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# Long-term behavioral consequences of prenatal MDMA exposure

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ABSTRACT

The current study sought to determine whether prenatal 3,4-methylenedioxy-*N*-methamphetamine (MDMA) exposure from E14–20 in the rat resulted in behavioral sequelae in adult offspring. Prenatal MDMA exposure results in increased dopaminergic fiber density in the prefrontal cortex, striatum and nucleus accumbens of young rats. Since these areas are critical in response to novelty, reward, attention and locomotor activity, we hypothesized that prenatal MDMA exposure would produce significant changes in the performance of tasks that examine such behaviors in adult rats. Adult rats prenatally exposed to MDMA exhibited greater activity and spent more time in the center during a novel open field test as compared to controls. This increased activity was not reflected in normal home cage activity. Prenatal exposure to MDMA did not affect feeding or food reward. It did not alter cocaine self-administration behaviors, nor did it have an effect on the locomotor response to amphetamine challenge. Finally, while prenatal MDMA did not affect performance in the radial arm maze or the Morris water maze (MWM), these animals demonstrated altered performance in a cued MWM paradigm. Prenatal MDMA exposure resulted in perseverative attendance to a hanging cue when the platform in the MWM was removed as compared to controls. Together, these data demonstrate that prenatal exposure to MDMA results in a behavioral phenotype in adult rats characterized by reduced anxiety, a heightened response to novelty, and "hyperattentiveness" to environmental cues during spatial learning.



## 1. Introduction

Much of the research on MDMA has focused on its long-term behavioral and neurologic consequences in those who abuse it. Recreational use of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") is most prevalent among adolescents and young adults. Outward symptoms of MDMA intoxication include acute hyperthermia, hyperactivity, euphoria and impulsive behavior. Repeated use of MDMA has been associated with depression, anxiety and deficits in learning and memory [1–6].

Pharmacologic studies have demonstrated that MDMA may be a putative serotonin (5-HT) neurotoxin. As with all amphetamine-like compounds, MDMA is taken up into the presynaptic terminals of monoaminergic neurons via the membrane-bound transporters. MDMA binds with varying affinity to these dopaminergic, noradrenergic and serotonergic transporters (DAT, NET and SERT, respectively) where it reverses the normal inward flow of monoamines at their respective transporters, releasing cytosolic monoamines into the extracellular space. The depletion of cytosolic 5-HT stores via this mechanism may be partially responsible for the long-term loss of phenotypic markers in cortical 5-HT axons as a result of MDMA exposure [7,8]. Many consider this long-term loss to be evidence of MDMA-induced 5-HT neurotoxicity.

Because many MDMA users are young women of childbearing age, there is an elevated risk for accidental fetal exposure. Modeling this phenomenon in rodents requires consideration of both the timing of administration and the dosage of MDMA. To date, three comprehensive reports have been published that examine the patterns of MDMA use in pregnant women [9–11]. A consensus across these studies is that MDMA use tends to be restricted almost exclusively to the first trimester, with most users ceasing by week 10. The first trimester in the human is characterized by the development and differentiation of the dopamine, norepinephrine, and serotonin systems. In rats, these systems begin to develop on embryonic day 13 (E13) [12]. The administration schedule used in these studies (E14–20) begins during early development and axonal sprouting, and ends around the time that these systems begin to form patent connections with target structures (including the striatum, nucleus accumbens, hippocampus, and frontal cortex). It also approximates the most common gestational period during which MDMA use occurs in the human. Finally, based

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upon interspecies scaling, it is possible to administer a dosage of MDMA that is equivalent, in magnitude, to those seen in human consumption [13]. The dose used in these studies (15 mg/kg) is significantly lower than those that have been shown to be neurotoxic, and falls within a range that is comparable to the quantity consumed during typical human use [14,15].

Unlike in adult animal models, prenatal exposure to MDMA in rats does not result in discernable changes in serotonin neurochemistry or neuronal morphology [16,17]. However, prenatal exposure to MDMA in rats results in a 5-fold increase in dopamine neuron fiber density in the prefrontal cortex (PFC), with smaller increases evident in the striatum (STR) and nucleus accumbens (NAc), by postnatal day 21 (P21) [17]. These same rats exhibit significant increases in spontaneous locomotor activity as measured during a 20 min novel cage test as compared to controls at P21.

Since these dopaminergic structures are critical in learning, attention, reward and locomotor activity [18–22], the current study sought to determine whether these neurochemical and neuroanatomical alterations from prenatal MDMA were associated with behavioral sequelae. Animals were assessed to determine whether prenatal MDMA exposure resulted in alterations in spontaneous or amphetamine-induced locomotor activity or learning tasks. The consequences of prenatal MDMA exposure on reward were assessed using both food and drug self-administration paradigms. Finally, these animals were assessed for deficits in spatial localization in the Morris water maze (MWM) and spontaneous alternation of a radial arm maze (RAM) task, which can reflect changes in learning and attention.

Based upon our prior neuroanatomical studies that demonstrated dopaminergic hyperinnervation of mesolimbic and mesocortical structures, we hypothesized that prenatal MDMA exposure would result in increased spontaneous and amphetamine-induced locomotion, and a decrease in anxiety-related behaviors in the open field paradigm. We also hypothesized that prenatal MDMA exposure would result in a lower threshold for self-administration, shorter intervals between lever presses for cocaine at a fixed dose, as well as perseverative lever pressing at lower systemic cocaine concentrations prior to extinguishing, as compared to controls. Finally, based on adult data which implicates MDMA in deficits in attention and memory, as well as evidence which suggests that the mesolimbic DA system is involved in spatial localization, we hypothesized that our animals would exhibit impaired working memory in both spontaneous alternation and MWM tasks [2–4,6,18].

## 2. Methods

### 2.1. Materials

MDMA HCl and cocaine HCl were provided by the NIDA Research Drug Supply System (RTI, Research Triangle Park, NC). Amphetamine $SO_4$ was purchased from Sigma Aldrich. Cocaine was dissolved in saline solution containing 1 unit/ml of heparin and then passed through a sterile 0.2-Am acetate filter prior to use in the self-administration studies. Heparin sodium was obtained from American Pharmaceutical Partners Inc (Schaumburg, IL).

### 2.2. Subjects

Timed-pregnant (embryonic day 10; E10) Sprague–Dawley rats (Zivic Miller, $n=18$) were acclimated to the AALAC approved facility for 4 days prior to the start of drug administration on E14. The dams were placed on a 12 h light/dark cycle (lights on 6:00 a.m.) in a temperature—(21 ° C) and humidity—(45%) controlled room. Dams were housed individually and food and water were available ad libitum. Protocols were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

### 2.3. Drug administration

On E14, dams were randomly assigned to one of two conditions. Each animal received a subcutaneous (s.c.) injection of either vehicle (VEH: 1 ml/kg saline), or MDMA (15 mg/kg), twice daily from E14 to E20. Dams delivered on E21 and the litters were culled on the following full postnatal day (P1) to eight (4 males, 4 females) offspring in the MDMA-exposed litters and ten (5 males, 5 females) offspring in the VEH-exposed litters. This measure was taken to compensate for lower birth weights in the MDMA pups associated with the drug's anorectic effects on the mother. It has been demonstrated that this approach produces a slower weight gain in the VEH pups as compared to the MDMA pups, equalizing their weights by P3 [23].

The offspring remained housed with the dam until weaning (P21), at which time they were removed and placed in individual cages. The rats were housed separately for the duration of the study. The animals were subjected to a 12 h light–dark cycle (lights on at 12:00 a.m.) and food and water were available ad lib. A single male from each litter was placed into one of four cohorts resulting in an $n$ of 8 for each condition (prenatal MDMA or VEH) in each cohort except for the self-administration cohort (cohort 4) which had an $n$ of 7 per condition ($N=62$).

### 2.4. Temperature and body composition

#### 2.4.1. Maternal temperature regulation

Hyperthermia has been implicated in many studies as a significant component of MDMA-induced neurotoxicity [24–26]. In order to eliminate the hyperthermic effects of MDMA administration as a variable, the ambient temperature was kept at 21 °C and maternal temperatures were recorded 0, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min post-injection. Temperature was monitored using a transponder reader system (DAS-6007, Bio Medic Data Systems Inc. Seaford, DE) following the protocol outlined by the manufacturer. A

**Table 1**
Timeline of behavioral assessment for each animal cohort

| | P35 | P40 | P45 | P50 | P55 | P60 | P65 | P70 | P75 | P80 | P85 | P90 | P95 | P100 | P105 | P120 | P125 | P130 | P135 | P140 | P145 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cohort 1 | Homecage activity | | | | Standard MWM | | | | NMR | | | | | | | AMPH challenge | | | HF diet | | |
| | P40–P50 | | | | P53–P57 | | | | P75 | | | | | | | P120 | | | P132–145 | | |
| Cohort 2 | Running wheel | | | | | Open field | | | NMR | | | | | | | | | | | | |
| | P36–P39 | | | | | P61–P62 | | | P75 | | | | | | | | | | | | |
| Cohort 3 | | RAM | | | Progressive ratio | | | Cued MWM | | | | | | | | | | | | | |
| | | P40 | | | P49–P58 | | | P69–P72 | | | | | | | | | | | | | |
| Cohort 4 | | | | | | | | | Cocaine self administration | | | | | | | | | | | | |
| | | | | | | | | | P54–P105 | | | | | | | | | | | | |

Note: Data are presented as the age (postnatal day) of the offspring at the time each assay was conducted. Cohorts 1–3 consisted of 8 VEH- and 8 MDMA-treated rats, each derived from separate litters. Cohort 4 consisted of 7 VEH- and 7 MDMA-treated rats derived from separate litters. P=postnatal day; MWM=Morris Water Maze; NMR=Nuclear Magnetic Resonance imaging; AMPH=amphetamine; RAM=radial arm maze; HF=high fat.

sterile IPTT-300 programmable temperature transponder was implanted subcutaneously in a longitudinal orientation above the right shoulder of each dam. Non-invasive temperature readings were taken by passing the reader over the implanted transponder. The transponder data was stored within the reader and transmitted to a PC via a PI-6000 Power Interface.

*2.4.2. Body composition analysis*

To address the concern that MDMA's anorectic effect on the dam may have an effect on the body composition of the offspring, nuclear magnetic resonance (NMR) (EchoMRI; EchoMedical Systems, Houston TX) was used to estimate total lean tissue, fat tissue and water in offspring on P75 (cohorts 1 and 2; $n$=32; Table 1) according to a previously published protocol [27]. Briefly, unanesthetized rats were placed in a restraint tube and inserted into the NMR for 30–45 s. The procedure is well tolerated in rats and does not cause significant distress [28]. The mean body weight and % body fat was calculated for each animal.

*2.5. Behavioral tasks*

*2.5.1. Activity*

Animals from cohorts 1 and 2 (Table 1) were subjected to tasks intended to determine whether the increased locomotor activity observed on P21, persisted in adult animals that had been prenatally-exposed to MDMA, and to determine whether locomotor changes were a reflection of a general elevation in activity level, or were specifically associated with a novel environment [17].

*2.5.1.1. Home cage locomotor activity.* To distinguish between anxiety-related behavior and changes in general locomotor activity, the home cage activity for VEH- ($n$=8) and MDMA-exposed ($n$=8) rats (cohort 1) was determined from P40 to P50 using the SmartFrame stainless steel cage rack frame (Hamilton-Kinder Scientific Company, Poway, CA) which was placed around each animal's shoebox homecage. Infrared photobeam interruption sensors (7X and 15Y) mounted in the frame detected movement which was recorded and analyzed using the HMM100 MotorMonitor software. Vertical and horizontal activity within the homecage was recorded for 48 h, and the events were collapsed into 60 minute bins. The data were analyzed as the average number of beam interruptions per group per hour.

*2.5.1.2. Running wheel activity.* Rats from cohort 2 were assessed for voluntary exercise in wall-mounted running wheels from P36 to P39. A stainless steel wheel suitable for rat voluntary exercise was placed within each animal's home cage. Infrared photobeam interruption sensors (Lafayette Instruments Company, Lafayette, IN) measured wheel revolutions, which was then converted to the total distance traveled and average velocity of each animal across 4 consecutive days. Distance was measured as cumulative meters, while velocity was recorded as average meters per minute.

*2.5.1.3. Open field.* On P61 or P62, rats from cohort 2 were placed individually in the Open Field, a 1 m×1 m black opaque 4-sided Plexiglas box with 30 cm tall black opaque walls surrounding each side. Trials lasted 15 min and the animals were not habituated to the apparatus prior to testing. All activity was recorded digitally and analyzed using Cleversystem TopScan Software (Virginia). Behaviors measured included: time spent in border areas, total distance, quadrant crossing, and average velocity. In addition to general activity levels, the amount of time spent along the periphery in the border areas, as opposed to the center areas, was used as an index of anxiety.

*2.5.2. Food reward*

Dopamine in the dorsal STR, NAc, and ventral tegmental area (VTA) is generally recognized to play a significant role in feeding behavior [29–32], especially with respect to highly palatable foods. This portion of the study sought to determine whether the altered dopaminergic innervation seen following prenatal exposure to MDMA resulted in a differential response to highly palatable foods or food-motivated behaviors as compared to controls. Separate cohorts of rats were used for these studies to eliminate any possible interaction between experimental conditions (Table 1).

*2.5.2.1. High fat diet.* Previous data demonstrate that diets high in fat elicit significant hyperphagia and obesity in rats. Therefore, subjects in cohort 1 were subjected to a high fat diet (HFD; Dyets, Inc., Bethlehem, PA, 4.41 kcal/g, 1.71 kcal/g from fat) or standard lab chow for 14 days (P132 to P145). Body weight and food intake were measured daily.

*2.5.2.2. Progressive ratio responding for sucrose.* Previous work indicates that rats pre-exposed to MDMA as adults show a marked increase in food motivated behavior under a progressive ratio schedule [33]. In order to determine whether prenatal exposure to MDMA would increase food-motivated behavior, we assessed progressive ratio response levels and breaking point for sucrose pellets in rats from cohort 3 on P58.

*2.5.2.2.1. Food restriction.* To encourage operant responding, the rats were restricted to 85% of their daily food intake for two days prior to the beginning of training. Following these two days, all rats had ad-lib access to food in the homecage.

*2.5.2.2.2. Apparatus.* The conditioning and testing procedures were conducted in four identical conditioning chambers constructed of aluminum end walls and clear Plexiglas sides and measuring 21.6×21.6×27.9 cm. A grid of 0.48 cm in diameter stainless steel bars, spaced 1.9 cm apart, served as the floor of each chamber. A food cup was located on one end wall of each chamber inside a 5×5 cm recessed opening. Two levers were located approximately 3 cm to the left and right of the food cup, level with the top of the opening. Only the right lever was active during this experiment. All experimental events were controlled and recorded by computers located in an adjoining room running ABET software (Lafayette Instruments; Lafayette, IN).

*2.5.2.2.3. Training.* Training was carried out from P48 to P57 in animals from cohort 3. Training was carried out in 1 hour sessions over eight consecutive days using the following schedule: During the first two days of training, a fixed ratio (FR1)-autoshaping procedure was employed, in which each lever press earned a single 45 mg sucrose pellet (TestDiet, Richmond, IN) and a noncontingent pellet was dispensed 600 s without any lever pressing. The rationale for this procedure is that the noncontingent pellet delivery facilitates the rapid acquisition of lever-pressing responses. All animals were then trained for 2 days using an FR1 schedule with no noncontingent pellet deliveries, followed by 2 days of FR2 training and 2 days of FR3 training, in which 2 or 3 presses were required to earn a sucrose pellet, respectively [34].

*2.5.2.2.4. Progressive ratio trial.* At the conclusion of the eight day fixed-schedule operant training regimen, animals were given a single test-session under a progressive ratio (PR2) schedule of reinforcement. One lever press initially resulted in the delivery of 1 sucrose pellet, and subsequent delivery of sucrose required an increase of 2 presses from the previously required number. In this way, the number of responses required to obtain a pellet increased on successive trials as follows: 1, 3, 5, 7, 9, 11, 13, 15, 17, etc. Each animal's breakpoint was defined by the number of lever presses which resulted in the final successful acquisition of a sucrose pellet followed by a 20 minute period during which the number of lever presses required for further sucrose delivery was not satisfied [34].

*2.5.3. Response to psychostimulants*

Because the mesolimbic dopaminergic system is associated with reward, we next attempted to determine whether the previously observed increase in DA fiber density in the PFC, STR, and NAc resulted in an increase in sensitivity to psychostimulants in either spontaneous or operant responding paradigms.

*2.5.3.1. Amphetamine challenge.* On P120, rats from cohort 1 (Table 1) were administered an intraperitoneal (i.p.) injection of amphetamine (1 mg/kg) 90 min prior to the onset of the dark cycle and home cage activity was recorded for 24 hour post-injection. Activity was again assessed via SmartFrame stainless steel cage rack frame (Hamilton-Kinder Scientific Company, Poway, CA) which was placed around each animal's shoebox homecage. Data from the first 2 h were analyzed in 1-minute intervals. Data across 24-h were analyzed in 1-h bins.

*2.5.3.2. Cocaine self-administration.* In a fourth cohort of animals (cohort 4; Table 1), one male from each litter (P42 to P45; MDMA $n=7$; VEH $n=7$) was implanted with an indwelling catheter into the right jugular vein under isoflurane anesthesia via a previously published protocol [35]. Self-administration behavior was examined from P54–P105.

*2.5.3.2.1. Apparatus.* Fourteen test chambers (modified chambers from Lafayette Instrument, Lafayette, IN) equipped with one active lever, one inactive lever, and one signal light, were placed inside a sound-attenuated wooden compartment (43×61×35 cm). House lights (7 W) within the chamber were illuminated for the duration of every session. Infusion pumps (model PHM-100, Med Associates, Georgia, VT) situated outside of the chamber served as the drug delivery mechanism. Unit dose of cocaine was regulated by the duration of the injection, which was given at a rate of infusion of 11.6 μl/s and a concentration of 5 μg/μl. Computers controlled the light and drug infusion and monitored lever presses using DIG-715 Smart Controller interfaces and a program written in Medstate Notation language (Med Associates Inc., St. Albans, VT).

*2.5.3.2.2. Training.* Beginning 12 days after surgery, rats were trained to self-administer cocaine HCl (0.5 mg/kg) using a fixed ratio (FR=1) schedule with a five second time out period (TO=5) following cocaine infusion. A signal light was illuminated whenever the pump was activated. Training at a fixed dose continued on consecutive days for 2.5 h per session until each rat demonstrated stable maintenance of self-administration. Self-administration was considered stable when there was no significant change of the mean and standard deviation (SD) of the inter-injection intervals over a minimum of 5 consecutive trials. Every session began with a drug infusion delivered by the experimenter (0.5 mg/kg), and computer-controlled injections (1.0 mg/kg) were given non-contingently if 60 min elapsed without a lever press.

*2.5.3.2.3. Priming threshold.* Cocaine reinstates self-administration behavior when a minimum threshold concentration is reached [28]. In order to measure this threshold, the level of cocaine in the body is titrated by gradually raising the concentration until the threshold level is achieved. Because environmental cues can induce spontaneous attempts to self-administer cocaine, drug-induced priming can become obscured. To extinguish purely environmentally-cued lever presses, each priming session began with a period in which lever-pressing did not result in cocaine infusion. During this time, the light was randomly illuminated but did not correspond with drug delivery. Following 30 min with no attempts to self-administer, the pump was activated and non-contingent injections of escalating doses of cocaine were administered every 2 min until self-administration behavior was reinstated. The non-contingent injections were calculated to raise the peak cocaine level by 20 μg/kg above the previous peak. This escalation resulted in a linear increase in the peak level of cocaine as a function of time (10 μg/kg per min).

The cumulative level of cocaine at any time during a session was calculated according to the following equations:

$$Lcc = Lpc \cdot (1 - k_{10} - k_{12}) + Lpp \cdot k_{21} + V_{01} \quad (1)$$

$$Lcp = Lpp \cdot (1 - k_{21} - k_{20}) + Lpc \cdot k_{12} \quad (2)$$

where Lcc=current central drug level, Lpc=the previously calculated drug level, Lcp=current peripheral level of the drug, Lpp=the previously calculated peripheral drug level, $V_{01}$=the amount of cocaine administered, $k_{10}$=the constant of elimination from the central compartment, $k_{12}$=the constant of distribution from the central to the peripheral compartment, $k_{21}$=the constant of redistribution from the peripheral to the central compartment, and $k_{20}$=the constant of elimination from the peripheral compartment [36]. The priming threshold value was calculated as the average of the cocaine levels resulting from the ultimate and penultimate priming injections. Only one priming threshold value was obtained from each session.

*2.5.3.2.4. Maintained self-administration.* After the priming threshold was reached, self-administration continued at unit doses between 750 and 12,000 nmols/kg. The mean time between lever presses (inter-injection interval; FR1 schedule) was calculated for each unit dose. Only one unit dose was administered per session.

*2.5.3.2.5. Extinction.* After stable self-administration had been demonstrated for at least 5 consecutive training sessions, test sessions commenced which consisted of a period of self-administration, followed by an extinction phase wherein lever-pressing did not result in a drug injection. Extinction criteria were satisfied when 30 min passed without an attempt to self-administer cocaine via either the active or inactive lever.

*2.5.4. Spatial learning, memory and attention*

Finally, separate experiments were conducted to determine whether pre-natal MDMA compromised spatial learning, memory and attention. In separate experiments, these were assessed by a 4-arm spontaneous alternation task as well as by fixed-platform and cued-platform MWM tasks (Table 1).

*2.5.4.1. Four-arm spontaneous alternations.* Under normal conditions, rats exhibit predictable exploratory behavior in novel environments, that depend on intact spatial working memory function [37]. On P40, rats from cohort 3 were placed in the center of a 4-arm radial maze (Lafayette Instruments) and allowed to explore freely for 15 min. Arm entries (defined by photobeam crossing at the end of each arm) were recorded as well as order of arm entries. Spontaneous alternations were calculated as described previously, where performance above chance levels is dependent on intact working memory processes [37]. Briefly, an alternation is defined as the number of successive non-repeat arm entries divided by the total possible number of entries, including potential repeats. The fewer number of successive repeat entries is used as an index of spatial working memory.

*2.5.4.2. Morris water maze*

*2.5.4.2.1. Apparatus.* The MWM consisted of a circular fiberglass pool (122 cm diameter, 75 cm height; Rowland Fiberglass Inc., Ingleside, TX) filled with water (17–19 °C, 43 cm deep). A clear glass platform (10.5 cm×10.5 cm; square) was submerged 1 cm below the water surface. The pool was situated in a room that contained extra-maze cues visible to the rats during testing (42 cm×76 cm posters printed with contrasting patterns and shapes). The pool water was dyed to a dark blue color with food coloring in order to allow significant contrast between the animals' white fur and the pool water and to render the platform invisible under the surface of the water. Latency to escape the water was calculated for each trial by overhanging digital video camera and computer controlled TopScan software (Cleversystem Inc., Reston, VA) and used as index of spatial learning and memory ability. Separate cohorts of rats were used for the standard MWM and the cued MWM experiments in order to eliminate any possible learning effect across experimental conditions (standard: cohort 1; cued: cohort 3).

*2.5.4.2.2. Fixed position platform.* Rats from cohort 1 ($n=8$ per group) were trained from P53–P56, and were tested on P57. At the onset of each trial, an individual rat (cohort 1) was placed into the water at one of four possible starting points (N, S, W, and E). The starting location for each trial was varied and all start locations were used in a given day and not repeated before each start location was used. A trial was terminated and the latency was recorded when the

rat found and climbed onto the platform for 5 s If the rat did not reach the platform within 1 min, the trial was terminated, and the rat was placed on the platform for 5 s. Each rat received two trials per day, 30 min apart, for four consecutive days. Training started each day 1 h into the rats' active phase and was performed in a well-lit room. Each trial was digitally recorded for subsequent path analysis utilizing Cleversystem TopScan software (Reston, VA). On the 4th and final day of testing, a probe trial was performed where the hidden escape platform was removed from the pool and each animal was allowed to swim for 60 s. The amount of time spent in the quadrant of the pool where the platform had been located was quantified.

*2.5.4.2.3. Cued platform.* Rats from cohort 3 ($n$=8 per group) were trained from P69–P72, and were tested on P73. This procedure was similar to the previous experiment with the exception that the platform position remained the same for each trial conducted within a single day, but was varied randomly between training days. Additionally, the platform position was always paired with an overhanging cue situated directly over the platform. This required the rat to attend to the moving cue, rather than orienting on fixed extra-maze cues as is done in Fixed Platform Test. In this way the rats were required to learn a new starting location at the beginning of each day, indicated by a visible overhanging cue. Each rat (cohort 3) was subjected 6 trials of 60 s per day on 4 consecutive days with a 60 second interval between trials. On the 5th day, rats were subjected to 4 trials of 60 s, immediately followed by a 5th trial in which the platform was removed from the pool and the overhanging cue was moved to the opposite quadrant. Path analysis, time spent in each quadrant, and latency to the platform was quantified by TopScan software (Cleversystem; Reston, VA).

### 2.6. Statistical analysis

The mean±SEM was calculated for each animal for each parameter and are provided in the results section. Significance was determined by the Student's $t$-test, a one or a two-way ANOVA with or without repeated measures when appropriate. Significant main effects or interactions were analyzed by post-hoc pairwise comparison using the Holm-Sidak correction. Significance was determined when $p<0.05$.

## 3. Results

### 3.1. Temp and body composition

#### 3.1.1. Temperature

Prior to injection ($t$=0) both MDMA and VEH rat dams had an initial temperature of 37.1 °C. VEH-exposed animals demonstrated a slight increase in temperature to 37.8 °C at 30 min which quickly returned to baseline. Administration of MDMA on E14 produced a significant hypothermic response that reached its lowest level at 60 min (35.5 °C) and slowly returned to baseline at 5 h post-injection (Fig. 1A). Rat dams developed a tolerance to the temperature dysregulating properties of MDMA such that by E17, there were no significant temperature changes from baseline (Fig. 1B). Later in the administration schedule (E18–E20) VEH-treated dams exhibited significant drops in body temperature post-injection of approximately 1 °C from baseline, whereas MDMA treated animals maintained their body temperature (Fig. 1C).

#### 3.1.2. Body composition

There were no differences in body weight (MDMA 504.19±12.3 g, VEH 500.71±9.12 g) or percent body fat (MDMA 11.1±0.54%, VEH 10.92±0.34%) in MDMA- versus VEH-exposed animals. This data confirms that the MDMA-induced anorexia in the dam does not result in significant metabolic consequences in the offspring.

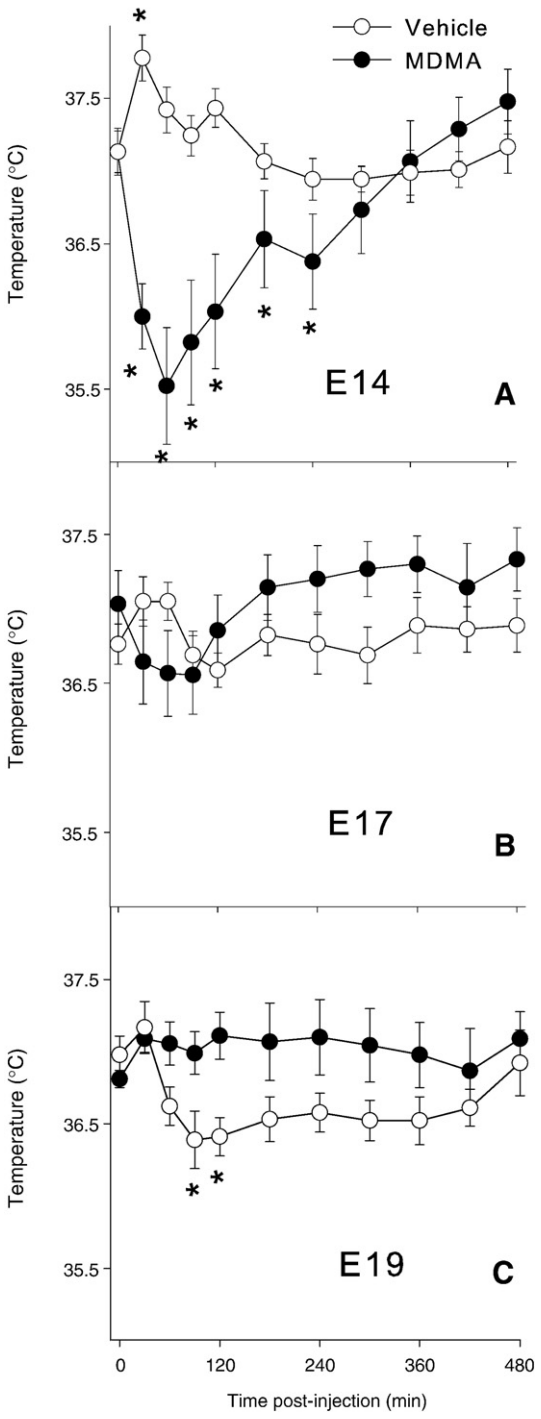


**Fig. 1.** Consequence of MDMA administration on rat dam core body temperature when kept at 21 °C. (A) On the first day of administration (E14), MDMA-exposed dams showed an initial drop in body temperature while dams receiving vehicle treatments exhibited a mild increase in temperature that resolved within the first hour after treatment. (B) By E17, MDMA administration did not result in significant fluctuation in temperature suggestion a tolerance to the temperature dysregulating effects of the drug. (C) In the following days (E18–20), MDMA-exposed dams maintained normal body temperature, while vehicle-treated dams showed reductions in body temperature after injections. These data demonstrate that MDMA induces thermal dysregulation rather than frank hyperthermia. (MDMA $n$=8; VEH $n$=8) *=$p<0.05$ vs time 0.

### 3.2. Activity

#### 3.2.1. Home cage locomotor activity

The results of the homecage locomotor activity test were not significant across groups (MDMA 34191.76±1971.03 beam breaks/h, VEH 34911.81±2042.162 beam breaks/h).

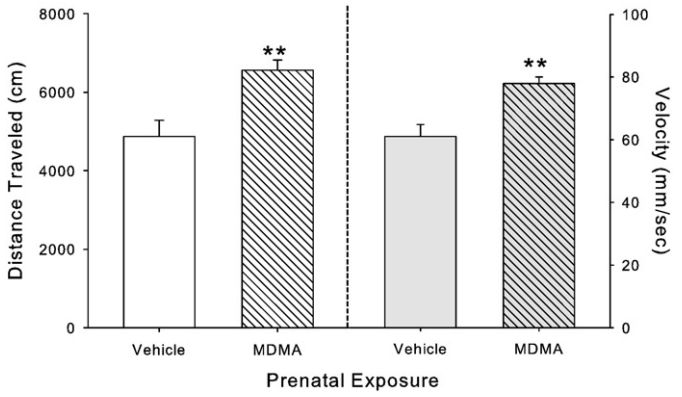


**Fig. 2.** Effects of MDMA on locomotor activity in an open-field. Adult rats prenatally exposed to MDMA locomoted more (MDMA 6559.7±259.7 cm, VEH 4876.5±412.1 cm; $t=3.456$; $p<0.004$) and moved at a greater velocity (MDMA 77.91±2.12 mm/s, VEH 61.05±3.78 mm/s; $t=3.889$; $p<0.002$) throughout the trial. This increase response to a novel environment may reflect a change in attentional processing. (MDMA $n=8$; VEH $n=8$) **=$p<0.01$.

### 3.2.2. Running wheel activity

Both VEH and MDMA rats exhibited a diurnal pattern of activity in the running wheel ($F_{(7,12)}=26.222$; $p<0.001$), however there was no distinguishable difference in the velocity (MDMA 6.63±0.29 m/min, VEH 6.41±0.89 m/min) or total distance traveled (MDMA 6159.5± 151.02 m, 5848.96±142.06 m) across groups again confirming that the results of the open field test do not represent an indiscriminate change in activity level.

### 3.2.3. Open field

The results of the open field test, as shown in Fig. 2, indicate that prenatal exposure to MDMA results in adult rats that moved 27.6% faster ($t=3.889$; $p<0.002$) and traveled 34.5% further ($t=3.456$; $p<0.004$) than VEH animals.

Additionally, MDMA animals crossed the quadrants of the apparatus 49.2% more ($t=3.552$; $p<0.003$), and spent more time in the central quadrants than VEH controls (9.7% vs. 5.54%; $t=2.188$; $p<0.046$; Fig. 3).

## 3.3. Food reward

### 3.3.1. High fat diet

There was no difference in the quantity of high fat chow consumed by MDMA- or VEH -treated animals (MDMA 34.72±0.66 g/day, VEH 33.95±0.72 g/day). Both groups gained a significant amount of weight (14.05%) during the study ($F_{(1,12)}=164.772$; $p<0.001$), however there was no effect of treatment over time in the amount of chow consumed or in the amount of weight gained (data not shown).

### 3.3.2. Progressive ratio for sucrose

All rats reliably increased lever-press responses for sucrose pellets regardless of drug exposure, however there was no difference in the progressive ratio breakpoints between the groups (MDMA 42.625± 10.5 presses, VEH 37.625±14.0 presses).

## 3.4. Response to psychostimulants

### 3.4.1. Amphetamine challenge

While we hypothesized that MDMA animals would be more sensitive to the locomotor effects of amphetamine as a result of the altered dopaminergic innervation of the STR, there was no statistical differences between the groups. Both VEH and MDMA-treated rats significantly increased their activity following AMPH injections (MDMA 409.8%, VEH 539.7%; $F_{(1,29)}=16.309$; $p<0.001$), however there was no difference in activity across groups over time following the AMPH administration (data not shown).

### 3.4.2. Cocaine self-administration

*3.4.2.1. Priming threshold.* All rats displayed reinstatement of drug-seeking behavior in response to priming doses of cocaine, however there was no significant difference in the cocaine level at which the animals began lever-pressing (the priming threshold; MDMA 2050± 333 nmol/kg, VEH 2600±379 nmol/kg).

*3.4.2.2. Maintained self-administration.* Rats from both groups demonstrated unit dose-dependent changes in the interval between lever presses (the inter-injection interval), however no significant differences were seen between the groups at unit doses of 750 nmol/kg (MDMA: 128±32 s, VEH 145±13 s), 1500 nmol/kg (MDMA: 234±22 s, VEH 274±38 s), 3000 nmol/kg (MDMA: 415±26 s, VEH 427±10 s), 6000 nmol/kg (MDMA: 687±45 s, VEH 695±32 s), or 12,000 nmol/kg (MDMA: 1086±25 s, VEH 1131±42 s).

*3.4.2.3. Extinction.* Finally, though it was hypothesized that MDMA-exposed animals would continue drug-seeking behavior longer than control animals, there was no distinguishable difference in the cocaine level at which the lever-pressing behavior ceased (the extinction threshold; MDMA 1188±477 nmol/kg, VEH 826±233 nmol/kg). There was also no significant difference in the number of lever presses made by the animals in the extinction phase (MDMA 31±8 presses, VEH 24±2 presses).

## 3.5. Spatial learning, memory, and attention

### 3.5.1. Radial arm maze

All rats exhibited spontaneous alternation performance above chance levels, indicating normal intact working memory. No differences in spontaneous alternation were observed between saline and MDMA-treated rats (MDMA 74.38±7.33%, VEH 77.69±5.54%), suggesting that spatial working memory is not compromised by prenatal exposure to MDMA.

### 3.5.2. Morris water maze

*3.5.2.1. Fixed position platform.* There were also no observable differences in a probe trial in the amount of time spent swimming in the quadrant which had previously contained a fixed position platform (MDMA 39.38±3.48 s, VEH 36.63±2.58 s), indicating, again, that spatial memory is not impaired as a result of MDMA exposure *in utero*.

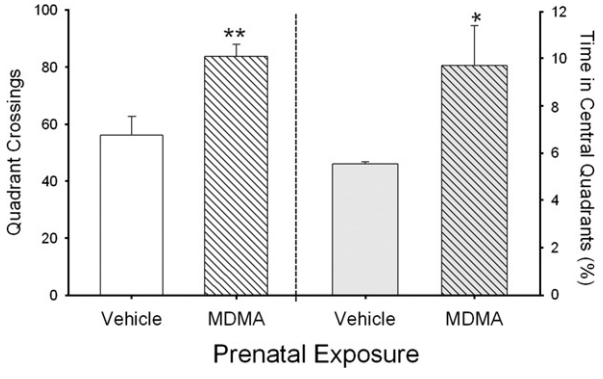


**Fig. 3.** Rats prenatally exposed to MDMA crossed the quadrants of the open field 49.2% more often (MDMA 83.75±4.24 crossings, VEH 56.13±6.52 crossings; $t=3.552$; $p<0.003$), and spent 75.1% more time in the central quadrants than VEH-treated controls (MDMA 9.7±1.69%, VEH 5.4±0.09% of total time; $t=2.188$; $p<0.05$). The increased crossing of quadrants and time spent in the exposed center area of the open field suggest that prenatal MDMA may reduce fear/anxiety in a novel environment. (MDMA $n=8$; VEH $n=8$) *=$p<0.05$, **=$p<0.01$.

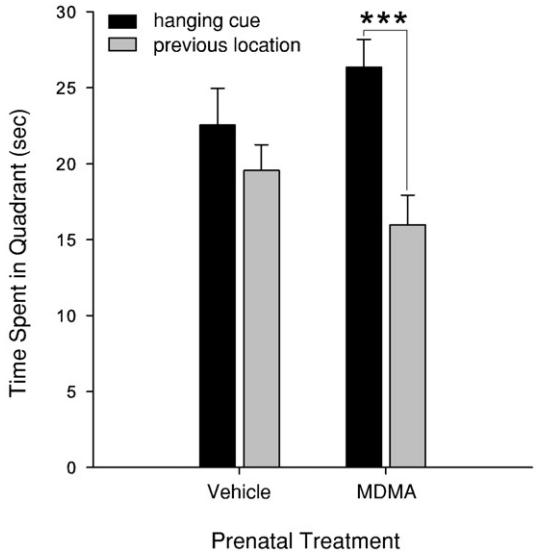


**Fig. 4.** In the probe trial of the cued MWM in which the platform was removed from the maze, rats prenatally exposed to MDMA spent approximately 43% of the time in the quadrant with the hanging cue, and only 26% of the time in the quadrant that had previously contained the hanging cue and platform (cue: 26.34±1.2 s, previous location: 15.97±1.7 s; $t=4.139$; $p<0.001$). Vehicle-treated rats spent equal time exploring both quadrants (cue: 22.54±2.43 s, previous location: 19.55±1.96 s; t=0.35; p=n.s.). These data imply that prenatal MDMA exposure increased attentiveness to the hanging cue whereas VEH rats attend to both the hanging cue as well as other environmental cues. (MDMA $n=8$; VEH $n=8$) ***=$p<0.001$.

*3.5.2.2. Cued platform.* There was a significant difference across groups during the probe trial in which the platform which had been associated with a hanging cue was removed. ANOVA revealed a significant 2-way interaction effect [$F_{(1,14)}=5.53$; $p<0.034$] that indicated that VEH animals made more visits to the previous location of the platform while MDMA animals made more visits to the cue's current location. When examined as a function of time, the VEH - exposed rats spent equal time exploring both the hanging cue, and the location where the platform had previously been located (Fig. 4). MDMA-exposed animals spent more time in the quadrant containing the hanging cue (43%), than in the quadrant that had previously contained the platform (26%) (Fig. 4). Thus, while spatial learning initially was indistinguishable between the 2 groups, the MDMA-exposed rats consistently attended to the overhanging cue in order to locate the platform, while saline-exposed rats did not display a preference for the local cue over other environmental information.

## 4. Discussion

The current study demonstrated that prenatal exposure to MDMA from E14–E20 results in a persistent behavioral phenotype in adult rats characterized by delayed habituation in a novel environment, a decrease in anxiety as measured by the open field test, and perseverative responding in a cued platform MWM test when compared to saline-exposed controls.

Hyperthermia has been shown to occur following MDMA administration [26,38,39]. By keeping the pregnant dams at 21 °C, no hyperthermia was observed. Rather, a mild hypothermic response was found which resolved after 2 days. This is consistent with other reports [40–43] that demonstrated that MDMA results in homeostatic temperature dysregulation which prevents the animal from maintaining a constant body temperature. Instead, the core temperature of the subject is influenced by ambient room temperature. Interestingly, while MDMA-treated dams were able to regain thermoregulation as indicated by reduced temperature fluctuation form the beginning to the end of drug administration, VEH-treated animals showed a mild hypothermic response in the last several days of treatment. This disparity was most likely caused by the stimulant effects of MDMA, which could have resulted in an increase in activity in drug-treated animals that allowed them to compensate for the decreased ambient temperature in the room. VEH-treated animals on the other hand demonstrated a lower level of activity during this period, resulting in the decrease in body temperature. While this control allowed for the dissociation of MDMA's thermal toxicity from its neurochemical action in the developing brain, it does not diminish the importance of investigating the consequences of MDMA-induced hyperthermia in the developing fetus. It does, however, demonstrate that hyperthermia is not necessary to produce long-term behavioral sequelae from prenatal MDMA exposure. Subsequent investigations examining the combined effects of the MDMA's pharmacologic action and its ability to induce hyperthermia in the appropriate environment are worthy of additional examination.

MDMA-induced anorexia in the dam is another potential source of teratogenicity that was controlled for in the current study. Maternal undernutrition has been shown to predispose offspring to weight gain and obesity [44] and can have significant impacts on brain development and subsequent behavior [45–47]. We found that a reduction in maternal weight gain occurs during the first few days of MDMA administration. To determine whether MDMA's anorectic effect on the dam altered body composition or ingestive behavior, we utilized NMR technology to estimate lean and fat tissue in MDMA and VEH-exposed offspring both before, and after, administering a high-fat diet. We did not observe any differences in body composition as determined by NMR, nor were there any changes in feeding patterns in response to the high fat diet. These results indicate that while MDMA induces transient weight loss in the pregnant dam, there is no evidence that this has any permanent consequence of the body composition or feeding behavior of the offspring.

No differences in cocaine self-administration acquisition, priming, or extinction were observed in MDMA-exposed animals when compared with controls. In addition, prenatal MDMA exposure did not increase the locomotor response of these adult animals to an amphetamine challenge. This is consistent with the finding of our previous study which found only small increases in TH+ neurite innervation of the striatum and nucleus accumbens [17]. This suggests that changes in dopamine-mediated locomotion and reward systems may be modest and difficult to detect, a problem which may be complicated by the small sample size necessitated by the intensive nature of the paradigms used in this study. Additional studies are needed to determine whether subtle changes may be present in the reward signaling circuitry.

Unlike the results of self-administration and amphetamine challenge experiments, prenatal MDMA exposure resulted in significant changes in behavior during the open field test. The open field test is an anxiogenic test that measures locomotor behavior and allows one to evaluate the anxiety and response to novelty in test subjects. Anxiety in the open field is generally associated with the freezing response and a greater proportion of time spent in the periphery [48]. Subjects prenatally exposed to MDMA exhibited increased locomotor activity as measured by increased velocity, total distance traveled, and the number of quadrant crossings and less time in the periphery during the open field test. Because homecage activity was not significantly different across groups, this data is consistent with a failure to acclimate to a novel environment, as opposed to a change in general activity level. This finding is also in agreement with our previous data from P21, which indicated that while control animals quickly habituated to a novel environment, MDMA-exposed animals continued to explore the new cage for the duration of the testing [17]. The mesocortical tract has been implicated in mediating such behaviors as risk-taking, exploration, novelty-seeking and impulsivity [20,49]. Alterations in this system can lead to hyperactivity in novel environments and changes in spatial processing [50]. Increased dopaminergic tone in the PFC has been shown to attenuate anxiety by reducing stress-related DA fluctuations in this region [51]. Changes in these

behaviors as measured in the open field are consistent with our previous finding of a 5-fold increase in DA neurite density in the PFC of juveniles after prenatal MDMA exposure, suggesting that increased mesocortical innervation may have significant effects on the response to novelty in an anxiogenic paradigm [17].

Prenatal treatment also resulted in differential performance on the cued MWM which requires the subject to associate an overhanging cue in order to learn the platform location. During the probe trial when the cue remained but the platform was removed, MDMA exposed rats perseverated on the hanging cue location while control animals spent equal time between the hanging cue location and the previous location of the platform. Because the negative results of the fixed position MWM test are not consistent with a global memory deficit, the best explanation for this disparity is that MDMA animals are more perseverative in their response in a task that permits more than one successful strategy. This behavior is also suggestive of changes in the mesocortical tract of the PFC. Insufficient or excessive DA activity in the PFC has been linked to impaired attention [52] and damage to the PFC can produce perseverative responding in animals and humans [53–55].

Behaviors such as novelty-seeking and perseverative responding are associated with an increased risk that an individual will experiment with, or compulsively abuse substances [56–58]. It has been established that the period of adolescence represents one of the most vulnerable times for the development of drug addiction [59]. Increased risk-taking, novelty-seeking, and impulsivity are modulated by the maturational changes in the mesocortical dopamine system during adolescence [60,61]. Testing subjects on tasks sensitive to alterations to the mesolimbic DA system during adolescence might be more illustrative in determining whether prenatal MDMA exposure makes these subjects more susceptible to the reinforcing properties of drugs like cocaine. So while no difference in behavioral responses to either amphetamine or cocaine were observed in the current study, the adolescent response to novelty and perseverative responding from prenatal MDMA exposure may be just as critical in the initiation of drug seeking behavior and compulsive drug use [56–58]. In future studies, it would be of significant interest to re-examine "reward"-motivated behaviors during this critical developmental period.

The link between an increased response to novelty and altered mesocortical transmission has also been demonstrated in another model. The Naples High-Excitability (NHE) rat is a rodent model for attention-deficit hyperactivity disorder (ADHD). These rats exhibit a hyper-responsivity to novelty, in the absence of an increase in basal locomotor activity [62]. Importantly, NHE rats also show a significant hyperinnervation of DA fibers in the PFC [63]. This lends support to our hypothesis that the behavioral changes observed in the open field and the cued MWM in MDMA-exposed rats may be related to this hyperinnervation of the mesocortical DA system.

Recent evidence suggests that methylphenidate (MPH; Ritalin®), a popular psychostimulant used to treat attention deficit disorders, can also facilitate cued learning in rats in a MWM task [64]. Given that the DAT is a common target of both MDMA and MPH, permanent changes in the dopaminergic system may play an important role in mediating the behavioral consequences we have observed following prenatal MDMA exposure. Testing drugs such as MPH to determine whether they normalize performance in the MWM would be an illustrative future experiment to determine whether the prenatal MDMA behavioral phenotype is similar to other rodent models of ADHD.

In conclusion, prenatal exposure to MDMA results in an impaired ability to acclimate to a novel environment, a decrease in anxiety-related behaviors, and perseverative responding in a working memory task. These behavioral findings are consistent with our prior determination of alterations in the mesocortical dopamine system following prenatal MDMA exposure. The subtle changes in behavior seen in this study may at first appear to be inconsistent with previous reports, which demonstrate substantial deficits in learning and memory in rats exposed neonatally to MDMA [65–67]. However the difference in drug administration schedule across the two paradigms results in exposure at very different stages of neural development. The model used in this study results in exposure during the human equivalent of the first trimester, which epidemiologic data demonstrates is the period associated almost exclusively with MDMA usage in pregnant women [9–11,68]. This disparity demonstrates how critical the timing of MDMA exposure can be in producing lasting behavioral alterations in the rat.

It is important to build upon the findings of increased response to novelty in the open field and perseverative responses in the cued MWM. Subsequent studies should address specific components of attention including distractibility, impulsivity and behavioral flexibility. Additionally, studies examining monoaminergic innervation in the adult rat following prenatal MDMA exposure will also be essential in determining whether these systems "normalize" over time, or whether they remain permanently altered in form and function. Continued investigation of the relationship between the altered neuroanatomical and neurochemical profile and the behavioral consequences of prenatal MDMA exposure will provide insight into its complex and enduring effects on the development and maturation of such subjects.

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