDMA. Values in the 2.5 and 10 mg MDMA/kg dosed male offspring were respectively 90.6 ± 28.4 and 99.6 ± 28.2 percent of control value, while values for females were 89.2 ± 24.8 and 101.6 ± 25.8 percent, respectively.

Brain 5-HT and 5-HIAA. As shown in Table 4, prenatal MDMA treatment did not affect 5-HT and 5-HIAA levels on PND 27 in the caudate nucleus, frontal cortex, or hippocampus of exposed male and female offspring. In contrast dams killed on postgestational day 27 following gestational treatment with MDMA at various doses up to 10 mg MDMA/kg manifested dose-dependent decreases in the content of 5-HT and 5-HIAA in discrete brain areas (Table 5). However, only the 5-HT reductions in the caudate nucleus and the hippocampus associated with the 10 mg/kg MDMA dose were statistically significant [CN, $F(2,27) = 4.57$, $p < 0.05$; Hipp, $F(2,27) = 4.84$, $p < 0.05$].

DISCUSSION

These results provide no evidence for a substantive effect of prenatal MDMA exposure upon offspring under the dosing conditions of the present study. There were no recognizable malformations in neonates, although since examination of uterine contents prior to delivery was not attempted, it cannot be stated that no anomalies or in utero deaths occurred. MDMA exposure also produced no effects on duration of gestation, litter size, time of appearance of physical landmarks, or on regional CNS content or uptake of serotonin or 5-HIAA in offspring. A variety of behavioral indices, including body righting, motor activity, passive avoidance and suckling behaviors, all thought to be modulated in part by the serotonergic system (32), were also unaffected by prenatal MDMA. Two behaviors did show some alteration in MDMA-exposed offspring. Ontogeny of negative geotaxis was slowed, but only in females, and then only for low, not high dose females. In contrast, olfactory discrimination was improved at the high MDMA dose in both sexes.

It is impossible to interpret these findings in the context of a single study. Given the large number of significance tests conducted, type I errors are not unlikely and the few positive effects could simply be stochastic error. Further, even if these occasional significant findings are real, it is possible that in the absence of cross-fostering at birth they were due not to direct drug effects on the fetus, but rather to being reared by dams with demonstrated damage to the serotonin system. Certainly successful replication of such findings will be required before they can be accepted as scientifically valid.

On balance, then, an MDMA dosing regime which caused damage to the maternal serotonin system had no substantial effect on the offspring. There could be many explanations for this lack of effect on the offspring. One obvious possibility is that MDMA was simply not given often enough at high enough doses to alter fetal serotonin. Even in dams only the high MDMA dose reduced serotonin levels, and then only to a modest degree. A related factor is the degree of placental metabolism of MDMA. If this is at all substantial, then it is probable that concentrations of MDMA or MDMA metabolites in fetal brain never attained toxic levels.

A second and more interesting possibility is that the fetal brain is somehow protected from MDMA toxicity. Parachloramphetamine (PCA), a known serotonergic neurotoxin, appears to be structurally and functionally similar to MDMA (50). It has been reported that rat pups do not suffer serotonin depletion from PCA administration if the drug is administered before the seventh postnatal day of life (16, 46, 47). It is known that uptake into serotonergic nerve endings is a necessary precondition for toxicity for both PCA (39) and MDMA (12). Conceivably, such uptake mechanisms are not completely functional prior to PND 7, a factor which would protect against MDMA neurotoxicity.

In conclusion, the findings of this study suggest that maternal doses of up to 10 mg/kg MDMA PO, given on alternate days, do not significantly alter behavior or neurochemistry in rat offspring. In fact, the dam is more at risk for alterations in central 5-HT content than are its offspring following such MDMA exposure.

TABLE 5

SEROTONIN (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA) CONTENT (μg/g TISSUE) IN THE BRAINS OF DAMS TREATED WITH VEHICLE OR MDMA DURING GESTATION ON ALTERNATE DAYS 6–18 AND SACRIFICED ON POSTGESTATIONAL DAY 27

Group (MDMA) mg/kg	Caudate Nucleus		Frontal Cortex		Hippocampus	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
0	39.35 ± 2.2	9.77 ± 1.0	45.15 ± 9.6	9.61 ± 1.7	36.12 ± 2.6	13.37 ± 0.9
2.5	35.26 ± 2.3	8.43 ± 1.0	45.00 ± 8.8	7.67 ± 1.4	34.55 ± 1.4	12.10 ± 0.8
10	33.30 ± 2.7*	9.04 ± 1.0	33.14 ± 6.3	6.51 ± 0.7	27.34 ± 1.1*	11.58 ± 0.8

Values are means ± SEM for 10 litters (1 pup/litter) per group.
*Significantly different from the appropriate control value, $p < 0.05$.

ACKNOWLEDGEMENTS

This research was supported in part by an appointment to the Oak Ridge Associated Universities (ORAU) Faculty Research Program at the National Center for Toxicological Research (NCTR) administered by ORAU through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration. We thank Ms. Rose Huber and Ms. Barbara Jacks for expert secretarial assistance in preparation of this manuscript.

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