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ORIGINAL INVESTIGATION

Comparison of the developmental effects of 5-methoxy-*N*, *N*-diisopropyltryptamine (Foxy) to (±)-3,4-methylenedioxymethamphetamine (ecstasy) in rats

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Abstract
Rationale We have previously shown that (±)-3,4-methylenedioxymethamphetamine (MDMA) treatment from postnatal days (P)11 to P20 leads to learning and memory deficits when the animals are tested as adults. Recently, the club drug 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) has gained popularity.
Objective Due to the similarities between MDMA and 5-MeO-DIPT and the substitution of 5-MeO-DIPT for MDMA, the purpose of this study was to compare the developmental effects of these drugs.
Methods Within a litter, animals were treated from P11 to P20 with either MDMA, 5-MeO-DIPT, or saline.
Results MDMA-treated animals showed increased anxiety in a measure of defensive marble burying, as well as deficits in spatial and path integration learning. 5-MeO-DIPT-treated animals showed spatial learning deficits; however, there were no deficits observed in spatial memory or path integration learning. 5-MeO-DIPT-treated animals also showed hyperactivity in response to a challenge dose of methamphetamine.
Conclusions The results show that treatment with either 5-MeO-DIPT or MDMA during development results in cognitive deficits and other behavioral changes but the pattern of effects is distinct for each drug.

Keywords Drug abuse · Anxiety · Behavior · Learning · Locomotor activity · Memory · Psychostimulant

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Introduction

Rats exposed to (±)-3,4-methylenedioxymethamphetamine (MDMA) from postnatal days (P)11 to P20, a period analogous to second to third trimester human brain development (Clancy et al. 2007a, b), have learning and memory deficits when tested as juveniles or adults (Broening et al. 2001; Cohen et al. 2005; Skelton et al. 2006; Vorhees et al. 2004, 2007a; Williams et al. 2003; reviewed in Skelton et al. 2008b). Recently, there have been reports of another club drug, 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT; Foxy or Foxy-methoxy), gaining popularity (US Drug Enforcement Agency 2003). 5-MeO-DIPT is a tryptaminergic hallucinogen originally characterized by Shulgin and Carter (1980) and has emerged as a "club drug" because it is used in the same venues as MDMA. 5-MeO-DIPT is taken as an alternate to or in combination with MDMA (Drug Enforcement Administration (DEA) 2004). Clinical reports of 5-MeO-DIPT ingestion include hallucinations, tachycardia, and hypertension, as well as confusion and agitation (Smolinske et al. 2005). Similar effects have been observed in MDMA users (Mas et al. 1999) with the exception being that 5-MeO-DIPT has a more pronounced hallucinogenic effect. Like MDMA, 5-MeO-DIPT appears to interact with the serotonergic (5-HT) system.

In adult mice, acute 5-MeO-DIPT exposure leads to an increase in head twitching, which is prevented by 5-HT_{2A} receptor antagonism (Fantegrossi et al. 2006). 5-MeO-DIPT has been shown to bind to the 5-HT transporter (SERT) and block 5-HT reuptake; however, unlike MDMA, 5-MeO-DIPT does not stimulate the release of 5-HT (Nagai et al. 2007; Nakagawa and Kaneko 2008). In adult rats, 5-MeO-DIPT treatment increases 5-HT turnover in the neostriatum and hypothalamus, while decreasing turnover in the prefrontal cortex (Williams et al. 2007). Adolescent exposure to 5-MeO-DIPT decreases 5-HT levels in the prefrontal cortex and hippocampus and results in Morris water maze (MWM) spatial learning deficits and deficits in a cross-shaped swimming maze using a win-stay lose-shift test procedure (Compton et al. 2006). In adult rats, 5-MeO-DIPT treatment also leads to deficits in reference memory in the Morris water maze and deficits in the acquisition of both spatial and path integration learning (Williams et al. 2007). The learning deficits seen in 5-MeO-DIPT-treated rats are similar to those of animals treated with MDMA suggesting possible overlapping modes of action (Able et al. 2006; Skelton et al. 2008a). Several systems are affected by both 5-MeO-DIPT and MDMA including but not limited to the serotonergic system and the hypothalamic–pituitary–adrenal (HPA) axis as demonstrated by increases in corticosterone (CORT) release (Green et al. 2003; Nash Jr. et al. 1988; Williams et al. 2007).

In developing animals, the impact of these drugs on the HPA axis may produce lasting detrimental changes. For example, the stress hyporesponsive period is a period of HPA axis quiescence that extends from ~P4–14 and is thought to protect the hippocampus from stress-induced increases in released glucocorticoids when corticoid receptors are being expressed at high levels (Sapolsky and Meaney 1986). As with MDMA (Schaefer et al. 2006a, 2007; Williams et al. 2006), 5-MeO-DIPT has been shown to increase CORT release from P3 to P19 (Schaefer et al. 2006b), raising the possibility that developmental 5-MeO-DIPT exposure might lead to later behavioral changes similar to those caused by MDMA. The increase in use of 5-MeO-DIPT and its overlapping pattern of use to MDMA (the latter one already established to have adverse developmental effects) raises concern over the consequences of 5-MeO-DIPT use during pregnancy. Accordingly, the purpose of this experiment was to compare the developmental effects of 5-MeO-DIPT with those of MDMA.

MDMA exposure in developing animals is known to have multiple long-lasting effects, including decreased locomotor activity, anxiety alterations, and spatial learning and memory and path integration learning deficits (Skelton et al. 2008b). For example, MDMA-induced learning and memory deficits emerge by P30 and persist until at least 1 year of age (Skelton et al. 2006). The Cincinnati water maze is a labyrinthine maze that assesses complex learning in rodents (Vorhees 1987). Recently, testing procedures have been modified to eliminate the availability of extra-maze cues in order to assess path integration ability separately from spatial learning. This is accomplished by testing animals in complete darkness using near infrared lighting. This test was included herein along with the Morris water maze (spatial learning and memory) and tests of anxiety and locomotion in order to characterize the long-term effects of developmental 5-MeO-DIPT.

Materials and methods

Subjects and treatments

Nulliparous female Sprague Dawley CD (IGS) rats were obtained from Charles River Laboratories (Raleigh, NC, USA) and acclimated to the vivarium (21±2°C; humidity 50±10%; 14:10 light dark cycle; lights on at 600 h) for a minimum of 1 week prior to breeding with males of the same strain and supplier. Food (Purina 5006) and filtered water were available ad libitum. Breeding occurred in hanging wire cages. The day a sperm plug was detected was designated as embryonic day 0. Gravid females were singly housed in polycarbonate cages (46×24×20 cm) and left undisturbed until parturition. Date of birth was considered P0 and litters were left undisturbed until P1. Litters were culled to eight pups with equal numbers of males and females on P1. From P11 to P20, one male/female pair within each litter was subcutaneously injected four times daily (2-h interdose interval) with ±MDMA HCl (10 mg/kg (51 mmol/kg) expressed as freebase), 5-MeO-DIPT (10 mg/kg (30 mmol/kg) expressed as freebase), or saline vehicle (SAL). The dose of MDMA was chosen because it has consistently been shown to induce learning and memory deficits in neonatal animals (Broening et al. 2001; Skelton et al. 2006; Vorhees et al. 2004, 2007a; Williams et al. 2003), while the 5-MeO-DIPT dose was selected based on data showing that this dose is effective in raising CORT levels in the developing animal (Schaefer et al. 2006b) as well as inducing learning deficits in adult animals (Williams et al. 2007). Six offspring from each litter were used in this study, i.e., one male and one female each receiving saline, MDMA, or 5-MeO-DIPT. Injections were administered in the dorsum and sites were varied to prevent irritation. Dosing weights are reported for the first and last dose of each day. Both MDMA and 5-MeO-DIPT were obtained from Research Triangle Institute (Research Triangle Park, NC, USA) and were verified to be >95% pure. Offspring remained with the dam until separated into same

sex pairs on P28. Body weights were recorded weekly following treatment. Temperature was not recorded as no changes in temperature occur in MDMA-treated animals during the preweaning period (Broening et al. 1995). Twenty litters were used; hence, there were 20 males and 20 females in each treatment group. The vivarium is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and protocols were approved by the Cincinnati Children's Research Foundation's Animal Use and Care Committee.

Elevated zero maze

Testing in the elevated zero maze (EZM; Shepherd et al. 1994) occurred on P29. We have previously shown that behavioral changes resulting from neonatal MDMA treatment are present by P30 (Skelton et al. 2006). The ring-shaped maze was elevated from the floor 72 cm and was 105 cm in diameter with a path width of 10 cm. The maze was partitioned into four quadrants, such that adjoining quadrants either had black walls that were 28 cm in height (closed area) or were open (with clear acrylic curb 1.3 cm in height). The room was illuminated by a single halogen lamp. To begin each test, an animal was placed in the center of one of the closed areas and its behavior recorded for 5 min with a camera that was mounted over the center of the maze and connected to a video recorder. Time in the open and the number of head dips were measured. Time in the open began when an animal had its two front paws and shoulders in the open area and a head dip was counted when the animal placed its head over the open quadrant side rail and looked over the edge.

Locomotor activity

Beginning approximately 1 h after EZM testing, animals were taken to a separate room and tested for spontaneous locomotor behavior in an automated activity monitor (Accuscan Electronics, Columbus, OH, USA). The apparatus was 41×41 cm and contained 16 photodetector–light-emitting diode pairs along each side spaced 2.5 cm apart and positioned 2.2 cm above the floor. Dependent measures were horizontal activity (sum of all photobeam interruptions occurring in the horizontal plane), distance moved (total, central, and peripheral), and repetitive beam breaks (consecutive photobeam interruptions at the same position).

Marble burying

Immediately following locomotor activity testing, animals were brought to another room and tested for marble burying (see Njung'e and Handley 1991; with minor modifications). Marble burying followed locomotor activity since preliminary data showed that rats buried more marbles after this experience. Eighteen blue marbles, 1.4 cm in diameter, were evenly spaced (3.5 cm from the sides and 7 cm apart in all directions) in six rows of three in a cage measuring 46×24×20 cm. Fresh wood chip bedding (5 cm deep) was placed in each cage and a filter top was placed over the cage. Animals were tested for 30 min; latency to begin burying and number of marbles buried at least 2/3 were measured.

Straight channel swimming

Immediately following marble burying, animals were tested for swimming ability in a straight water channel. The channel was 15×244 cm long and filled to a depth of 35 cm with room temperature water (22±1°C). Rats were placed at one end facing the wall and allowed a maximum of 2 min to find an escape ladder located at the opposite end. Four consecutive timed trials were given and escape latency was recorded for each trial. This task acclimates animals to swimming, measures motivation to escape (swim speed), and teaches them that escape is possible.

Morris water maze

On P30, animals began testing in the MWM in three phases (6 days/phase) consisting of four trials per day for 5 days to learn the location of the hidden platform followed by a single probe trial on day 6 with the platform removed (Vorhees and Williams 2006). The maze was a black stainless steel tank 210 cm in diameter with extramaze cues on the walls. During phase 1, a 10×10-cm platform was submerged 2 cm below the water in the SW quadrant halfway between the center and wall. Rats were placed in the maze in one of four distal start locations as defined previously (Vorhees and Williams 2006) and allowed 2 min to locate the platform. Upon reaching the platform, the rat was given a 15-s intertrial interval on the platform. If a rat failed to find the platform within 2 min, it was placed on the platform for 15 s. For phase 2 (P37–43), which began the second day after the completion of phase 1, the 10×10-cm platform was relocated to the NE quadrant and the converse starting positions were used. For phase 3 (P44–50), a 5×5-cm platform was placed in the adjacent NW quadrant and the four start positions were adjusted accordingly. A camera mounted over the center of the maze was attached to a computer with video tracking software (Smart®, San Diego Instruments, San Diego, CA, USA). The dependent variables analyzed were latency, path length, cumulative distance, and swimming speed. On probe trials, the variables analyzed were platform site crossings (crossovers), path length, mean directionality,

average distance from the platform, and latency to cross the platform site.

Cincinnati water maze

The Cincinnati water maze has been described previously (Vorhees 1987) with modification (Herring et al. 2008). The apparatus is a 9-unit asymmetric multiple T maze. The walls were 51 cm high with a channel width of 15 cm and the maze was filled with room temperature water to a depth of 25±1 cm. Testing was performed under near infrared lighting to eliminate distal cues. Each animal was placed in the start position and allowed to search for the goal (escape ladder) for 5 min. If an animal did not find the escape, it was removed from the water and returned to its home cage. Two trials per day were given with a 5-min minimum intertrial interval on those trials where an animal failed to locate the escape. Errors, latency to escape, and start returns were recorded for each trial by an observer viewing a closed circuit monitor from an adjacent room. An error was defined as a head and shoulder entry into any arm of one of the T units. If an animal entered the crossing channel of a T but failed to fully enter either the right or left arm before exiting, this was counted as an error provided the animal crossed a line dividing the stem from the crossing channel of the T. Animals were tested for ten consecutive days from P51 to P60.

Locomotor activity with challenge

On P61, a 30-min rehabituation interval was provided in the locomotor chambers prior to pharmacological challenge. Following this, animals were briefly removed and given a 1-mg/kg dose of (+)-methamphetamine HCl (expressed as freebase and >95% pure) and placed back in the test chambers for an additional 2 h. Dependent variables were the same as above.

Statistical methods

Because each experiment used a split-litter design, offspring were matched on multiple factors by virtue of being littermates (Kirk 1995). To ensure that litter effects were controlled, litter was treated as a random factor (block) in a completely randomized block analysis of variance (ANOVA). In this model, treatment and sex were between factors within each block and litter was the blocking factor. Measures taken repetitively on the same animal were treated as repeated measure factors. For maze testing, the ANOVA was a two-between one-within randomized block model. In this model, treatment had three levels (MDMA, 5-MeO-DIPT, or SAL), and sex had two levels, while test interval or day had five or ten levels (day) depending on the test. Data were analyzed using SAS Proc Mixed (SAS Institute, Cary, NC, USA). Covariance structures for each data set were checked for best fit and several models compared. Autoregressive (AR(1)) covariance structure was optimal in most cases; however, in a few cases, compound symmetry was the better fit. Kenward–Rogers method of adjusted degrees of freedom was used; these do not match those used in standard ANOVAs and can be fractional. Significant interactions were analyzed using slice-effect ANOVAs at each level of the repeated measure factor. Step-down Bonferroni a posteriori tests were used to determine differences between treatment groups. Significance was considered at $p \leq 0.05$ and trends at $p \leq 0.10$. Data are presented as the least square (LS) means ± LS standard error of the mean (SEM).

Results

Body weights

Prior to drug treatment, there were no differences in body weights between groups. During drug administration, main effects of treatment ($F(2,89)=213.03$, $p<0.0001$), sex ($F(1,88.9)=13.03$, $p<0.001$), and day ($F(19,1,710)=295.16$, $p<0.0001$), as well as a treatment × day ($F(38,1,783)=22.09$, $p<0.0001$) interaction were observed (Fig. 1). MDMA treatment decreased body weight compared to SAL ($p<0.001$) beginning on P12 and throughout the remainder of the dosing period, while 5-MeO-DIPT treatment decreased body weights compared to MDMA- ($p<0.001$) and SAL-treated ($p<0.001$) animals beginning by the last dose on P11 and persisting throughout the dosing period.

Following dosing, there were main effects of treatment ($F(2,42.6)=7.06$, $p<0.01$), sex ($F(1,42.3)=159.75$, $p<0.0001$), and week ($F(6,271)=1,116.74$, $p<0.0001$) for body weights. The decreased body weights of 5-MeO-DIPT-treated animals persisted throughout behavioral testing compared to SAL- ($p<0.01$) and MDMA-treated ($p<0.05$) animals [(P28 weights (g): SAL=102.8+5.5; 5-MeO-DIPT=84.7+5.8; MDMA=92.7+5.6) and (P63 weights (g): SAL=316.7+6.2; 5-MeO-DIPT=302.1+6.6; MDMA=320.5+6.2)]. Body weights did not differ between MDMA- and SAL-treated animals after dosing. No treatment × week interaction was observed. All animals gained weight throughout testing, and males weighed more than females.

Elevated zero maze

There were no main effects of treatment on latency to enter the first open zone, time spent in the open, or head dips (not shown).

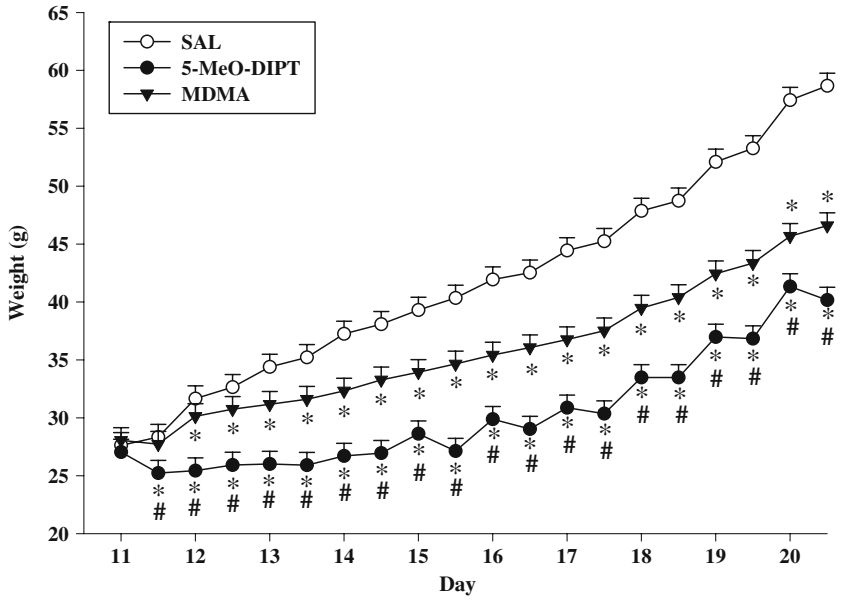

Fig. 1 MDMA and 5-MeO-DIPT administration leads to reductions in body weight during dosing. Body weight (LS mean ± LS SEM) of offspring exposed to MDMA, 5-MeO-DIPT, or SAL from P11 to P20. Data represent the first and last dose of each day. During dosing, body weights were reduced in MDMA- and 5-MeO-DIPT-treated animals soon after treatment began compared to SAL-treated animals. Data are averaged across sex. *$p<0.05$ vs. SAL, #$p<0.05$ vs. MDMA

Locomotor activity

For measures of horizontal activity and total distance traveled, there were no significant treatment effects or interactions of treatment. A main effect of interval was observed ($F(11,909)=99.83$, $p<0.001$) for horizontal activity with animals ambulating less as time progressed. A similar effect was seen for total distance (not shown). Similarly, there were no significant treatment effects on regional activity (peripheral vs. central) or on the repetitive beam-break measure of focused movement.

Marble burying

There were main effects of treatment on marbles 2/3 buried ($F(2,110)=8.54$, $p<0.001$) and latency to start burying ($F(2,110)=28.31$, $p<0.001$; Fig. 2). MDMA treatment increased the number of marbles buried compared to 5-MeO-DIPT and SAL ($p<0.05$) treatment while 5-MeO-DIPT- and SAL-treated animals did not differ. 5-MeO-DIPT treatment increased the latency to bury the first marble compared to SAL and MDMA treatment ($p<0.05$) while MDMA treatment decreased latency to bury compared to SAL treatment ($p<0.05$).

Straight channel swimming

No effect of treatment was observed for latency to swim the straight channel (latency: SAL 17.3±0.7 s, MDMA 16.4±0.8 s, 5-MeO-DIPT 15.9±0.7 s). A trial effect was observed ($F(3,330)=138.31$, $p<0.001$); latencies decreased across trials.

Morris water maze

Path length during platform trials (top) and average distance from the platform site on probe trials (bottom) are illustrated for each phase of the test in Fig. 3. During phase 1, main effects of treatment were observed for latency to find the platform ($F(2,94.1)=13.52$, $p<0.001$), path length ($F(2,94.4)=16.07$, $p<0.001$), and cumulative distance from the platform ($F(2,94)=13.46$,

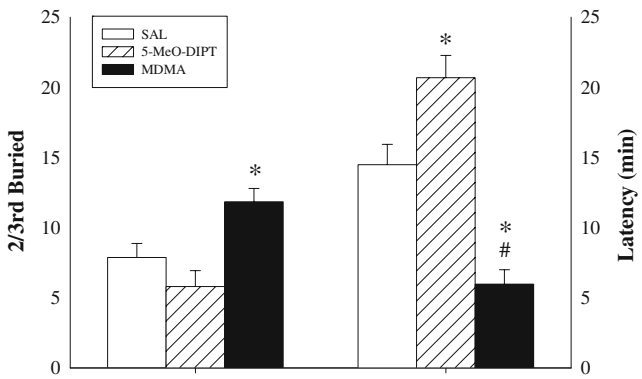

Fig. 2 Increased anxiety in MDMA-treated animals in a marble burying task. P11–20 MDMA-treated animals buried more marbles (LS mean ± LS SEM) and initiated burying sooner than SAL- and 5-MeO-DIPT-treated animals. 5-MeO-DIPT-treated animals initiated burying later than SAL- and MDMA-treated animals. Data are averaged across sex. *$p<0.05$ vs. SAL, #$p<0.05$ vs. 5-MeO-DIPT

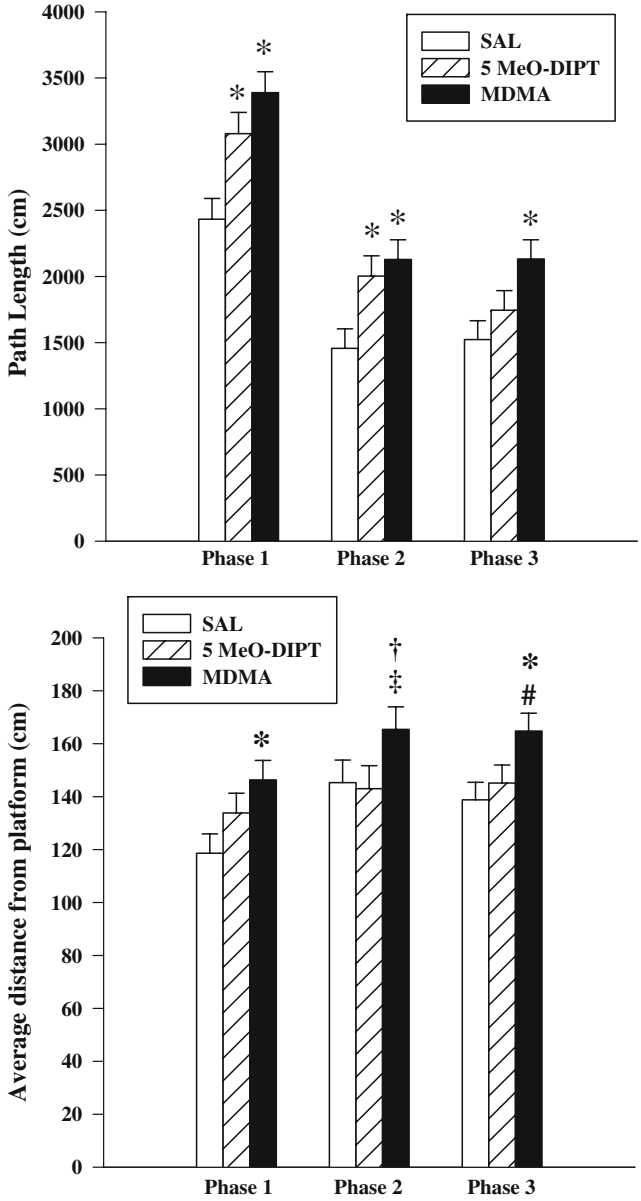

Fig. 3 MDMA and 5-MeO-DIPT treatment induces spatial learning deficits in the MWM. *Top*: MDMA- and 5-MeO-DIPT-treated animals show deficits in path length (LS mean ± LS SEM) to the goal in phases 1 and 2 of the Morris water maze. In addition, MDMA-treated animals showed increased path lengths during phase 3 of the MWM. *Bottom*: MDMA-treated animals had greater average distances from the platform site than SAL- or 5-MeO-DIPT-treated animals. Data are averaged across sex. *$p<0.05$ vs. SAL; †$p<0.10$ vs. SAL; #$p<0.05$ vs. 5-MeO-D IPT; ‡$p<0.10$ vs. 5-MeO-DIPT

$p<0.001$). Both MDMA and 5-MeO-DIPT treatment increased latency, path length, and cumulative distance compared to SAL-treated animals ($p<0.001$ for all comparisons). There were no differences observed between MDMA- and 5-MeO-DIPT-treated animals. There were no differences observed in swimming speed and there was no effect of sex on any measure. A main effect of day was observed (e.g., for latency ($F(5,421)=139.98$, $p<0.001$)) with animals improving during successive days of testing. During the probe trial for phase 1, a main effect of treatment was observed for average distance from the platform ($F(2,91)=5.64$, $p<0.01$) with MDMA-treated animals further from the platform site compared to SAL-treated animals. No differences were observed between 5-MeO-DIPT and SAL-treated animals. There were no differences seen for time or distance in the target quadrant or on crossovers.

During phase 2, main effects of treatment were observed for latency ($F(2,82.1)=8.29$, $p<0.001$), path length ($F(2,82.2)=8.52$, $p<0.001$), and cumulative distance ($F(2,82)=8.10$, $p<0.001$). Both MDMA and 5-MeO-DIPT treatment increased latency, path length, and cumulative distance compared to SAL-treated animals ($p<0.05$ for all measures). No differences were observed between MDMA- and 5-MeO-DIPT-treated animals. There was no effect of treatment on swimming speed and no effect of sex on any measure examined. There was a main effect of day for all measures (e.g., latency ($F(5,354)=75.69$, $p<0.001$)) with all groups improving during successive days of testing. During the probe trial, there was a main effect of treatment for average distance from the platform ($F(2,76)=3.60$, $p<0.05$), with MDMA-treated animals showing a trend ($p<0.10$) toward increased distance from the platform site compared to SAL- and 5-MeO-DIPT-treated animals. No differences were observed in time or distance in the target quadrant or on crossovers.

During phase 3, main effects of treatment were observed for latency ($F(2,82.3)=6.50$, $p<0.01$), path length ($F(2,82.6)=5.75$, $p<0.01$), and cumulative distance ($F(2,82.6)=5.62$, $p<0.01$). MDMA treatment increased latency compared to 5-MeO-DIPT and SAL treatment, while 5-MeO-DIPT-treated animals did not differ from SAL-treated animals. For path length and cumulative distance, MDMA treatment increased these measures compared to SAL treatment, while no differences were observed between MDMA- and 5-MeO-DIPT-treated animals or SAL- and 5-MeO-DIPT-treated animals. There was a main effect of day for all measures (e.g., for latency, $F(5,372)=27.87$, $p<0.001$) and sex did not have an effect on performance on any measure. No treatment effects were found on swimming speed. For the probe trial, a main effect of treatment ($F(2,98)=5.84$, $p<0.01$) was observed for average distance from the platform with MDMA-treated animals having increased distances compared to SAL- and 5-MeO-DIPT-treated animals. No differences were observed between SAL- and 5-MeO-DIPT-treated animals. MDMA-treated animals had decreased distance traveled in the target quadrant ($F(2,98)=7.33$, $p<0.01$) compared to 5-MeO-DIPT- and SAL-treated animals. No differences in crossovers were observed.

Cincinnati water maze

The results are illustrated in Fig. 4. For latency, a main effect of treatment ($F(2,127)=8.71$, $p<0.001$) and a treatment × day interaction ($F(18,974)=2.38$, $p<0.01$) were observed. MDMA treatment increased latency to find the goal compared to 5-MeO-DIPT ($p<0.05$) and SAL ($p<0.001$) treatment. Beginning on day 4, MDMA treatment increased latency to the goal compared to SAL ($p<0.05$) treatment and increased latency compared to 5-MeO-DIPT ($p<0.05$) treatment starting on day 7 of testing. 5-MeO-DIPT treatment did not increase latency to find the platform compared to SAL on any day of testing. There was a main effect of sex ($F(1,126)=5.76$, $p<0.001$) with females having shorter latencies than males (LS means ± SEM males=250.28±9.08 s; females=225.2±9.14 s) and a main effect of day ($F(9,954)=24.03$, $p<0.001$) with animals having decreased latencies on successive days.

For errors, a main effect of treatment ($F(2,105)=9.63$, $p<0.001$) and a treatment × day ($F(18,886)=1.74$, $p<0.05$) interaction were observed (Fig. 4, middle panel). Similar to latency, MDMA treatment increased errors compared to 5-MeO-DIPT ($p<0.05$) and SAL ($p<0.001$) treatments, while 5-MeO-DIPT- and SAL-treated animals performed similarly. Specifically, MDMA treatment increased errors compared to SAL treatment beginning on day 4 of testing and increased errors compared to 5-MeO-DIPT treatment beginning on day 7 of testing. Males committed more errors than females (LS means ± SEM for males=54.2±2.3, females=48.0±2.3; $F(1,105)=5.94$, $p<0.05$) and animals committed fewer errors on successive days of testing ($F(9,831)=22.58$, $p<0.001$).

For start returns, a main effect of treatment ($F(2,94.9)=12.10$, $p<0.001$) and a treatment × day interaction ($F(18,863)=1.66$, $p<0.05$) were observed. MDMA-treated animals returned to the start more compared to both 5-MeO-DIPT- and SAL-treated animals ($p<0.001$ for both comparisons), while 5-MeO-DIPT-treated animals did not differ from SAL-treated animals. In MDMA-treated animals, start returns increased compared to SAL-treated animals beginning on day 4 of testing, and MDMA-treated animals had increased start returns beginning on day 5 compared to 5-MeO-DIPT-treated animals. Start returns declined as testing progressed ($F(9,796)=13.72$, $p<0.001$). There was no effect of sex on start returns. No interactions of sex × treatment were observed for any measure in the CWM.

Locomotor activity with methamphetamine challenge

For horizontal activity, there were main effects of treatment prior to ($F(2,89.3)=3.20$, $p<0.05$) and following ($F(2,94.6)=3.75$, $p<0.05$) MA challenge (Fig. 5). For the period prior to MA challenge, MDMA-treated animals showed reductions in horizontal activity compared to SAL-treated animals ($p<0.05$). No differences were observed between SAL and 5-MeO-DIPT treatments or 5-MeO-DIPT and MDMA treatments. Following methamphetamine challenge, 5-MeO-DIPT-treated animals showed a significantly larger increase in horizontal activity compared to SAL- and MDMA-treated animals (both $p<0.05$). No differences were observed between MDMA- and SAL-treated animals, i.e., they exhibited the typical methamphetamine-induced increase followed by return to baseline. Similar effects were observed for total distance, central vs. peripheral distance, and focused movements (not shown).

Discussion

The purpose of this experiment was to compare the effects of exposure to MDMA vs. 5-MeO-DIPT after P11–20 treatment

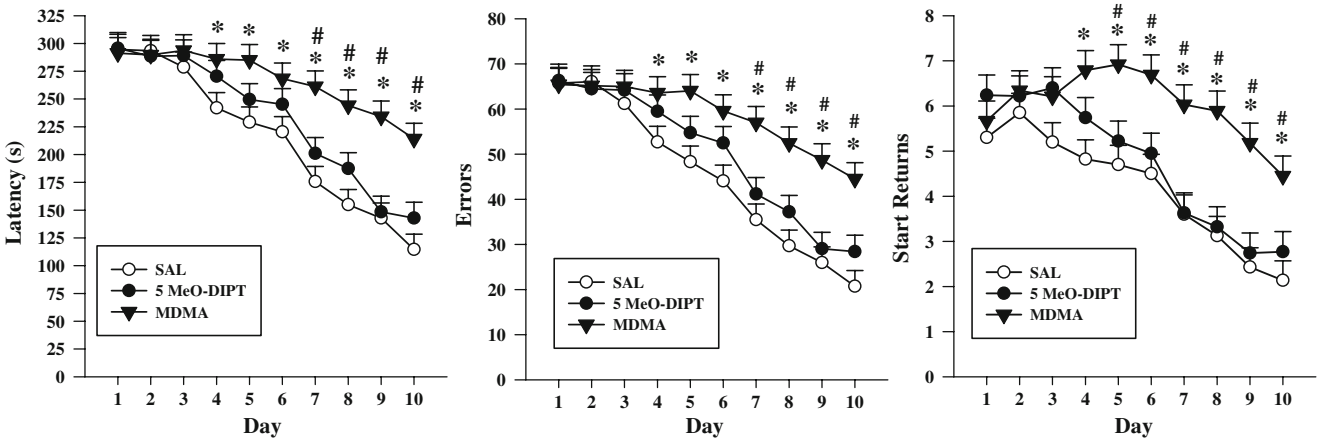

Fig. 4 MDMA treatment induces deficits in the Cincinnati water maze. MDMA treatment from P11 to P20 led to an increase in latency to reach the goal, errors, and start returns (LS mean ± LS SEM). 5-MeO-DIPT treatment did not alter CWM behavior. Data are averaged across sex. *$p<0.05$ vs. SAL; #$p<0.05$ vs. 5-MeO-DIPT

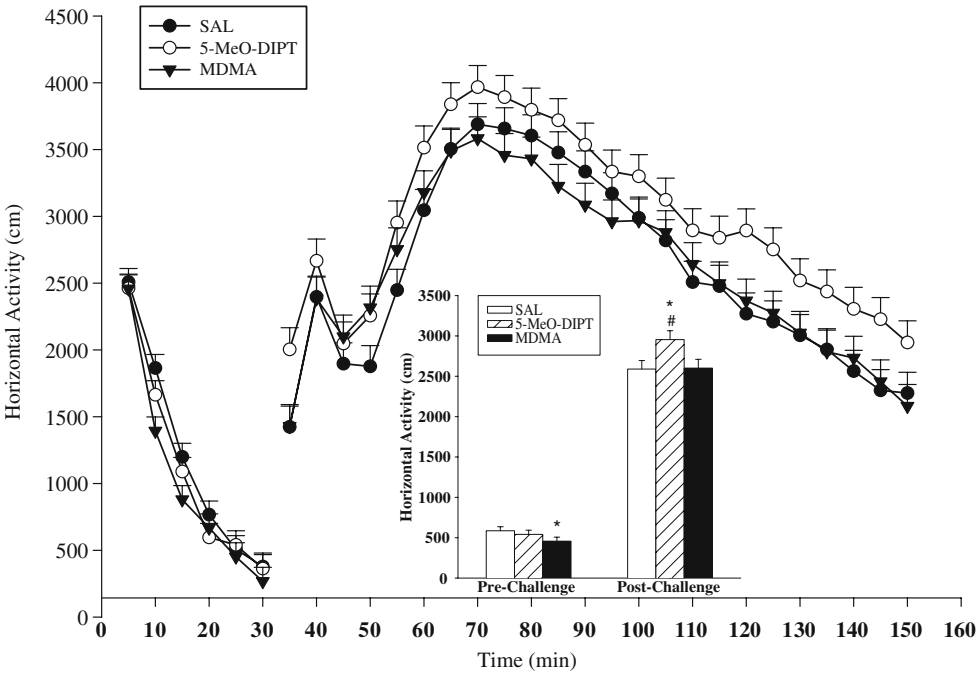

Fig. 5 5-MeO-DIPT treatment increases hyperactivity induced by methamphetamine challenge. 5-MeO-DIPT-treated animals had exaggerated increases in horizontal activity following methamphetamine administration compared to the increases seen in SAL- and MDMA-treated animals. Prior to testing, MDMA-treated animals were hypoactive compared to SAL-treated animals. No differences were observed between SAL- and MDMA-treated animals following methamphetamine treatment. *Inset*: Main effects of treatment. Data are averaged across sex. *$p<0.05$ vs. SAL; #$p<0.05$ vs. MDMA

using the same milligrams per kilogram dose of each. Consistent with previous findings, MDMA induced spatial learning and memory deficits in the MWM (Vorhees et al. 2007a). MDMA also induced deficits in CWM maze performance under near infrared light. A salient difference was that the CWM effect after MDMA was larger here than that reported previously (Vorhees et al. 2007a). This difference is attributable to the change from red light used previously to near infrared light used herein. Under red light, rats may use spatial cues to solve the task, whereas in the present experiment, extramaze cues were eliminated by using near infrared light that was not visible to rats, thereby leaving only egocentric methods to find the escape, i.e., path integration (Etienne and Jeffery 2004). Hence, the present data provide additional support for the idea that developmental MDMA treatment causes both path integration as well as spatial learning deficits.

Spatial learning impairments were observed in both MDMA- and 5-MeO-DIPT-treated animals. MDMA-treated animals had larger deficits compared to 5-MeO-DIPT-treated animals based on two findings: (1) the MDMA group had deficits in all three phases of the MWM, while 5-MeO-DIPT animals had deficits in the first two phases but not in phase 3, and (2) MDMA-treated animals showed reference memory deficits on probe trials on all three phases whereas 5-MeO-DIPT-treated animals did not. During phase 3, the platform was reduced in size and moved to an adjacent quadrant, requiring greater precision in locating the platform than in phases 1 and 2 (Vorhees and Williams 2006). It is interesting that 5-MeO-DIPT-treated animals showed deficits during the first two phases while performing similarly to SAL-treated animals during phase 3. There could be qualitative or quantitative reasons for this disparity. Quantitatively, it may be that 5-MeO-DIPT-treated animals are simply less affected at the same nominal dose as the MDMA-treated animals since it is not known whether the two drugs are equipotent. Efficacy may also not be equivalent because the molar dose of MDMA (51 mmol/kg) was higher than for 5-MeO-DIPT (30 mmol/kg). The substantial reduction in weight gain in the 5-MeO-DIPT group, however, suggests that it may be as or more potent than MDMA, although we cannot rule out the possibility that the body weight effects of 5-MeO-DIPT were the result of peripherally mediated toxicity and it may be less potent than MDMA centrally. In addition, this dose of 5-MeO-DIPT has been shown to increase CORT levels in developing animals to comparable degrees as methamphetamine, which also induces learning and memory deficits following developmental exposure. Given this, the absence of effect of 5-MeO-DIPT on CWM performance and its effects on MWM performance during phases 1 and 2 with catch-up during phase 3 suggests that 5-MeO-DIPT may have significantly less central effect on cognitive development than MDMA. Qualitatively, it may be that 5-MeO-DIPT-treated rats do not have a spatial learning deficit and that their poorer performance on phases 1 and 2 of the MWM are the result of their having difficulty learning the prerequisite subordinate skills necessary to solve the maze as has been shown previously for other treatments (Bannerman et al. 1995; Cain 1997; Saucier et al. 1996; Williams et al. 2002). If the effect of 5-MeO-DIPT is on task-specific subordinate skill learning, this too could explain why as the animals proceeded through the MWM test, they acquired

these skills and showed no differences on phase 3 or on probe trials.

As shown previously, MDMA treatment did not alter elevated zero maze indices of anxiety (Cohen et al. 2005). Similarly, 5-MeO-DIPT-treated animals showed no differences on this task. In another measure of anxiety, 5-MeO-DIPT treatment increased the latency to defensive marble burying compared to SAL treatment, suggesting that these animals were less anxious than SAL-treated animals. Contrary to the 5-MeO-DIPT effect, MDMA treatment increased defensive marble burying and reduced the latency to begin burying, suggesting increased anxiety in this task. The results of this task indicate that MDMA and 5-MeO-DIPT treatment have opposite effects on anxiety. Interestingly, in adult animals treated with MDMA, an increase in time spent in the open arms of the elevated zero maze was observed while no differences were observed in defensive marble burying (Skelton et al. 2008a). In adult animals that received 5-MeO-DIPT, no differences in marble burying were observed. Taken together, the results of these experiments suggest that multiple tests should be used to determine anxiety levels, as each may tap somewhat different aspects of emotional responses. Furthermore, the differences in effects of neonatal vs. adult administration of drugs make prediction of outcomes difficult for different ages.

In adults, 5-MeO-DIPT treatment caused mild deficits in path integration learning (Williams et al. 2007), while developmental administration led to no changes in this measure. The path integration deficits in Williams et al. (2007) may be different because of the maturational state of the brain during which the drug was given, but the exact reason for this difference requires further investigation. For MDMA, neonatal exposure leads to both path integration and spatial learning and memory deficits while adult exposure leads to primarily path integration deficits, with spatial learning mostly unaffected and some reference memory deficits (Able et al. 2006; Skelton et al. 2008a; Sprague et al. 2003). These differences could be related to the fact that the hippocampus is still developing during the P11–20 administration period (Rice and Barone Jr 2000), making the developing animal more sensitive to spatial learning disruptions than the adult animal. It is also possible that test order played a role in path integration learning in the 5-MeO-DIPT-treated animals that prevented deficits from being observed. Training in the MWM may have alleviated a path integration deficit through training. This reinforces the hypothesis that P11–20 MDMA exposure induces greater path integration learning deficits compared to 5-MeO-DIPT. Finally, it is possible that the animals were examined before path integration deficits fully developed. Animals began testing in the CWM prior to adulthood, while the 5-HT system is still developing. Depletion of 5-HT during similar periods to the model used for this study led to decreases in synaptic density on P30, while densities were similar to control on P60 (Mazer et al. 1997), suggesting that there is a rapid recovery of 5-HT synapses during this period. It is possible that this remodeling leads to aberrant 5-HT function and learning and memory deficits. Testing the effects of developmental 5-MeO-DIPT administration at different points in the animals' lives in future experiments might shed light on this question. Similar studies have been conducted with MDMA (Skelton et al. 2006) and methamphetamine (Vorhees et al. 2007b) and suggest that no amelioration of the deficits with age occurs, but whether this applies to 5-MeO-DIPT remains to be seen.

Treatment with 5-MeO-DIPT from P11 to P20 leads to an exaggerated hyperactivity following methamphetamine challenge. This suggests that 5-MeO-DIPT treatment alters either serotonergic or dopaminergic function (Leussis and Bolivar 2006; Viggiano et al. 2003). The hyperactivity in 5-MeO-DIPT-treated animals following methamphetamine administration compared to MDMA- and SAL-treated animals suggests that there is aberrant development of excitatory striatal systems. Interestingly, there were no alterations in MDMA-treated offspring, which is somewhat surprising given that MDMA treatment from P11 to P20 leads to alterations in 5-HT signaling (Crawford et al. 2006). However, in that experiment, the releasable pool of 5-HT in the neostriatum was not assessed, only total tissue concentration; it may be that the releasable pool is critical in determining the effect of 5-HT on locomotion. When we examined the spontaneous locomotor behavior immediately prior to the drug challenge, we showed hypoactivity in the MDMA-treated animals as we have seen previously (Cohen et al. 2005; Vorhees et al. 2007a); however, when locomotor effects were examined earlier on P30, no differences were noted. Whether this difference in locomotor activity is related to the age of the animal or to some other effect is unknown, however, in our previous study, locomotor activity was assessed at approximately P60, which is when we see the effects in this study. Taken together, there appears to be a delay in the hypolocomotion induced by neonatal MDMA treatment. Similarly, there also appears to be a delay in sex-related differences in MWM learning. In this study, during all three phases of MWM learning, we showed no sex-related differences in learning, but when we start testing at a later age (e.g., P60), sex-related differences were present (Williams et al. 2003). Therefore, sex differences in spatial learning and memory may be related to the hormonal state of the animal.

Persistent weight loss was observed in MDMA- and 5-MeO-DIPT-treated rats. While the overall weight loss could be indicative of general toxicity, we have previously shown that the slower weight gain observed in MDMA-treated

animals does not account for the learning and memory deficits (Williams et al. 2003). Also, the lack of deficits in swimming speed suggests that animals were not physically impaired in a manner that would reduce swimming ability.

In conclusion, 5-MeO-DIPT induces learning deficits in the Morris water maze in animals treated from P11 to P20 but the effects are less severe than those caused by MDMA at the same nominal dose. Furthermore, based on these and previous data, it appears that 5-MeO-DIPT treatment from P11 to P20 induces greater learning deficits in the Morris water maze than adult treatment. The MDMA data show that P11–20 treatment induces severe path integration deficits in addition to impairing spatial navigation and reference memory with effects on anxiety measures that suggest increased anxiousness.

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