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(±)-3,4-Methylenedioxymethamphetamine treatment in adult rats impairs path integration learning: A comparison of single versus once per week treatment for five weeks

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA) administration (4×15 mg/kg) on a single day has been shown to cause path integration deficits in rats. While most animal experiments focus on single binge-type models of MDMA use, many MDMA users take the drug on a recurring basis. The purpose of this study was to compare the effects of repeated single-day treatments with MDMA (4×15 mg/kg) once weekly for 5 weeks to animals that only received MDMA on week-5 and saline on weeks 1–4. In animals treated with MDMA for 5 weeks, there was an increase in time spent in the open area of the elevated zero-maze suggesting a decrease in anxiety or increase in impulsivity compared to the animals given MDMA for 1 week and saline treated controls. Regardless of dosing regimen, MDMA treatment produced path integration deficits as evidenced by an increase in latency to find the goal in the Cincinnati water maze. Animals treated with MDMA also showed a transient hypoactivity that was not present when the animals were re-tested at the end of cognitive testing. In addition, both MDMA-treated groups showed comparable hyperactive responses to a later methamphetamine challenge. No differences were observed in spatial learning in the Morris water maze during acquisition or reversal but MDMA-related deficits were seen on reduced platform-size trials. Taken together, the data show that a single-day regimen of MDMA induces deficits similar to that of multiple weekly treatments.

Keywords

MDMA; Morris water maze; Cincinnati water maze; path integration learning; spatial learning

Introduction

While use of ±3,4-methylenedioxymethamphetamine (MDMA) has declined recently, prevalence data show that 20% of 25–26 year olds in the United States have tried MDMA

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(Johnston et al., 2007). In the European Union, MDMA use varies widely among countries with a range of 0.6–8.8% of 15–34 year olds reporting use (EMCDDA., 2004). It is known that MDMA exposure to humans, rats, and nonhuman primates leads to reductions in brain serotonin (5-HT) levels (Green et al., 2003). In adult rats, it has been shown that 5-HT is reduced in the prefrontal cortex, neostriatum, and hippocampus (structures important for learning and memory) following 4 doses of 15 mg/kg of MDMA administered over a single day (Able et al., 2006). MDMA exposure also leads to moderate decreases in neostriatal dopamine levels long after treatment (Able et al., 2006; Cohen et al., 2005; Commins et al., 1987; McGregor et al., 2003).

Cognitively, MDMA exposure in humans leads to impairment of verbal, prospective, and working memory (Kalechstein et al., 2007; Zakzanis and Campbell, 2006). Central executive and decision making skills are also altered in persistent MDMA users (Bolla et al., 1998; Klugman and Gruzellier, 2003; Parrott et al., 1998), and abstaining from MDMA does not ameliorate these effects (Zakzanis and Campbell, 2006). Similar to humans, MDMA administration in rats also leads to cognitive deficits. For example, MDMA treatment produced reference memory deficits on probe trials in the Morris water maze (MWM) (Able et al., 2006; Sprague et al., 2003). With a short retention time of 15 min, MDMA-treated animals showed deficiencies in a test of novel object recognition (Morley et al., 2001), however with a longer retention interval of 1 h no deficits were seen (Morley et al., 2001; Able et al., 2006). Taken together, these findings imply that MDMA treatment may affect the function of the hippocampus, which is supported by the observed reduction in 5-HT levels. The Cincinnati water maze (CWM) is a multiple T-maze that requires a combination of path integration and spatial learning abilities when run under lighted conditions. A previous study showed that a single day treatment regimen of MDMA leads to deficits in the CWM, as evidenced by increases in latency to find the escape and number of errors (Able et al., 2006). While this study was able to detect learning and memory deficits following a single day of administration, this regimen does not mimic the pattern of human abusers (i.e., weekend binges). Another substituted amphetamine, fenfluramine (FEN), also affects CWM performance (Skelton et al., 2004; Williams et al., 2002a), however these animals do not show any deficits in the MWM (Skelton et al., 2004; Williams et al., 2002a). Taken together, these data suggest that path integration learning is an important form of cognitive deficit induced by substituted amphetamines that deserves further study.

While many studies have been conducted using an acute model of administration, few studies have examined a chronic abuse model of MDMA use. In one study 10 mg/kg MDMA was administered two times per day (4 h interdose interval, i.e., 10×2 mg/kg) every 5 days from postnatal day (P)35–60 (Piper and Meyer, 2004). In a subsequent study the dose frequency was changed from 2 to 4 doses with interdose intervals of 1 h and with a lowered dose of 5 mg/kg/dose (i.e., 5×4 mg/kg)(Piper et al., 2005). The 10×2 mg/kg MDMA regimen lead to decreases in novel object recognition with a 15 min retention interval, whereas no deficits were seen with the 5×4 mg/kg frequent dose regimen (Piper et al., 2005; Piper and Meyer, 2004). The 10×2 mg/kg dose regimen of MDMA also led to decreases in anxiety in the elevated plus maze, while no changes in these measures were observed with the 5×4 mg/kg MDMA dose regimen (Piper et al., 2005; Piper and Meyer, 2004).

The purpose of the present study was to extend Able et al.'s (2006) previous findings using an exposure regimen that better mimics the adult chronic MDMA abuser. This was accomplished by administering MDMA (15 mg/kg × 4 doses/day) or SAL once a week for 5 consecutive weeks. In order to directly compare the effects of a repeated dosing model with the more commonly used acute model, one group was dosed with SAL for 4 weeks and MDMA on the fifth week and the other group with MDMA each week for all five weeks (along with saline

controls that received SAL all 5 weeks). Behavioral tests were conducted in the same order and time after last treatment as in Able et al. (2006).

Materials and methods

Subjects

Male Sprague-Dawley, CD IGS rats (225–250 g) were obtained from Charles River Laboratories (Raleigh, NC). The rats were allowed to acclimatize to the colony room for one week prior to the day of MDMA administration. The colony room was maintained at a temperature of $21 \pm 1^\circ\text{C}$ ($50 \pm 10\%$ humidity) with food and water available freely, except during MDMA treatment. The animals were initially housed in pairs in cages measuring $46 \times 24 \times 20$ cm prior to drug treatment, then singly housed during and following drug treatment (Able et al., 2006). The Cincinnati Children's Research Foundation's Institutional Animal Care and Use Committee approved the research protocol. The vivarium was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Methylenedioxymethamphetamine administration

$\pm 3,4$ -Methylenedioxymethamphetamine HCl (MDMA, expressed as the freebase and greater than 95% pure) was obtained from the National Institute on Drug Abuse through its provider Research Triangle Institute, Research Triangle Park, NC. Sixty-three animals were assigned to one of three treatment groups ($n = 21/\text{treatment}$) as follows: (1) MDMA, $15 \text{ mg/kg} \times 4/\text{day}$, one day per week for 5 weeks (MDMA $\times 5$); (2) isotonic saline $\times 4/\text{day}$, one day per week for 4 weeks and $15 \text{ mg/kg} \times 4$, MDMA on the last week (MDMA $\times 1$); and (3) isotonic saline $\times 4/\text{day}$, one day per week for 5 weeks (SAL). On all treatment days, the interdose interval was 2 h. All treatments were separated by 7 days. On each day of MDMA treatment, animals were maintained in smaller $28 \times 16 \times 12$ cm polycarbonate cages in a room outside of the home suite that was at an ambient temperature of $22 \pm 1^\circ\text{C}$. MDMA or SAL was given subcutaneously in the dorsum and the site of injection was varied to prevent irritation to the skin. The experiment was performed with three cohorts of 21 animals each (7 animals per treatment per cohort). In order to ensure that animals did not die from the hyperthermia induced by MDMA exposure, body temperatures were monitored from the beginning of MDMA administration until 2 h after the last dose.

Temperature monitoring

Prior to MDMA treatment, rats were implanted via injection with subcutaneous temperature transponders (IPTT-200, Biomedic Data Systems, Seaford, DE) under light isoflurane anesthesia. This was done to address two issues. Firstly, the subcutaneous temperature probes alleviate the stress of rectal temperature measurements and the physical manipulation required to obtain such recordings (Balcombe et al., 2004). Secondly, temperature probes were used to determine if cooling intervention was required to keep an animal's body temperature from becoming lethal as previously described (Able et al., 2006). Cooling was utilized to ensure that animals did not die as a result of MDMA-induced hyperthermia; a procedure that is similarly observed in humans (Green et al., 2003).

On each day of MDMA administration, temperatures were taken immediately before the first dose and subsequently every 30 min until 2 h after the last dose. If the temperature of an animal reached or exceeded 40°C , it was placed in a cage that contained shallow room temperature water and body temperatures were monitored every 10 min until they fell below 40°C , at which time the animal was removed and returned to its treatment cage. For animals in the MDMA $\times 5$ group, 10 rats required cooling during week 1, 5 during week 2, 8 during weeks 3 and 4, and 11 during week 5. For animals in the MDMA $\times 1$ group, 10 rats required cooling on the fifth week when MDMA was administered. No rats in the SAL group required cooling.

Body Weight Measurements

In order to determine the impact of MDMA administration on body weight, animals were weighed the day before and 24 h following each drug treatment day. Once the animals began behavioral testing, body weights were obtained weekly.

Behavioral Methods

The behavioral tests began 5 days after the last day of treatment in order to be consistent with the testing procedure used previously (Able et al., 2006). All behavioral testing was done blind to the treatment group of the animals and tests were performed during the light portion of the light/dark cycle (14 h of light, lights on at 600 h). Cognitive tasks were performed in the same order that we previously used to assess the effect of a single day of MDMA administration (Able et al., 2006). Behavioral equipment was cleaned between animals with 70% ethanol.

Elevated Zero Maze (Day 5 following last MDMA treatment)

The first behavioral task was the elevated zero maze (Shepherd et al., 1994) as modified by (Williams et al., 2003). This task assesses anxiety and was used since others showed that MDMA treatment induces changes in anxiety-related behavior (Morley et al., 2001; Piper and Meyer, 2004). 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 in quadrants, so that adjoining quadrants either had black walls that were 28 cm in height (closed area) or a clear acrylic curb 1.3 cm in height (open area). 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 2 front paws and shoulders in the open area, whereas a head dip was counted when the animal placed its head over the open quadrant side-rail.

Spontaneous locomotion

Beginning approximately 1 h after elevated zero maze testing, animals were taken to a separate room and tested for spontaneous locomotor behavior in an automated activity monitor (Accuscan Electronics, Columbus, OH). The apparatus was 41 × 41 cm and contained 16 photodetector-LED pairs along each side spaced 2.5 cm apart and positioned 2.2 cm above the floor of the test chamber. To begin the test, an animal was placed in the center of the apparatus and allowed to explore for 1 h. The dependent variables were horizontal activity (sum of all photobeam interruptions occurring in the horizontal plane), center distance, and repetitive beam breaks (the sum of consecutive photobeam interruptions at the same position).

Marble Burying

Immediately following locomotor activity testing, animals were brought to yet 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 than without it. Eighteen blue marbles were used, 1.4 cm in diameter (blue produced greater burying compared to clear marbles in a preliminary study), and were evenly spaced (3.5 cm from the sides and 7 cm apart in all directions) in 6 rows of 3 (a template was used to position the marbles within the cage) 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 used to cover the cage. Animals were given 30 min of exposure to the marbles and latency to begin burying and number of marbles buried at least 2/3 were measured.

Straight channel swim (Day 6 following last MDMA treatment)

Animals were tested for swimming ability in a straight water channel as described previously (Williams et al., 2002b). The straight 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 of the channel facing the wall and allowed a maximum of 2 min to locate an escape ladder at the opposite end. Four consecutive timed trials were given and escape latency was recorded for each trial. This task measures the motivation of animals to escape water as well as swimming ability and speed. The straight channel also acclimatizes animals to swimming and introduces them to the fact that escape from the water is possible. Experience has shown that this is an important procedure when used prior to the assessment of cognitive ability in water mazes because it reduces the time spent adjusting to a swimming environment.

Cincinnati water maze (Day 7–12 following last MDMA treatment)

On the day following the straight channel, the animals began CWM training. The CWM consists of 9 black acrylic T's with the long arms forming the main channel of the maze as described previously (Vorhees, 1987). The walls were 51 cm high with a channel width of 15 cm and the maze was filled with room temperature water brought to a depth of 25 ± 1 cm. Testing was performed under a single 25 W red light as before (Able et al., 2006). An 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 when the animal did not locate the escape. Errors and latency to escape were scored for each trial. An error was defined as a whole body entry into one of the short arms of a T. If an animal entered the crossing channel of a T but failed to fully enter either the right or left arm of the T before exiting, this was counted as an error provided the animal crossed an imaginary line dividing the stem from the crossing channel of the "T". The animals were tested for 6 consecutive days.

Morris water maze (Day 14–33 after the last MDMA treatment)

Morris water maze (MWM) training began two days after the CWM and was executed in three consecutive phases: acquisition (days 14–19), reversal (days 21–26), and shifted platform with reduced platform size (shifted-reduced; days 28–33). Each phase consisted of 4 trials per day for 5 days to find the hidden platform followed by a single probe trial on day-6 with the platform removed (Vorhees and Williams, 2006). Briefly, the apparatus was a black stainless steel tank 210 cm in diameter with various extramaze cues available for animals to use to navigate. During the acquisition phase a 10 × 10 cm platform was submerged 2 cm below the water in the SW quadrant of the maze. 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 the reversal phase, which began the second day after the completion of acquisition, the 10 × 10 cm platform was relocated to the NE quadrant of the tank and the converse starting positions of the acquisition phase were used. For the shifted-reduced phase, 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). The dependent variables analyzed were latency, path length, and mean directionality. On probe trials the variables analyzed were platform crossings, path length, mean directionality, average distance from the platform, and latency to cross the platform.

Novel object recognition (Days 35–39)

Novel object recognition began after the last phase of the MWM and was conducted as described previously (Clark et al., 2000) with modification. Briefly, circular (91 cm in diameter with 51 cm high walls) polyethylene arenas were used and animals were habituated to the arenas for 10 min per day for 4 days. On the fifth day, novel object recognition testing took place (day 30 after dosing) and was divided in two phases. The familiarization phase entailed placing two identical objects (blue porcelain flower pots) 25 cm from the sides of the arena and 41 cm apart, on center. The rats were placed in the arena between the two objects and given a maximum of 10 min to accumulate 30 s of object exploration. Object exploration was defined as the animal standing within 1 cm and oriented towards the object. According to Clark et al.'s (2000) definition, exploration of the object occurred if the animal sniffed or pawed the object, however climbing on the object was not counted. The retention phase began 1 h after familiarization. A new object (pink mug) was introduced in combination with an identical copy of the original object. As in the familiarization phase, the animals had 10 min to complete 30 s of object exploration. A video camera was placed over the arena, and behavior was scored using a computer program provided by Dr. Robert E. Clark. Time exploring the new object during the test phase was analyzed.

Novel place recognition (40 days after last drug administration)

Novel place recognition began one day following the test phase of novel object recognition and occurred under the same conditions as novel object recognition with the exception that during the test phase identical copies of the same object as used during familiarization were used; however one object was placed 90° clockwise compared to its original location. As with novel object recognition, animals were given 10 min to obtain 30 s of observation time and retested 1 h later.

Locomotor activity with challenge (Day 42)

A 30 min re-habitation 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 (MA; expressed as freebase) and placed back in the test chambers for an additional 2 h. Dependent variables were horizontal activity and repetitive beam breaks (as described above).

Neurotransmitters and tissue collection

In order to verify the 5-HT depletion usually seen with MDMA treatment, monoamine levels were measured in selected brain regions. Briefly, three days after completion of locomotor activity with MA challenge (45 days after last drug treatment), the animals were killed by decapitation and the brains removed. From each brain, the hippocampus, neostriatum, and prefrontal cortex were dissected over ice, rapidly frozen on dry ice, and stored at -80°C until assayed by HPLC.

Each tissue sample was weighed, homogenized in 50 volumes of 0.2 N perchloric acid buffer and centrifuged at $10,000 \times g$ for 5 min. 20 μl aliquots of each sample were injected in a C18 column (5 μm , 100×2 mm) connected to an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN) with a reference electrode at +0.60 V oxidation potential. The mobile phase contained 35 mM citric acid, 54 mM sodium acetate, 50 mg/L disodium ethylenediamine tetraacetate, 70 mg/L octane sulfonic acid sodium salt, 6% methanol, 6% acetonitrile, at a pH of 4.0. The flow rate was 0.28 ml/min. Chromatograms were obtained and integrated, and the neurotransmitter concentrations were calculated from standard curves generated for each neurotransmitter analyzed.

Statistics

Data for the elevated zero maze, novel object recognition, neurotransmitters, and straight channel swimming times were analyzed using general linear model factorial analyses of variance (ANOVA; Proc GLM, SAS Institute, Cary, NC). Treatment was a between-subject factor and trial (straight channel) was a within-subject factor. The Greenhouse-Geisser correction was used in instances in which the variance-covariance matrices were significantly non-spherical. Body weight, body temperature, MWM, CWM, and locomotor activity data were analyzed using mixed linear model ANOVAs (Proc Mixed, SAS Institute, Cary, NC) in order to better fit covariance assumptions in models with repeated measures. The SAL and MDMA×1 data were combined for body weights and temperatures for the first four dosing periods since these groups both received saline. Each model was checked using best fit statistics against covariance matrix models. Autoregressive (AR(1)) covariance structure was optimal for both mazes and both activity tests. Mixed ANOVAs using the Kenward-Roger method for calculating degrees of freedom provide adjusted degrees of freedom and do not necessarily match those used obtained with general linear models and can be fractional and different even between factors within the same test. Significant interactions were further analyzed using ANOVA slice effect tests at each level of the repeated measure factor. Based on our previous findings, we hypothesized that MDMA would impair maze performance. Accordingly, on these tests the Hochberg method to conduct planned nonorthogonal contrasts comparing each treatment group to the control group was used. Marble burying scored number of marbles covered and therefore was analyzed nonparametrically using the Wilcoxin rank test. Significance was set at $P < 0.05$; trends at $P < 0.10$.

Results

Body Weight

There were effects of treatment ($F(1,65)=5.26$, $p<0.05$), week ($F(3,187)=419.18$, $p<0.001$), and treatment × week ($F(3,187)=8.87$, $p<0.001$) on body weight prior to each day of treatment during the dosing period (Table 1). Since the MDMA×1 group received saline during weeks 1–4, their data were merged with those of the SAL group after determining that they were not significantly different nor did they approach being different. On week 5 a significant effect of treatment was observed ($F(1,63)=15.63$, $p<0.001$). Step-down Bonferroni tests showed that the effect of MDMA began to emerge during week-2; specifically, the MDMA×5 group showed a non-significant reduction in body weights compared to SAL-treated animals ($p<0.10$). On weeks 3–5, MDMA×5 animals showed significantly reduced body weights compared to SAL-treated animals ($p<0.001$).

Body weights were also taken 24 h following MDMA administration each week. A main effect of treatment ($F(1,64.6)=13.86$; $p<0.001$) and week ($F(3,187)=366.83$, $p<0.001$) and a treatment × week interaction ($F(3,187)=5.30$; $p<0.01$) were observed. During the second through fourth week of dosing, MDMA×5 animals had lower body weights than the animals treated with saline ($p<0.01$), i.e., lower than both the SAL and the MDMA×1 group treated with saline during weeks 1–4. On the fifth week after treatment, a main effect of treatment was observed ($F(2,59)=11.99$, $p<0.001$). At this point, both the MDMA×5 ($p<0.001$) and MDMA×1 animals ($p<0.01$) showed reduced body weights compared to SAL controls (data not shown).

Following the five week dosing period, body weights were recorded weekly during the six weeks of behavioral testing. There was no main effect of treatment on body weight during this period, however effects of week ($F(5,291)=330.39$, $p<0.001$) and treatment × week ($F(10,291)=6.17$, $p<0.0001$) were observed (Table 1). All animals gained weight during behavioral testing regardless of treatment, however, both groups of MDMA-treated animals weighed significantly less than the SAL controls during the first week of testing ($p<0.05$ for both the

MDMA×1 and MDMA×5 groups versus SAL). No differences were observed for body weights among the groups during the remaining weeks of behavioral testing.

Body Temperature

As with body weights during the first four weeks of dosing, the MDMA×1 and SAL-treated animals were combined. After the first dosing day (Week-1), there were significant effects of treatment ($F(1,61.2)=102.81$, $p<0.0001$), time ($F(16,830)=10.18$, $p<0.001$), and treatment $\times$ time ($F(16,830)=12.62$, $p<0.0001$). The MDMA×5 group had increased body temperatures compared to SAL-treated animals beginning 120 min following the first dose and this effect continued throughout the dosing period (Figure 1A). For Week-2, the significant effects were treatment ($F(1,60.1)=66.36$, $p<0.0001$), time ($F(16,843)=14.79$, $p<0.0001$), and treatment $\times$ time ($F(16,843)=14.79$, $p<0.0001$). The MDMA×5 group showed increased body temperatures 30 min following treatment and this persisted throughout the dosing period compared to SAL-treated animals (Figure 1B). For Week-3, the MDMA×5 group showed a similar result with treatment ($F(1,61.2)=141.05$, $p<0.0001$), time ($F(16,843)=13.91$, $p<0.0001$), and treatment $\times$ time ($F(16,843)=12.56$, $p<0.0001$) effects observed. Again, the MDMA×5 group had elevated body temperatures compared to SAL-treated animals beginning 30 min after the first dose and continuing throughout the dosing period (Figure 1C). For Week-4 there were significant effects of treatment ($F(1,63.3)=173.95$, $p<0.0001$), time ($F(16,861)=16.56$, $p<0.0001$), and treatment $\times$ time ($F(16,861)=13.34$, $p<0.0001$). The MDMA×5 group showed increased body temperature beginning 60 min following treatment and continuing through the dosing period compared to SAL-treated animals (Figure 1D). On Week-5 there were significant effects of treatment ($F(2,65.9)=70.99$, $p<0.0001$), time ($F(16,849)=11.73$, $p<0.0001$), and treatment $\times$ time ($F(32,885)=5.48$, $p<0.0001$). For the MDMA×5 group, body temperatures were elevated beginning 60 min following the first dose and remained elevated throughout the dosing period compared to the SAL group. The MDMA×1 group had elevated body temperatures compared to the SAL group beginning 90 min following the first dose and continuing throughout the dosing period (Figure 1E). A comparison of the MDMA×1 and MDMA×5 groups showed no overall effect between these groups, however time ($F(16,623)=7.07$, $p<0.0001$) and time $\times$ treatment ($F(16,623)=1.98$, $p<0.05$) were significant. Stepdown Bonferroni comparisons revealed that the MDMA×5 group had elevated body temperatures compared to MDMA×1 group beginning at 60 min following the first dose and this difference persisted until 150 min following the first dose (Figure 1E).

Elevated Zero Maze

A main effect of treatment ($F(2,58) = 10.68$, $p<0.0001$) was observed in the time spent in the open quadrants (Figure 2, top). Post hoc pairwise comparisons revealed that the MDMA×5 group spent more time in open than the MDMA×1 or SAL animals ($p<0.05$), while no differences were observed between the MDMA×1 and SAL animals. For head dips, a significant effect of treatment was also observed ($F(2,58) = 15.87$, $p < 0.0001$); post hoc comparisons showed that the MDMA×5 group had more head dips compared to the SAL or MDMA×1 groups ($p<0.05$), while there were no differences between the MDMA×1 and SAL groups (Figure 2, bottom). No differences were noted for stretch attends (Figure 2, bottom).

Locomotor Activity

For horizontal activity there were significant main effects of treatment ($F(2,59.8)=6.40$, $p<0.01$) and interval ($F(11, 558)=179.12$, $p<0.0001$) (Figure 3). MDMA treatment, regardless of regimen, decreased the horizontal activity compared to SAL treatment. No differences were observed between MDMA×5 and MDMA×1 animals. Similarly, for the measure of total distance, significant effects of treatment ($F(2, 59.5)=4.77$, $p<0.01$) and interval ($F(11, 548) =141.67$, $p<0.001$) were observed. MDMA treatment decreased the total distance traveled

compared to SAL-treated animals; and no differences were seen between the MDMA×5 and the MDMA×1 groups (not shown). There was no treatment effect on time spent in the center of the chamber, although there was an interval effect ($F(11, 451)=53.98, p<0.0001$) with animals spending less time in the center as the test progressed. There was no effect of MDMA treatment on rearing, although there was an interval effect ($F(11, 559)=3.34, p<0.001$) with the animals rearing less as the test progressed.

Marble Burying

No significant effects of MDMA treatment were observed for marble burying. [Means±SEM of marbles buried: SAL: 2.9 ± 0.3 ; MDMA×1: 3.2 ± 0.4 ; MDMA×5: 2.9 ± 0.3]

Straight Channel Swimming

There was no effect of MDMA treatment on latency to reach the end of the straight channel. An effect of trial was observed ($F(3,177)=21.36, p<0.0001$); all animals improved performance across successive trials [Means±SEM across trials (in s): SAL: 14.8 ± 1.3 ; MDMA×1: 15.0 ± 1.0 ; MDMA×5: 14.8 ± 0.9].

Cincinnati water maze

Significant main effects of treatment ($F(2,83.3) = 3.04, p < 0.05$) and day ($F(5,249) = 93.63, p < 0.0001$) were observed for latency to reach the escape (Figure 4, top). No significant interactions were obtained. Hochberg comparisons showed that both MDMA-treated groups had longer latencies to reach the escape than SAL controls (both $p < 0.02$). There were no significant differences between the MDMA×1 and MDMA×5 groups. For errors, significant main effects of treatment ($F(2,49.2) = 2.53, p<0.05$) and day ($F(5,190)=80.34, p<0.0001$) were also obtained (Figure 4, bottom). Hochberg comparisons showed that both the MDMA×1 and MDMA×5 groups made more errors than SAL controls (both $p < 0.04$).

Morris water maze-acquisition

No effect of treatment was observed for latency, path length, or mean directionality during the acquisition phase of the MWM. An effect of day (for latency ($F(4,177)=88.57, p<0.001$)) was observed in all measures showing that all animals improved performance as the test progressed. No interactions of day and treatment were observed. For the probe trial, no treatment effects were observed for platform crossings, path length, mean directionality, average distance from the platform or latency to cross the platform (not shown).

Morris water maze-reversal

No effect of treatment was observed for latency, path length, or mean directionality during the reversal phase of the MWM. An effect of day was observed (for latency ($F(4,148)=75.36, p<0.001$)) in all measures showing that all animals improved performance as the test progressed. No interactions of day and treatment were observed. For the probe trial, no treatment effects were observed on any measure.

Morris water maze, shifted-reduced

No main effect of treatment was observed for latency or mean directionality during the shifted-reduced phase of the MWM. A main effect of treatment was observed for path length ($F(2,60.4) = 11.70, p < 0.05$), with MDMA-treated animals, regardless of regimen, traveling a longer path to reach the platform compared to SAL-treated controls (means in cm: SAL 943.33 ± 99.20 , MDMA×1 1286.27 ± 96.8 , MDMA×5 1160.92 ± 99.20). Again, day effects were observed (for latency ($F(4,179)=28.79, p<0.001$)) in all measures with all animals improving as the test progressed. For the probe trial, no treatment effects were observed on any measure.

Novel object and novel place recognition

There were no effects of treatment on exploration time of the two identical objects during familiarization. Similarly, there were no treatment effects on time spent exploring the novel object during testing; all groups showed novelty preference (~70%). Similarly, no effects were found during novel place testing.

Locomotor with challenge

Prior to MA challenge, there was no main effect of treatment on horizontal activity during the 30 min habituation period, however there was a main effect of time ($F(5,184) = 99.85$, $p < 0.0001$) such that all animals ambulated less as time progressed (not shown). Following challenge with MA (1 mg/kg), there was no effect of treatment, however time ($F(23,1334) = 53.65$, $p < 0.0001$) and time $\times$ treatment ($F(46,1334) = 3.27$, $p < 0.0001$) effects were observed (Figure 5). Both MDMA-treated groups, regardless of regimen, showed increased horizontal activity during the first 15 min following challenge than did SAL controls. All three groups' activity converged thereafter.

Neurotransmitters

In the prefrontal cortex (Figure 6A), there were main effects of treatment on 5-HT ($F(2,57) = 37.12$, $p < 0.001$) and 5-HIAA ($F(2,57) = 27.09$, $p < 0.001$). MDMA treatment lowered 5-HT levels regardless of regimen compared to SAL-treated animals, but MDMA $\times$ 1 animals had lower 5-HT levels than MDMA $\times$ 5 animals ($p < 0.05$). For 5-HIAA, a similar pattern was seen with MDMA $\times$ 1 animals having lower levels than MDMA $\times$ 5 animals ($p < 0.05$), and all MDMA-treated animals had lower 5-HIAA levels compared to SAL-treated animals. Dopamine levels were not affected by MDMA treatment in the prefrontal cortex.

In the hippocampus (Figure 6B), a main effect of treatment was observed for both 5-HT ($F(2,57) = 36.95$, $p < 0.0001$) and 5-HIAA ($F(2,57) = 35.35$, $p < 0.0001$), with MDMA treatment lowering the levels of both compared to SAL controls. Post hoc pairwise step-down comparisons also showed that the MDMA $\times$ 1 group had lower 5-HT ($p < 0.05$) and 5-HIAA ($p < 0.05$) levels compared to the MDMA $\times$ 5 group.

In the neostriatum (Figure 6C), there was a main effect of treatment for 5-HT ($F(2,57) = 2.82$, $p < 0.05$) and 5-HIAA levels ($F(2,57) = 6.72$, $p < 0.01$). Post hoc pairwise comparisons showed that both 5-HT ($p < 0.05$) and 5-HIAA ($p < 0.01$) levels were lower in MDMA $\times$ 1 compared to SAL animals while no differences were seen in the MDMA $\times$ 5 compared to SAL animals. Neostriatal dopamine levels were also significantly lower in MDMA-treated animals, regardless of regimen, compared to SAL-treated animals ($F(2,57) = 4.48$, $p < 0.02$). For DOPAC, there were decreased levels in the neostriatum in the MDMA $\times$ 1 group compared to the SAL group ($F(2,57) = 2.59$, $p < 0.04$).

Discussion

It was previously shown that acute MDMA treatment on a single day leads to deficits in path integration or egocentric learning in the CWM (Able et al., 2006). Accordingly, the first purpose of the present experiment was to determine if a repeated exposure model would lead to similar or more severe path integration effects. The second purpose was to determine the short-term effects of an acute MDMA treatment regimen on other behaviors; therefore behavioral testing began 5 days after the last MDMA treatment. It was recognized that investigation of the long-term effects of MDMA would also be worthwhile and it has been shown that MDMA administration to neonates impairs learning and memory up to 1 year following treatment (Skelton et al., 2006). Moreover, in adult animals MDMA treatment has been shown to cause lasting behavioral changes that are also evident several months after drug

treatment (McGregor et al., 2003). In the present study, MDMA treatment once a week for five weeks led to CWM performance deficits as evidenced by longer latencies and increased number of errors and it was demonstrated that a single day treatment with MDMA also produces CWM deficits, thereby replicating prior findings (Able et al., 2006). Interestingly, no differences in learning were observed between the MDMA $\times$ 1 and MDMA $\times$ 5 groups, suggesting that a single, acute exposure to MDMA is as detrimental as multiple exposures over a longer period of time. Another substituted amphetamine, fenfluramine, produces similar deficits in the CWM following a single day of exposure (Skelton et al., 2004; Williams et al., 2002a). This effect of MDMA is more pronounced for learning in the CWM compared to learning in the MWM. For example, previously it was shown that MDMA induced deficits in spatial memory (Able et al., 2006; Sprague et al., 2003), a finding not obtained in the present study. However, in this study MDMA-treated animals did show difficulty locating the platform when the platform size was reduced by 75% as demonstrated by the increased path lengths during the reduced platform trials. It is possible that the training in the CWM had a transfer effect on the performance of the animals in the MWM, as it has been shown that non-spatial pre-training can ameliorate some types of deficits in the MWM (Cain, 1997), but not all (Williams et al., 2002b). It may therefore be beneficial to examine the effects of test order on animals exposed to MDMA, although for fenfluramine, test order has been shown to have no effect (Skelton et al., 2004; Williams et al., 2002a). Or it may be that the reduced platform trials represent a reference memory impairment because this phase of the test places a premium on remembering the location of the platform more precisely than the other phases since the platform is 75% smaller than the one used during acquisition or reversal.

The CWM procedure has recently been modified so that the maze is run in complete darkness instead of with a 15 W red light as used for this study. The use of visible red light presumably added a spatial component to the task; however, with the MDMA-treated animals displaying more subtle spatial learning deficits, it is unlikely that the effect was due to poorer spatial navigation. When the CWM is run under infrared light, the animals do not have access to distal, spatial cues, and therefore must rely upon self-direction cues or what is commonly referred to as egocentric or path integration learning. When the CWM is run under infrared lighting, methamphetamine neurotoxicity induces deficits in the CWM (Herring et al., 2008). The neuroanatomical origin of path integration learning is not completely understood, although it appears to depend in part upon the entorhinal cortex (Moser et al., 2008). Other areas are also likely to be important for this type of learning. For instance, the mammillary bodies and dorsal tegmental nucleus may form a loop in which head direction cells, a major component of path integration learning, play an important role (Song and Wang, 2005; Sharp et al., 2001; Frohardt et al., 2006). Other regions that have been suggested to play a role in path integration learning include the medial prefrontal cortex, anterior thalamus, hippocampus, and striatum (Potegal, 1972; Cook and Kesner, 1988; Blair and Sharp, 1996; Wolbers et al., 2007). Monoamines, namely 5-HT and 5-HIAA, were decreased in the prefrontal cortex and neostriatum in this study, although the effect on the entorhinal cortex was not examined. After the present experiment was conducted, MA treatment has been found to reduce 5-HT levels in the entorhinal cortex (unpublished observation).

The only measure in which multiple weekly treatments with MDMA had a unique effect compared to the MDMA $\times$ 1 group was in the elevated zero maze. MDMA exposure over several weeks produced an anxiolytic effect, while a single exposure to MDMA produced no anxiety differences relative to SAL-treated animals. An anxiolytic effect was also observed in adolescent rats treated over multiple weeks with MDMA (Piper and Meyer, 2004). However, there was no effect of MDMA treatment in a test of defensive behavior, marble burying, or in the locomotor chambers where animals, regardless of treatment, spent similar times in the center region, which is generally regarded as another index of anxiety. These data suggest that the elevated zero maze and marble burying tests may measure different aspects of anxiety.

Alternatively, it is possible that the effects seen in the elevated zero maze may be related to the initial hypoactivity seen in MDMA-treated animals, however, this is unlikely due to the lack of effect in the MDMA×1 group, which was also hypoactive during locomotor activity testing but showed no changes in the elevated zero maze. Further, if hypoactivity was a factor then one might expect decreased time in the open rather than increased time, since the animals are first placed in the closed quadrant. In addition, the MDMA×5 animals, while hypoactive, spent a similar amount of time in the center of the locomotor chambers compared to SAL-treated animals, suggesting that hypoactivity alone did not account for the difference in anxiety levels in the elevated zero maze. It has been suggested that increased time in the open arms of the elevated plus-maze, which is similar in function to the elevated zero maze, reflects increased impulsiveness rather than decreased anxiety (Harro, 2002). It has also been shown that there is an increase in impulsiveness in MDMA users compared to drug naïve humans (Morgan, 1998). However, a recent study has shown that MDMA administration in rats on a single day did not increase premature lever pressing in an operant task (Saadat et al., 2006), suggesting that MDMA administration in rats does not increase impulsive behavior under schedule-controlled conditions.

The hypoactivity in the initial test of spontaneous locomotion seen in the MDMA-treated groups was not present during the re-habituation period prior to the MA challenge test. This was probably attributable to familiarization to the testing environment and recovery from the short-term effects of the drug. The hyperactivity seen in both MDMA-treated groups following MA challenge suggests an alteration in dopaminergic and/or serotonergic function, as both have been implicated in the control of locomotor activity (Viggiano et al., 2003; Leussis and Bolivar, 2006). The decrease of both neurotransmitters in the neostriatum suggests that the alterations may be related to sensitization of the postsynaptic receptors for these systems, although the effect seen here with higher doses of MDMA does not appear as large as those induced by typical intermittent drug sensitization regimens.

Interestingly, the hyperthermia induced by MDMA treatment had a more rapid onset after the first week of MDMA exposure than when animals were naïve to the effects of MDMA. This suggests a subtle sensitization to MDMA and/or some stress-related effect in these animals, since the MDMA×1 group also showed a more rapid increase in temperatures than animals exposed to MDMA on week 1 (c.f., Figure 1A and Figure 1E). It would be interesting to observe if measures of stereotypy or locomotor activity increased following this dosing regimen as an additional confirmation of sensitization. Following a binge of MDMA (7.5 mg/kg 3 times and 2 h apart), a challenge dose of MDMA two weeks later did not alter DA release in the nucleus accumbens or the caudate nucleus however 5-HT release in these regions was reduced (Baumann et al., 2008).

While the primary focus of MDMA studies is often on serotonergic effects, recent experiments have shown that MDMA treatment also causes moderate long lasting depletions of DA (Able et al., 2006; Cohen et al., 2005; McGregor et al., 2003). The changes in DA appear delayed, as no effect of MDMA on DA levels 7 days after treatment (Green et al., 2003) have been reported, whereas in the aforementioned experiments, DA decreases became apparent following a prolonged period of drug abstinence (weeks after initial exposure).

In the MDMA×5 group, 5-HT levels were higher than in the MDMA×1 group in all brain regions examined. This increase in 5-HT is most likely due to partial recovery of the 5-HT system after the initial MDMA insult. This explanation is plausible since a binge dose of MDMA results in a decrease in 5-HT release to subsequent MDMA exposure (Baumann et al., 2008), which likely reduces the efficacy of subsequent MDMA treatments. While the diminished 5-HT release may not directly affect 5-HT levels, it is likely indicative of changes in neuronal response to subsequent doses of MDMA. It may be that the MA challenge

administered to the rats prior to tissue collection in this study also had an effect on the neurotransmitter reductions observed. However, similar reductions in neurotransmitter levels were observed in the previous study when no challenge was administered (Able et al., 2006). In combination, these findings suggest that low dose MA challenge does not alter dopamine levels.

While behaviorally relevant doses may be an appropriate means of determining drug dose, this is not how human users take these drugs (Parrott et al., 1998). On the contrary, many of these drugs are used in binges in order to maintain or reinstate a 'high.' When administering psychostimulants to animals, several factors should be considered to better model human exposure. For example, one must consider the amount of MDMA obtained in a single use (tablet), the number of tablets taken on any given occasion, the frequency of use per day, and ADME (absorption, disposition, metabolism, and excretion). Comparison of drug effects could be made on a strict weight-adjusted (mg/kg) basis, as has been suggested (Baumann et al., 2007), however this may underestimate the bioavailability of the drug. Self-administration methods could be used to determine dose, but these methods do not always mimic the human condition accurately. For instance, with alcohol, animals will not self-administer high levels of alcohol, which is quite different than humans (Cunningham et al., 2000). Heroin self-administration in rats can go as high as 3 mg/kg (Dai et al., 1989), well above the range of most human heroin users (0.06–0.3 mg/kg) yet less than what heavy users take (6 mg/kg) (Uchtenhagen et al., 1999). Also, it should be noted that while self-administration studies examine the dose at which the drug becomes sufficiently rewarding to reinforce responding, it is not a model of chronic abuse. Another approach is the use of scaling models that take into account differences in body size, disposition, excretion, metabolic rate, and related factors (Lin, 1998). If one uses such an interspecies scaling formula: $\text{Dose}_{\text{human}} = \text{Dose}_{\text{animal}} \times (\text{Weight}_{\text{human}}/\text{Weight}_{\text{animal}})^{0.7}$ [(Mordenti and Chappell, 1989), see also (Green et al., 2003) for drugs of abuse], and assumes a 70 kg human, 300 g rat, and a human dose of 100 mg, then a rat would require an equivalent dose of 7.3 mg/kg. Some models of interspecies scaling suggest that the exponent should be 3/4 (West et al., 2002) or 2/3 (White and Seymour, 2005), which would suggest that the mg/kg basis for a rat would be between 8.5 and 5.2 mg/kg, respectively. It should be noted that others have cautioned that the predictive metabolic rate for some drugs using interspecies scaling may not always be accurate (Mahmood, 1999), although this conclusion was based on a small study rather than on a large number of species as in White and Seymour (2005) which supports this approach. It has also been suggested that since rats metabolize MDMA differently than humans, with rats producing more active metabolites than humans, that this may limit the ability of interspecies scaling to correctly model human exposure (de la Torre and Farre, 2004). Another consideration for the animal model is the level of drug in plasma (Cho et al., 2001). In adult humans, the elimination half-life of MDMA is 8–9 h (de la Torre et al., 2004), whereas in adult rats (i.v.) it is 73 min (Cho, 1990). MDMA users who attended a "rave" the previous night showed a range of plasma MDMA levels of ~0.1–0.9 mg/L up to 10 h following drug ingestion (Irvine et al., 2006). In 11 day old rats, a 20 mg/kg dose produces plasma levels of 1 mg/L after 10 h (Williams et al., 2004), suggesting a degree of comparability. It should also be noted that MDMA and MA users take these drugs over a wide range of doses, sometimes exceeding a 1000 mg/day (Cho, 1990; Derlet and Heischouer, 1990; Scholey et al., 2004; Kouimtsidis et al., 2006).

In conclusion, the present results indicate that acute (binge) doses of MDMA have the same effects on egocentric learning as multiple, weekly exposures. These results may predict one type of human cognitive deficit that should be investigated in clinical studies of current and former regular MDMA users.

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Figure 1. MDMA induced hyperthermia

Body temperatures (Mean $\pm$ SEM) during DMA administration. Animals were dosed 4 times daily every 2 h (demarcated by arrows) and temperatures were taken every half hour following the first dose. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs SAL. ### $p < 0.001$ MDMA $\times$ 1 vs MDMA $\times$ 5.

Figure 2. Increased open zone time in the elevated zero maze in animals exposed to MDMA once a week for five weeks

MDMA×5 exposure led to increases in time in open quadrants (top) and head dips (bottom left) compared to animals that were exposed to MDMA×1 or SAL, however no differences were noted in stretch attends (bottom right). $p < 0.05$.

Figure 3. MDMA-induced reduction in locomotor activity

Mean $\pm$ SEM horizontal activity (cm) measured every 5 min for 1 h. A main effect of MDMA treatment was observed in which both the MDMAx5 and MDMAx1 groups showed reduced activity compared to SAL. a. $p < 0.01$ SAL vs MDMAx1, b. $p < 0.01$ SAL vs MDMAx5

Figure 4. MDMA-induced deficits in Cincinnati water maze performance

MDMA treatment once/week for 5 weeks (MDMA×5) or saline once/week for 4 weeks and MDMA on week-5 (MDMA×1) resulted in increased latency (top) and errors (bottom) in both MDMA groups compared to the SAL group. a. $p < 0.05$ SAL vs MDMA×1, b. $p < 0.05$ SAL vs MDMA×5

Figure 5. Methamphetamine-induced exaggerated hyperactivity in MDMA-treated groups
MDMA-5 and MDMAx1 groups showed a larger increase in activity from 0–15 min following challenge with 1 mg/kg of (+)-MA than the SAL group. **p<0.01, *** p<0.001 compared to SAL.

Figure 6. MDMA-induced depletions in 5-HT and DA

Neurotransmitter levels following MDMA administration in the (A) prefrontal cortex, (B) hippocampus, and (C) neostriatum. MDMA treatment, regardless of regimen, resulted in 5-HT and 5-HIAA depletions but no changes in DA were observed in the prefrontal cortex compared to the SAL-treated animals and MDMA×1 animals had lower levels of 5-HT and 5-HIAA compared to MDMA×5 animals. In the hippocampus MDMA treatment, regardless of regimen, resulted in 5-HT and 5-HIAA depletions compared to the SAL-treated animals and MDMA×1 animals had lower levels of 5-HT and 5-HIAA compared to MDMA×5 animals. In the neostriatum, DA was reduced by MDMA treatment, regardless of regimen, but only the

MDMA×1 group caused depletions in DOPAC, 5-HT, and 5-HIAA compared to SAL-treated animals. * $p<0.05$ vs SAL; # $p<0.05$ vs MDMA×1.

Table 1

Body weights (g) prior to the first MDMA administration of the day on each of the 5 week dosing periods (top) and then weekly following the 5 week dosing periods (bottom). During the dosing period the SAL and MDMA×1 treatments were combined since they both received SAL from weeks 1–4.

During dosing period						
Group	Week 1	Week 2	Week 3	Week 4	Week 5	
SAL	315.4±4.7	355.91±4.7	390.57±4.7	423.29±4.7	442.3±4.7	
MDMA×5	297.36±6.8	331.8±6.8 [†]	358.1±6.8 ^{***}	381.27±6.8 ^{***}	394.9±6.8 ^{***}	
Following MDMA administration						
Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
SAL	473.4±9.6	484.3±9.6	497.9±9.6	514.1±9.6	532.3±9.6	549.5±9.6
MDMA×1	442.2±9.6 [*]	462.7±9.6	482.8±9.6	502.4±9.6	520.9±9.6	539.6±9.6
MDMA×5	432.2±9.8 [*]	458.8±9.8	480.9±9.8	505.5±9.8	523.5±9.8	540.9±9.8

Mean±SEM

*
p<0.05

p<0.001

[†]
p<0.10