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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.