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Prenatal 3,4-methylenedioxymethamphetamine (ecstasy) alters exploratory behavior, reduces monoamine metabolism, and increases forebrain tyrosine hydroxylase fiber density of juvenile rats

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA; ecstasy) use has risen among women of childbearing age. Consequently, there is a substantial risk for fetal exposure from women who are, or become pregnant while abusing MDMA. However, attempts to demonstrate that prenatal MDMA results in neurochemical alterations in rat models have failed. MDMA administration to neonatal rats (third trimester equivalent) results in significant and persistent neurochemical and behavioral alterations, yet human epidemiologic data suggest that the vast majority of prenatal exposure is limited to the first trimester. The following study was conducted to reexamine the potential for prenatal MDMA administration to produce lasting postnatal neurochemical and behavioral alterations using a new rodent model. Pregnant rats were administered twice-daily injections of MDMA (15 mg/kg sc) or saline from embryonic days (E) 14–20. Prenatally exposed pups were examined on postnatal days (P) 3 and 21. At P3, MDMA offspring showed reductions in the dopamine metabolite homovanillic acid which persisted through P21, along with reductions in the serotonin (5-HT) metabolite, 5-HIAA. Prenatally exposed MDMA animals at P21 also had reduced dopamine and 5-HT turnover in the nucleus accumbens. Increases in tyrosine hydroxylase fiber density were found in the frontal cortex, striatum and nucleus accumbens of MDMA animals. In addition, prenatal MDMA significantly increased locomotor activity of P21 pups in a 20-min novel cage environment. These findings provide the first evidence of lasting neurochemical and behavioral alterations following prenatal MDMA. Further investigation is warranted to elucidate possible mechanisms of action and to monitor children gestationally exposed to MDMA.

Keywords: MDMA; Neurotoxicity; Ecstasy; Prenatal; Dopamine; DOPAC; HVA; 5-HT; 5-HIAA; Tyrosine; Litter; Culling; Hydroxylase; Habituation; Juvenile; Frontal cortex; Striatum; Nucleus accumbens; Neurochemistry

1. Introduction

Among adolescents and college students 3,4-methylenedioxymethamphetamine (MDMA; ''ecstasy'') use has increased steadily [38]. Consequently, there is a substantial risk of prenatal exposure from women who are, or become, pregnant while abusing MDMA. The limited epidemiologic data suggests that women who become pregnant while using MDMA continue to consume the drug until learning of their pregnancies. This typically results in discrete, early gestation fetal MDMA exposure. While the epidemiologic data is very limited, and may not fully characterize all abusing populations, current data suggest that most usage among pregnant women ceases early in pregnancy. Indeed, less than 3% of reported MDMA exposures occur in second or third trimester [11,20].

In adult animal and human studies, MDMA has been shown to damage 5-HT neurons [3,13,19,21,24,26–31,35,39,43,47,48]. Animals exposed to MDMA show decrements that persist in several 5-HT axon terminal markers including serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tryptophan hydroxylase (TPH), and the serotonin transporter (SERT) [43]. This suggests that damage is exclusive to the terminals, leaving associated

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cell bodies and axons of passage intact [27]. MDMA also has significant neurotoxic consequences on the dopaminergic system. Nonhuman primates exhibit significant reductions in dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), the dopamine transporter (DAT), and in vesicular monoamine transporter type 2 (VMAT-2) in the caudate nucleus and putamen [32].

Surprisingly, the few prenatal animal studies conducted have failed to demonstrate any lasting neurobiological alterations from MDMA [1,6,42]. Alternative models utilizing *neonatal* rather than *prenatal* MDMA exposures have demonstrated alterations in serotonergic systems as well as learning decrements [4,22]. These authors assert that perinatal exposures in the rat are analogous to third trimester development in humans, however, epidemiologic data suggest that most human gestational exposures occur exclusively during the first trimester [11,20]. Based upon the paucity of data for models of prenatal MDMA exposure, and the inability of the current neonatal models to simulate early gestational exposures, we systematically reexamined whether in utero MDMA exposure produces lasting changes in neurochemistry, neuronal morphology, and behavior in offspring exposed to MDMA.

We chose to expose pregnant rats to MDMA during a period that is developmentally equivalent to the human first trimester (embryonic days [E] 14–20). According to the limited, existing reports of MDMA use during pregnancy [11,20] the majority of fetal exposure occurs during the first trimester. This gestational period spans the stages from initial axonal sprouting from the VTA, raphe and substantia nigra through the establishment of patent connections to target structures (striatum, nucleus accumbens, and frontal cortex) [2]. Dopamine and 5-HT systems begin to develop and differentiate in humans during the first 4–7 weeks of pregnancy [2,9,40,44] and in rats, the equivalent development of these systems begins at about E13. The mesolimbic and nigrostriatal dopamine systems conclude their development by E16, which is the equivalent of week 7.4 in humans, well within the first trimester [2]. Such evidence suggests that our drug exposure approximates the period equivalent to human first trimester neurodevelopment.

Following the above administration schedule we provide the first report of neurochemical and behavioral alterations in prenatally MDMA-exposed neonatal and juvenile rats.

2. Materials and methods

2.1. Subjects

Timed-pregnant Sprague–Dawley albino rats (Zivic-Miller, $n = 8$ per treatment) were received on E10 (conception by the vendor was considered E0). Subjects were placed on a 12-h light/dark cycle schedule in a temperature controlled room (21 °C). Beginning at E14, dams received either ± MDMA (15 mg/kg sc) or saline (SAL; 1 ml/kg) at 12-h intervals each day through E20. This dosage would be considered equivalent in magnitude to those used by humans [33].

Dams were weighed and rectal temperature taken daily prior to their first injection. Temperature was also taken daily 2- and 6-h post first injection. This enabled us to evaluate the presence of any temperature dysregulation as a result of MDMA administration. Dams generally delivered late on E21 (also considered postnatal day [P] 0), and the following full postnatal day was then considered P1. Offspring remained group housed with the dam throughout the entire study.

The animal facility was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and complied with all Federal animal care and use guidelines. Protocols were all approved by the Institutional Animal Care and Use Committee of Rush-Presbyterian-St. Luke's Medical Center.

A pair of male and female offspring from each litter was used for: (1) quantitation of monoamine neurochemistry at P3; (2) quantitation of monoamine neurochemistry at P21; (3) behavioral and immunohistochemical assessment at P21.

2.2. Weight gain

Our control method was based upon a modification of Williams et al. [46]. As a method to normalize pup weights across treatments postnatally, control litters were culled on P1 to 10 pups (5 females and 5 males) and MDMA litters were culled to 8 pups (4 females and 4 males). The hypothesis for this pseudo-pair-feeding control was that increased nipple competition for SAL pups would produce a slower weight gain, allowing the weights of MDMA pups to "catch up" [46].

2.3. Behavior

Prior to sacrifice on P21, rats to be used for immunohistochemistry were evaluated for spontaneous locomotor activity in a novel cage environment.

Computerized photobeam cage activity monitors (San Diego Instruments, San Diego, CA) were placed in a darkened, sound attenuated room. Three rats were individually tested in 20-min sessions with each of the three cages separated by partitions. The rats were brought in and simultaneously placed into the cages with data collection beginning 30 s later. Activity cage monitors measured photobeam breaks and divided such movements into ambulatory (beam breaks at successive sensors) or fine motor movements (successive beam breaks at the same sensor). Data were parsed into blocks of 15 s for a total of 80 time blocks over 20 min. All animals received fresh industrial washed cages from the vivarium for each session to prevent scent carryover effects to subsequent subjects.

Table 1
Postnatal day (P) weights of animals exposed prenatally to ± MDMA or saline (SAL)

	P1	P3		P10		P21	
	Male/Female[a]	Male	Female	Male	Female	Male	Female
SAL	7.6 ± 0.21	9.33 ± 0.23	8.89 ± 0.18	25.85 ± 0.48	26.76 ± 0.44	61.71 ± 2.94	61.20 ± 2.26
MDMA	6.3 * ± 0.19	9.50 ± 0.23	9.17 ± 0.26	25.22 ± 1.03	23.83 ± 1.01	63.67 ± 2.23	61.00 ± 1.61

At P0 SAL litters were culled to 10 (5 male, 5 female) and MDMA litters to eight (4 male, 4 female). Values are expressed in grams (mean ± S.E.M.).
[a] Weights were not recorded with gender until P3.
* $P < 0.01$ between groups.

2.4. Neurochemistry

At P3 or P21, rats were processed following a modified version of our previously published method [16–18] for analysis of brain monoamines. Briefly, rats were anesthetized with an intraperitoneal injection of ketamine/xylazine (285 mg/kg/9.5 mg/kg) and transcardially perfused with ice-cold saline + 0.1% sodium heparin (at P3: 10 ml/min for 5 min; at P21: 15 ml/min for 5 min) to remove endogenous circulating monoamines from blood vessels. The subject was then decapitated, brain quickly removed and placed in a frozen stainless steel brain blocker (Stoelting, Wood Dale, IL). Using razor blades, the brain was sectioned into 1–2 mm slabs with the frontal cortex (FC), nucleus accumbens (NAc), striatum (STR), and brainstem dissected out and pooled bilaterally. Dissection of P3 NAc was not conducted as reliable landmarks were not visible on the saline-perfused fresh tissue. Tissues were placed in vials with 200 μl of an antioxidant solution (perchlorate and sodium metabisulfite) and immediately frozen on powdered dry ice and stored at − 80 °C. At the time of analysis samples were thawed and homogenized using an ultrasonic tissue homogenizer (Biologics, Gainesville, VA). The suspension was spun at 5000 × g for 20 min in a refrigerated centrifuge. The supernatant was reserved for high-performance liquid chromatography (HPLC) analysis and the pellet quantified for protein content.

Samples were separated on a Microsorb MV C-18 column (5 μm, 4.6 × 250 mm, Varian, Walnut Creek, CA) and simultaneously examined for DA, DOPAC, HVA, 5-HT and 5-HIAA. Compounds were detected using a 12-channel coulometric array detector (CoulArray 5200, ESA, Chelmsford, MA) attached to a Waters 2695 Solvent Delivery System (Waters, Milford, MA) under the following conditions: Flow rate of 1 ml/min; detection potentials: 50, 175, 350, 400, 525 mV; scrubbing potential: 650 mV. The mobile phase consisted of a 10% methanol solution in distilled H_2O containing 21 g/l (0.1 M) citric acid, 10.65 g/l (0.075 M) Na_2HPO_4, 176 mg/l (0.8 M) heptanesulfonic acid, and 36 mg/L (0.097 mM) ethylenediaminetetraacetic acid at a pH of 4.1. Unknown samples were quantified against a six point standard curve with a minimum r^2 of .97. Quality control samples were interspersed with each run to ensure HPLC calibration.

2.5. Immunohistochemistry

Animals were deeply anesthetized with an intraperitoneal injection of ketamine/xylazine and transcardially perfused with 100 ml of ice-cold 0.9% saline + 0.1% sodium heparin solution followed by 100 ml of a 4% paraformaldehyde solution (in 0.1 M PBS, pH 7.4) at a rate of 15 ml/min. Brains were removed and placed in the same 4% paraformaldehyde solution overnight at 4 °C. Brains were then sequentially placed, until saturated, in a 20% and then a 30% sucrose solution (w/v in 0.1 M PBS, pH 7.4) until cut. Brains were frozen on a chuck and cut into 50 μm coronal slices on a sliding microtome and stained for tyrosine hydroxylase immunoreactivity (THir) as described previously [37].

2.5.1. Striatum and nucleus accumbens

The optical density of TH immunoreactivity was quantified within the striatum and nucleus accumbens bilaterally using the Scion Image 1.60c (Frederick, MD) analysis system by averaging the mean of the entire striatum, nucleus accumbens core or shell on all stained sections through each structure (50 μm sections every 300 μm). Optical density values were used to determine relative differences in TH staining between groups.

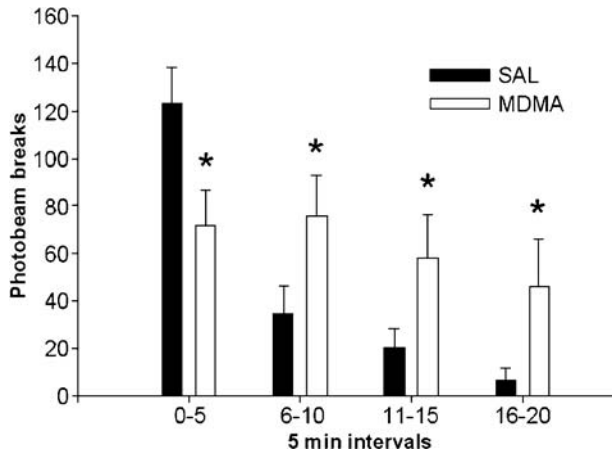

Fig. 1. Prenatal MDMA from E14–E20 produces a lack of habituation to a novel cage environment in 21-day-old rat pups as measured by persistent locomotor activity throughout the 20-min testing period. * $P < 0.01$.

2.5.2. Frontal cortex

Stereologic fiber density analysis utilizing hemispheric probes was used to obtain an unbiased estimate of monoaminergic axon density in frontal cortex [25]. This method has been reported to be relatively insensitive to intersubject variability and is thought to reliably reflect axon density. Comparable sections were chosen from each experimental subject and matched for level by using standard landmarks. All samples were captured and analyzed from coded slides by a single blinded investigator. Digital images of TH-stained sections were visualized using an Olympus BX-60 microscope (Melville, NY) at 100 × magnification using a CCD video camera (HV-C20, Hitachi, San Jose, CA) with a computer interface running Stereo Investigator 5.04.3 (Microbrightfield, Williston, VT). A total of 500 random sites were chosen on each stained section by computer. A 7-μm radius virtual hemisphere was placed at each sampling site (with a 2 μm guarding zone between the surface of the section and the outer edge of the hemisphere). The operator moved through the entire focal plane of the hemisphere counting any fiber that intersected with the boundary of the hemisphere. The number of TH-ir axons intersecting the hemispheric boundaries at each sampling site were quantified and summed for the entire section.

2.6. Statistical methods

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) 11.0 (Chicago, IL). Data were analyzed using a two-way ANOVA with treatment group (SAL vs. MDMA) and gender as between-subjects factors. (Statistics were also compared using gender as a within subjects factor. The presence of significant differences did not differ as a result of the method of analysis.) P values less than 0.05 were considered statistically significant.

3. Results

3.1. Maternal temperature

Dams did not exhibit a hyperthermic response to MDMA. Rather, the dams demonstrated a 1.8 °C drop in rectal temperature at 2-h postinjection [$F(1,11) = 4.813$, $P < 0.05$] that returned to baseline by 6 h on E14. By E17, even this small hypothermic response disappeared and was no longer significant as compared to controls [$F(1,11) = 0.771$, $P = ns$]. Controls showed no change in core temperature at any of the times measured (pre, 2 h post, 6 h post).

3.2. Maternal weight gain

Both MDMA and SAL exposed rat dams gained weight during gestation. Maternal weight gain proceeded slower in the MDMA exposed rat dams. Weights increased by 12% in the MDMA dams and 21% in the SAL dams from E14-E20 [$F_{(1,16)} = 11.911$, $P < 0.01$].

Table 2
Effects of prenatal MDMA exposure on brain structures at P3 and P21

		DA		DOPAC		HVA		HVA/DA	
		P3	P21	P3	P21	P3	P21	P3	P21
FC	SAL	1.18 ± 0.27	0.17 ± 0.01	0.27 ± 0.02	0.14 ± 0.01	0.92 ± 0.04	0.42 ± 0.02	1.51 ± 0.32	2.57 ± 0.16
	MDMA	0.76 ± 0.32	0.18 ± 0.01	0.22 ± 0.02	0.15 ± 0.01	0.83 ± 0.07	0.42 ± 0.03	2.18 ± 0.49	2.50 ± 0.21
STR	SAL	18.86 ± 0.92	56.58 ± 1.98	1.31 ± 0.08	16.21 ± 0.83	1.78 ± 0.07	9.64 ± 0.46	0.10 ± 0.01	0.17 ± 0.01
	MDMA	17.47 ± 1.69	53.16 ± 1.43	1.25 ± 0.10	13.60** ± 0.49	1.44 * ± 0.12	8.13 * * ± 0.24	0.08 ± 0.00	0.15 * ± 0.00
NAc	SAL	–	43.10 ± 2.62	–	11.22 ± 1.11	–	7.06 ± 0.32	–	0.17 ± 0.01
	MDMA	–	47.15 ± 1.94	–	10.22 ± 0.58	–	6.28 ± 0.33	–	0.13 * ± 0.01
BS	SAL	3.54 ± 0.12	2.40 ± 0.44	1.06 ± 0.04	0.79 ± 0.10	2.47 ± 0.06	0.83 ± 0.08	0.70 ± 0.02	0.43 ± 0.04
	MDMA	3.79 ± 0.25	1.83 ± 0.31	1.12 ± 0.06	0.76 ± 0.07	2.43 ± 0.13	0.78 ± 0.09	0.65 ± 0.03	0.48 ± 0.03

		5-HT		5-HIAA		5-HIAA/5-HT	
		P3	P21	P3	P21	P3	P21
FC	SAL	1.19 ± 0.06	0.71 ± 0.70	0.99 ± 0.10	4.10 ± 0.18	0.82 ± 0.04	2.47 ± 0.16
	MDMA	1.11 ± 0.07	1.79 ± 0.09	1.02 ± 0.15	3.74 ± 0.17	0.90 ± 0.11	2.16 ± 0.14
STR	SAL	1.31 ± 0.13	3.14 ± 0.11	1.63 ± 0.23	9.66 ± 0.38	1.21 ± 0.08	3.09 ± 0.11
	MDMA	1.07 ± 0.16	2.92 ± 0.10	1.28 ± 0.21	8.22 * * ± 0.23	1.23 ± 0.11	2.84 ± 0.07
NAc	SAL	–	3.48 ± 0.11	–	7.39 ± 0.34	–	2.14 ± 0.11
	MDMA	–	3.71 ± 0.09	–	6.39 * ± 0.21	–	1.73 * * ± 0.06
BS	SAL	4.67 ± 0.25	8.17 ± 0.88	8.95 ± 1.37	17.80 ± 1.83	1.82 ± 0.17	2.20 ± 0.06
	MDMA	4.37 ± 0.35	9.05 ± 1.00	7.49 ± 1.32	19.61 ± 2.12	1.65 ± 0.17	2.18 ± 0.06

Values represent nanograms of compound per milligram of protein content (mean ± S.E.M.).
P3 NAc was not examined due to lack of prominent dissection landmarks at this age; P21 NAc values represent males only as females were not significantly different.

* $P < 0.05$.

** $P < 0.01$.

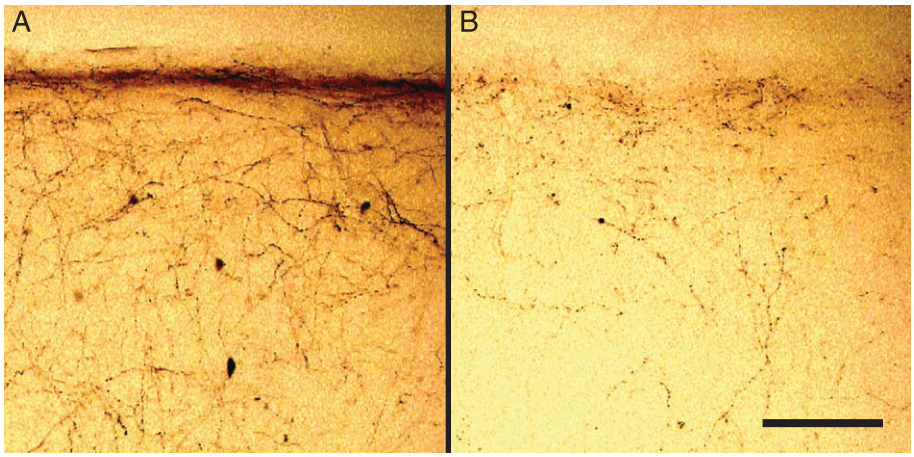

Fig. 2. Prenatal MDMA (A) from E14–E20 produces a significant increase in tyrosine hydroxylase immunoreactive fibers in the frontal cortex as compared to saline controls (B) at P21. Note: While TH stains for both DA and noradrenergic neurons, the pattern of innervation observed is consistent with DA innervation. Scale bar: 200 μm.

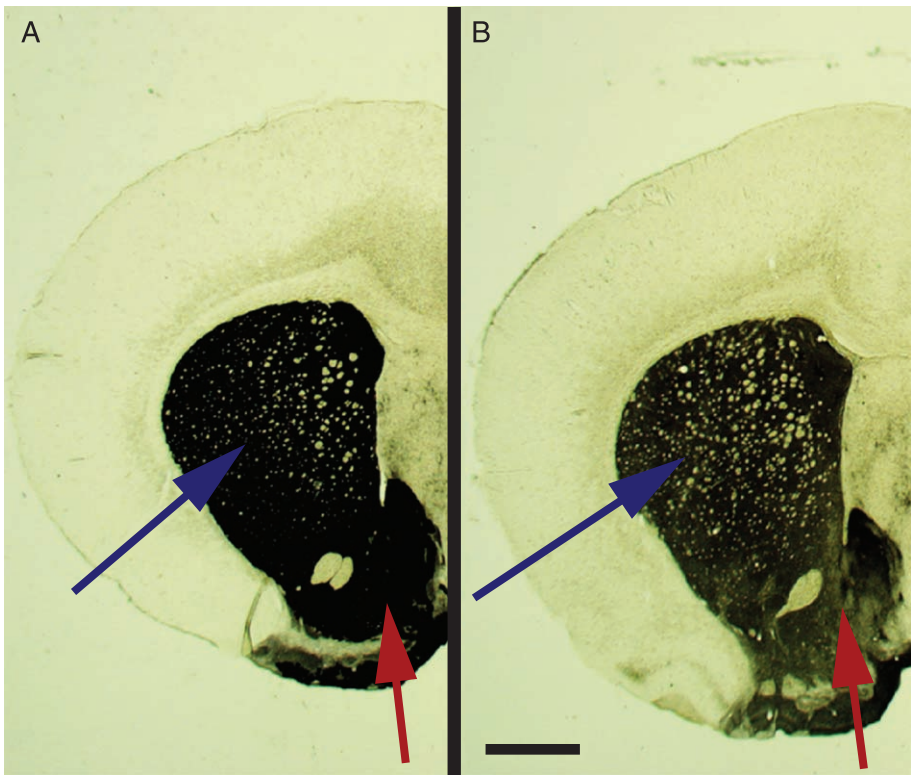

Fig. 4. Prenatal MDMA (A) from E14–E20 produces a significant increase in tyrosine hydroxylase stained fibers in the striatum (blue arrow) and the nucleus accumbens core and shell (red arrow) vs. saline controls (B). Scale bar: 1 mm.

3.3. Litter size and pup weights

MDMA exposure did not affect litter birth size or gender distribution. At P1, MDMA pups weighed 17% less than SAL pups. However, once SAL litters were culled to 10 pups per litter and MDMA litters were culled to 8 pups, the weight disparity between the groups disappeared by P3 (Table 1).

3.4. Behavior

P21 rats exposed to MDMA prenatally from E14–E20 demonstrated a 35% increase in total ambulatory activity over 20 min in a novel cage environment as compared to those exposed to prenatal SAL injections [$F(1,58) = 7.21$, $P < 0.01$]. There were no differences in fine motor movements (which would be suggestive of grooming or

stereotypy). Through post hoc examination of locomotor activity over time (parsed into 5 min blocks), it was evident that the MDMA exposed pups continued to explore the new cage, unlike controls, whose activity fell precipitously after the first 5 min of the 20-min trial (Fig. 1).

3.5. Neurochemistry

All neurochemical measures, group means and S.E.M.s are expressed in Table 2.

3.5.1. Frontal cortex

Prenatally exposed MDMA rats at P3 or P21 exhibited no changes in monamines or their metabolites. At P21, however there was a dramatic and significant fivefold increase in TH-ir fiber density in the frontal cortex from prenatal MDMA exposure [$F(1,32) = 14.991$, $P < 0.001$] (Fig. 2). There were no gender differences observed in any frontal cortex measures.

3.5.2. Striatum

Prenatal MDMA exposed rat pups exhibited a 19.3% reduction in the DA metabolite homovanilic acid (HVA) [$F(1,21) = 7.019$, $P < 0.02$] at P3. This HVA reduction was still present (15.7% reduction) at P21 [$F(1,32) = 8.933$, $P < 0.005$] (Fig. 3). In addition, by P21 a 16.1% reduction in DOPAC was evident [$F(1,32) = 7.844$, $P < 0.01$] as was a significant 11.76% reduction in DA turnover [HVA/DA; $F(1,32) = 4.854$, $P < 0.05$]. The 5-HT metabolite, 5-HIAA, was reduced by 14.91% at P21 [$F(1,32) = 10.982$, $P < 0.002$] and 5-HT levels were not altered. Optical density analysis of TH-ir fibers in STR at P21 revealed that prenatal MDMA exposure increased fiber density by 7.8% [$F(1,29) = 4.692$, $P < 0.05$] (Fig. 4). No gender differences were evident.

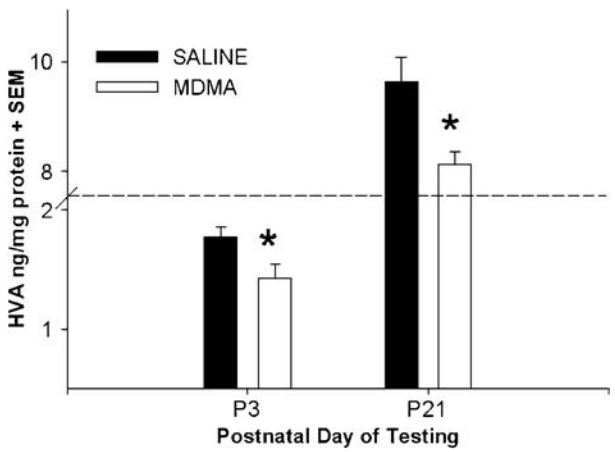

Fig. 3. Prenatal MDMA from E14–E20 produces reductions in homovanillic acid (HVA) in the striatum at P3 that persist through at least P21. * $P < 0.05$.

3.5.3. Nucleus accumbens

P21 dopamine and 5-HT activity was significantly reduced in prenatally exposed male pups, but not female pups. DA and 5-HT turnover ratios were reduced in MDMA exposed males by 23.5% and 19.16%, respectively vs. control males [HVA/DA: $F(1,15) = 8.506$, $P < 0.01$; 5-HIAA/5-HT: $F(1,15) = 11.965$, $P < 0.01$]. 5-HIAA was also significantly reduced in MDMA exposed male pups by 13.5% [$F(1,15) = 6.535$, $P < 0.05$]. In addition, TH-ir fiber density was increased in the core of the NAc by 9.2% [$F(1,29) = 6.686$, $P < 0.01$] (Fig. 4) and in the shell of the NAc by 7.7% [$F(1,29) = 4.938$, $P < 0.05$] in both males and females.

3.5.4. Brainstem

No differences in monoamine neurochemistry were evident in the brainstem at P3 or P21.

4. Discussion

Prenatal MDMA exposure from E14–E20 results in significant neurochemical and behavioral alterations in rats that persist at least through P21. These data represent the first report of its kind following a prenatal MDMA exposure. It has been suggested that MDMA induced neurotoxicity is an epiphenomenon of stimulant-induced hyperthermia. These findings are not, however, a result of MDMA-induced hyperthermia, as pregnant rat dams did not display a hyperthermic response to MDMA when housed in a 21 °C environment. Additional evidence suggests that MDMA administration alters homeostatic temperature regulation and does not specifically induce hyperthermia. As an example, animals administered MDMA and placed in a cold environment exhibit marked hypothermia as compared to controls. Conversely, animals placed in a warm room display a hyperthermic response [7,8,19,41]. By keeping animals in a relatively cool room (21 °C), we were able to prevent a hyperthermic response to MDMA.

While prenatal MDMA did result in birth weight reductions for neonatal rat pups, our postnatal culling procedure normalized group weight disparities by P3 (based upon modifications of the method reported by Williams et al. [46]). While this control procedure did not protect fetuses from maternal nutritional deficiencies during gestation, the neurochemical, morphological and behavioral observations described here have never been observed in other studies [5,12,45,49] utilizing pair-fed pregnant dam controls, suggesting that our findings are MDMA-specific.

By P3, prominent reductions in HVA were evident in the STR. By P21 several target structures including STR and NAc demonstrated decreased DA and 5-HT turnover as well as decreased DA and 5-HT metabolite levels suggesting either decreased release or increased re-uptake in the STR and NAc. Since dopaminergic terminal field loss is typically associated with increased DA turnover (e.g., HVA/DA) [10], the decrease in DA and 5-HT turnover observed supports

the concept that there may be a proliferation in synaptic terminals in target structures.

Such findings are reinforced by the observation that by P21, TH-ir fiber density in the STR and NAc were significantly increased in MDMA pups. Also at P21, frontal cortex TH-ir fiber density was dramatically increased (five-fold) from prenatal MDMA exposure. There are several potential mechanisms that may potentially explain MDMA-induced increases in TH-ir in the FC, STR and NAc: (1) prenatal MDMA increased numbers of substantia nigra (SN) and/or ventral tegmental area (VTA) DA neurons, providing increased innervation of target structures, (2) prenatal MDMA increased axon collaterals in FC, STR and NAc without increased numbers of TH neurons in the SN and VTA or (3) a normal neuronal population is increasing its phenotypic expression of TH. Future investigations will hopefully demonstrate that one or more of these hypotheses may explain the observed increases in the TH-ir fiber density.

Prenatal MDMA exposure also caused an increase in locomotor activity and a lack of habituation to a novel cage environment at P21. There was a significant reduction in the locomotor activity of MDMA animals during the first 5 min of the session (Fig. 1). Freezing is a standard fear response in the rat. This reduction in locomotor activity may highlight an initial heightened fear response in prenatally exposed MDMA animals. Future investigations to visually monitor rat behavior in the novel cage environment may provide additional insight into these group differences.

Decreases in DA metabolism and significant increases in TH fiber density in the FC have also been observed in the spontaneously hypertensive rat (SHR), a Wistar–Kyoto mutant that is an established model of attention deficit/hyperactivity disorder (ADHD) [15,36]. The SHR does not become hypertensive until puberty, yet it displays significant decrements in attention and learning prior to becoming hypertensive. This suggests, that perhaps, common environmental perturbations during gestation may explain the common neurochemical, morphologic and behavioral features between the prenatal MDMA exposed and the SHR rat. One possibility is that MDMA, a sympathomimetic, may increase maternal blood pressure resulting in a hypertensive pregnancy similar to the SHR model. These findings warrant further behavioral testing of the prenatally exposed MDMA rat to determine the presence of learning and/or attention deficits.

It is curious that prior prenatal MDMA studies conducted by others have not demonstrated significant findings [1,6,42]. These studies demonstrated no changes associated with the dopamine or 5-HT system in any structure. These reports used varying dosages and schedules, examined animals at different postnatal time points, and examined different brain structures. In order to evaluate the drug amounts administered in these other studies, we converted their administration schedules into cumulative doses. A cumulative dose is defined as the amount of drug administered to the pregnant dam over the entire study. The cumulative doses in these studies ranged from 17.5 mg/kg to 160 mg/kg (in our studies, the cumulative dose was 210 mg/kg). With the exception of a single study [14], no one has shown any changes associated with prenatal MDMA exposure. This study demonstrated persistent increases in regional cerebral glucose metabolism at P90 but did not attempt to demonstrate any neurotransmitter system specific changes. Taken together, the few studies examining a true prenatal MDMA exposure may not have demonstrated significant findings due to low cumulative dose administration, methodologic issues associated with brain dissection, the choice of structures to be examined, and postnatal time of analysis.

While no prior evidence exists demonstrating prenatal MDMA-induced neurodevelopmental alterations, there are currently two research groups actively engaged in the development of neonatal rodent MDMA models [4,22]. These groups have both demonstrated that postnatal exposure to MDMA can produce neurochemical and receptor/transporter changes associated with the 5-HT system. Both groups also contend that the rodent neonate is analogous to late fetal gestation in humans. The first model exposed neonatal rats to MDMA from P11 to P20 [4]. This resulted in reductions in 5-HT and increases in norepinephrine (NE) in the hippocampus at P105. Behavioral deficits in sequential learning and spatial memory tasks at P105 were also noted but did not correlate with the observed neurochemistry. The second MDMA model employs a twice daily 10 mg/kg injection from P1 to P4 and found that hippocampal SERT binding was lower in MDMA exposed animals at both P25 and P60. Parietal cortex showed no differences in SERT binding at P25 from MDMA exposure, but it was significantly lower by P60. By 9 months of age these MDMA-treated rats displayed decreases in SERT-immunoreactive fiber density in the primary visual and somatosensory cortices, and increases in the dorsal caudate–putamen and nucleus accumbens shell [23]. While these studies demonstrate significant and persistent neurodevelopmental consequences from neonatal, third trimester equivalent MDMA exposures, existing epidemiologic data demonstrate that greater than 95% of pregnant MDMA users limit their consumption to the early phases of pregnancy [11,20]. These epidemiologic data are limited however to the size and the demographics of the populations studied, suggesting the potential for underreported use patterns. For example, patterns of cocaine use by pregnant women demonstrated that the more educated middle-class population ceased use during pregnancy, whereas less educated women often continued to abuse cocaine throughout their pregnancies [34].

While it is evident that prenatal MDMA can induce altered neurochemistry, neuronal morphology and behavior there are many interesting avenues of exploration that can be pursued as a result of these studies. Does MDMA-induced hyperthermia in the pregnant dam exacerbate or

alter the persisting neuronal changes observed? Would different gestational exposures result in different patterns of alterations? Is MDMA's direct pharmacologic action responsible for the observed changes or could indirect effects such as vasoconstriction or oxidative stress be responsible? Can neuroprotective agents reduce the alterations observed from prenatal MDMA exposure? Are such animals more susceptible to postnatal neurotoxic insults putting them at greater risk for the development of neurodegenerative diseases?

The present data demonstrate that in rodents MDMA exposure during a period of brain development correlating with human first trimester mesolimbic and nigrostriatal development produces lasting effects on DA and 5-HT neurochemistry, TH-ir fiber density, and behavior. These changes are not a result of hyperthermia or postnatal differences in pup weight. Further studies to elucidate the mechanisms of such changes are warranted, as is the continued monitoring of children gestationally exposed to MDMA.

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