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3,4-Methylenedioxymethamphetamine in Adult Rats Produces Deficits in Path Integration and Spatial Reference Memory

Jessica A. Able, Gary A. Gudelsky, Charles V. Vorhees, and Michael T. Williams

From the Neuroscience Graduate Program (JAA, GAG, CVV, MTW), University of Cincinnati College of Medicine; Division of Neurology (JAA, CVV, MTW), Cincinnati Children's Research Foundation; and College of Pharmacy (GAG), University of Cincinnati, Cincinnati, Ohio.

Abstract

Background— $\pm$ 3,4-Methylenedioxymethamphetamine (MDMA) is a recreational drug that causes cognitive deficits in humans. A rat model for learning and memory deficits has not been established, although some cognitive deficits have been reported.

Methods—Male Sprague-Dawley rats were treated with MDMA (15 mg/kg $\times$ 4 doses) or saline (SAL) (n = 20/treatment group) and tested in different learning paradigms: 1) path integration in the Cincinnati water maze (CWM), 2) spatial learning in the Morris water maze (MWM), and 3) novel object recognition (NOR). One week after drug administration, testing began in the CWM, then four phases of MWM, and finally NOR. Following behavioral testing, monoamine levels were assessed.

Results— $\pm$ 3,4-Methylenedioxymethamphetamine-treated rats committed more CWM errors than did SAL-treated rats. $\pm$ 3,4-Methylenedioxymethamphetamine-treated animals were further from the former platform position during each 30-second MWM probe trial but showed no differences during learning trials with the platform present. There were no group differences in NOR. $\pm$ 3,4-Methylenedioxymethamphetamine depleted serotonin in all brain regions and dopamine in the striatum.

Conclusions— $\pm$ 3,4-Methylenedioxymethamphetamine produced MWM reference memory deficits even after complex learning in the CWM, where deficits in path integration learning occurred. Assessment of path integration may provide a sensitive index of MDMA-induced learning deficits.

Keywords

MDMA; Morris water maze; Cincinnati water maze; sequential learning; spatial learning

The compound $\pm$ 3,4-methylenedioxymethamphetamine (MDMA) is a drug of abuse that is widely used throughout the world and is frequently used in the context of night-clubs and raves. Fourteen percent of 19- to 30-year-olds in the United States are reported to have tried MDMA in their lifetime (Johnston et al 2003). In the United Kingdom, it is estimated that 500,000 young adults use MDMA every week (Green 2004), and this is especially problematic, since the health and cognitive risks associated with MDMA have not yet been fully elucidated. It is known that MDMA depletes serotonin (5-HT) in the brains of humans, nonhuman primates, and rats (Green et al 2003). Concurrent with the 5-HT depletions, humans and nonhuman

primates demonstrate cognitive deficits (Morgan 1999; Taffe et al 2001). For humans, these impairments involve a wide range of cognitive functions that include difficulties in verbal, prospective, and working memory (Bhattachary and Powell 2001; Gouzoulis-Mayfrank et al 2000, 2003; McCardle et al 2004; Reneman et al 2000; Thomasius et al 2003; Verkes et al 2001) and central executive and decision-making skills (Heffernan et al 2001). Interestingly, these deficits do not disappear with sustained abstinence (Morgan et al 2002). A confound of human research is that most users of MDMA use other drugs as well. However, among chronic polydrug users of MDMA, a predictor of working memory and abstract reasoning deficiencies is use of MDMA rather than the other drugs (Verdejo-Garcia et al 2005). In contrast to the human data, MDMA administration to rats has not produced a clear set of cognitive changes.

There have been a number of behavioral measures performed in rats to assess cognitive ability following MDMA. For example, rats previously administered MDMA have problems forming a conditioned place preference for ethanol (Cole et al 2003). $\pm 3,4$ -Methylenedioxymethamphetamine-treated animals show impairment in active avoidance learning (Ho et al 2004), demonstrate problems with novel object recognition (NOR) with a 15-minute retention delay (Morley et al 2001), and have deficits on spatial memory trials but not on learning trials in the Morris water maze (MWM) (Sprague et al 2003). Contrary to the aforementioned findings, no change in passive or active avoidance learning following MDMA was noted (Timar et al 2003), and Morley et al (2001) showed no NOR deficits when the retention delay interval was 60 minutes. These findings are difficult to interpret because of the use of different rat strains and doses. For example, Morley et al (2001) administered 4×5 mg/kg over 2 days in Wistar rats as opposed to our dose of 4×15 mg/kg given to Sprague-Dawley rats on 1 day. Therefore, the consistency of these effects remains uncertain.

Morris water maze testing is frequently used to test spatial memory and has been a very effective assay in screening for hippocampal damage (Morris et al 1982). Sprague et al (2003) used the MWM paradigm to examine spatial deficits in rats 7 days after administration of MDMA. They found small differences on probe trial performance, while learning was similar between MDMA-treated and control animals. These results suggest that MDMA-treated animals forget the platform position more rapidly than the control animals. The Cincinnati water maze (CWM), a test of path integration learning, has proved useful in revealing memory deficits in rats administered fenfluramine (FEN), another amphetamine analog and 5-HT releasing drug (Morford et al 2002; Williams et al 2002; Skelton et al 2004), whereas no differences were noted during the learning or the reference memory (probe) phases of the MWM after fenfluramine treatment. Based on these findings, the CWM may be a useful test for one type of memory impairment following MDMA administration.

The first goal of this study was to use the CWM to test for cognitive deficits in rats given a known 5-HT-depleting regimen of MDMA and compare the results to performance on the MWM and NOR. The NOR was included based on the data of Morley et al (2001); however, a different procedure was implemented here in which animals were equated for total time attending to each of the test objects (Clark et al 2000). This was done to ensure that drug-induced changes in attending time did not influence the assessment of recognition memory.

Methods and Materials

Subjects

Male Sprague-Dawley rats (225–250 g) were obtained from Charles River Laboratories (Raleigh, North Carolina). The rats were allowed to acclimate to the colony room for 1 week prior to the day of drug administration. The colony room was maintained at a temperature of 21°C to 22°C with food and water available ad libitum. The animals were initially housed in pairs in cages measuring $45.7 \times 23.8 \times 20.3$ cm prior to drug administration, then singly during

and following drug administration. For the duration of the dosing period, animals were maintained in smaller $27.9 \times 16.5 \times 12.1$ cm polycarbonate cages in a room outside of the home suite at an ambient temperature of $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The Cincinnati Children's Research Foundation's Institutional Animal Care and Use Committee approved the research protocol under which this experiment was conducted. The vivarium was accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

MDMA Administration

$\pm 3,4$ -Methylenedioxymethamphetamine HCl (expressed as the freebase and obtained from the National Institute on Drug Abuse through its provider, Research Triangle Institute, Research Triangle Park, North Carolina) or the vehicle, isotonic saline (SAL), was administered to animals randomly assigned to treatment groups once every 2 hours for a total of four doses on a single day. Drug was given subcutaneously in the dorsum and the site of injection was varied to prevent irritation to the skin. Forty animals were assigned to one of two treatment groups, either 15 mg/kg MDMA or SAL ($n = 20/\text{treatment}$). The experiment was performed with two cohorts of 20 animals; each with 10 MDMA-treated and 10 SAL-treated animals.

Temperature Monitoring

Prior to MDMA treatment, the rats were implanted via injection with subcutaneous temperature transponders (IPTT-200, Biomedic Data Systems, Seaford, Delaware). This was done to address two major problems. Firstly, the subcutaneous temperature probes were implemented to alleviate the stress of rectal temperature measurements and any physical manipulation of the animal that occurs with these recordings. A multitude of studies demonstrate that standard laboratory procedures such as handling, cage changing, and temperature measurement can increase corticosterone, body temperature, and heart rate as reviewed in Balcombe et al (2004). Secondly, temperature probes were used to determine if cooling intervention was required to keep the animal's body temperature from becoming lethal (Williams et al 2002). Temperatures were taken immediately before the first injection and subsequently every 30 minutes until 4 hours after the last injection. If an animal's temperature reached or exceeded 40°C , it was placed in a holding cage containing shallow cool water until body temperature fell below 38°C . Six out of 20 MDMA-treated animals required cooling during the dosing period. Cooling was utilized to ensure that animals did not die as a result of MDMA-induced hyperthermia, an important and likely biasing effect considering MDMA-related deaths in humans are uncommon (Green et al 2004).

Straight Channel

Swimming in a straight water-filled channel where almost no learning is required may be used as a general measure of swimming ability and motivation to escape from water. Here we used it as a control procedure to ensure that the treatment did not cause a motor impairment that could interfere with learning. On the third day after drug administration, the animals were tested for swimming ability in a straight water channel (Williams et al 2002). The straight channel is 244 cm long and was filled with 35 cm of room temperature water ($22^{\circ}\text{C} \pm 1^{\circ}\text{C}$). The rats were placed at one end of the channel facing the wall and allowed a maximum of 2 minutes to locate an escape ladder at the opposite end. Four consecutive timed trials were given and escape latency was recorded for each trial.

Cincinnati Water Maze

On the day following the straight channel, the animals began CWM training. The CWM was developed by Vorhees (1987) and is a modification of the Biel maze. The CWM consists of nine black acrylic T's, the long arms of which form the main channels of the maze (Figure 1). The arms of the T's and the channel are 15.2 cm wide and the walls are 50.8 cm high. The

water was 25 ± 1 cm deep and maintained at room temperature ($22^{\circ}\text{C} \pm 1^{\circ}\text{C}$). Testing was performed under red light to limit the use of “extramaze” spatial cues. To begin each trial, an animal was placed in the start position (position B as defined by Vorhees 1987) and allowed to find the escape ladder at position A (Figure 1). Two trials per day were given with a 5-minute limit per trial and a minimum 5-minute intertrial interval. Errors and latency to escape were scored for each trial. An error is defined as a whole body entry into one of the short arms of a T. The animals were tested for 6 consecutive days (i.e., 4–9 days after drug administration).

Morris Water Maze

The Morris water maze apparatus was a black stainless steel tank 210 cm in diameter. Cardinal positions of north, east, west, and south were assigned in a compass fashion to delineate four separate quadrants, and extramaze cues were placed on three walls of the room surrounding the tank. Morris water maze training began 2 days following the completion of CWM training and was performed in four phases: acquisition, acquisition probe, reversal, and reversal probe. During the acquisition phase, a 10×10 cm platform was submerged 2 cm below the water in the southwest quadrant of the maze. The platform was constructed from clear acrylic with a fiberglass screen glued to the top for traction. In each trial, the rats were placed in the maze in one of four start positions as defined previously (Williams et al 2003) and allowed a maximum of 2 minutes to find the platform. Upon reaching the platform, the rat was given a 15-second intertrial interval on the platform. If the rat failed to find the platform within 2 minutes, it was placed on the platform for 15 seconds. Four trials were administered each day for 5 days (i.e., days 12–16 after dosing). On the sixth day (day 17 after dosing), a 30-second probe trial was administered with no platform. For the reversal phase, which began the second day after the completion of acquisition, a smaller 5×5 cm platform was placed in the northeast quadrant of the tank and the converse starting positions of the acquisition phase (i.e., south, west, southeast, and northwest) were used. As during acquisition, the animals had a maximum of 2 minutes to find the platform with a 15-second interval between trials and were given four trials a day for 5 days (i.e., days 19–23 after dosing), with one 30-second probe trial on the sixth day (day 24 after dosing). A camera mounted over the center of the maze was attached to a Poly-Track video system (San Diego Instruments, San Diego, California) and the latency, path length, and first bearing were obtained from the recorded tracings of the animal. Latency to the platform, path length (cm), and first bearing were the measures quantified in both phases of the MWM training.

Novel Object Recognition

Novel object recognition testing took place on the second day following the end of the reversal phase of MWM training. Circular polyethylene arenas measuring 91 cm in diameter with 51 cm high walls were used for this test. Each animal was given 10 minutes per day for 4 days (days 26–29 after dosing) to habituate to the arena. The test arena was cleaned between animals with 70% ethanol. Novel object recognition testing took place on the fifth day (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 minutes to accumulate 30 seconds of object exploration (Clark et al 2000). Object exploration was defined as the animal standing within 1 cm and oriented toward the object. According to the Clark et al (2000) definition, exploration of the object was occurring if the animal sniffed or pawed the object; however, climbing on the object was not counted. The retention phase began 1 hour after familiarization. A new object (pink mug) was introduced in combination with a copy of the original object. As in the familiarization phase, the animals had 10 minutes to complete 30 seconds of object exploration. A video camera was placed over the testing arena, and behavior was scored using a computer program generously provided by Robert E. Clark, Ph.D. (Clark et al 2000). Time exploring the new object during the test phase was analyzed.

Neurotransmitters and Tissue Collection

To verify the 5-HT depletion usually seen with MDMA treatment, monoamine levels were measured in selected brain regions. Briefly, 3 days after completion of novel object recognition (35 days after drug), the animals were killed by rapid decapitation and the brains were 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 high-pressure liquid chromatography (HPLC).

For HPLC procedure, each tissue sample was weighed, homogenized in 50 volumes of .2 N perchloric acid buffer, and centrifuged at $10,000 \times g$ for 5 minutes. Twenty-microliter aliquots of each sample were injected in a C18 column ($5 \mu\text{m}$, $100 \times 2 \text{ mm}$) connected to an LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, Indiana) with a reference electrode at +.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, and 6% acetonitrile, at a pH of 4.0. The flow rate was .28 mL per minute. Chromatograms were obtained and integrated, and the neurotransmitter concentrations were calculated from standard curves generated for each neurotransmitter analyzed.

Statistics

The MWM, CWM, straight channel, and temperature data were analyzed using a mixed factor analysis of variance (ANOVA) with the SAS GLM procedure (SAS, Cary, North Carolina). Treatment was a between-subject factor, and day (MWM and CWM), trial (straight channel), and time (temperature) were within-subject factors. The Greenhouse-Geisser correction was used in instances in which the variance-covariance matrices were significantly nonspherical. The novel object and neurotransmitter data were analyzed using Student *t* tests for independent samples (two-tailed). To determine if cooling affected any of the parameters, *t* tests were used to compare the MDMA animals that were cooled with those that were not cooled. Significance was set at a level of $p < .05$, and trends were noted at a level of $p < .10$.

Results

Temperature Monitoring

Both the SAL- and MDMA-treated animals began the experiment with comparable body temperatures. There was a significant main effect of treatment, $F(1,34) = 43.11$, $p < .0001$; time, $F(20,680) = 5.89$, $p < .0001$; and the interaction of treatment $\times$ time, $F(20,680) = 15.89$, $p < .0001$. Analysis of the interaction showed that following the first injection, body temperatures of the MDMA-treated animals steadily increased, and at 90 minutes, temperatures were significantly increased relative to SAL-treated animals, $p < .05$ (Figure 2). Temperatures of the MDMA-treated animals remained elevated throughout the remainder of the dosing and temperature collection period (i.e., 4 hours after the last injection). Slight temperature dips within the MDMA-treated group occurred around 300 minutes and 450 minutes after the first dose because several animals were cooled at these times.

Straight Channel

The latency to escape did not differ significantly between treatment groups. The mean latency $\pm$ SEM across trials was $15.32 \pm .88$ seconds for SAL-treated animals and $14.29 \pm .84$ seconds for MDMA-treated animals.

Cincinnati Water Maze

For the number of errors, there was a significant effect of day, $F(5,185) = 59.80$, $p < .0001$, and an interaction of treatment $\times$ day, $F(5,185) = 2.53$, $p < .0001$. Analysis of the interaction

showed that MDMA-treated animals made more errors on days 4 and 5, $p < .05$, and a trend for more errors on day 6, $p < .10$, relative to the SAL-treated animals (Figure 3). Latencies demonstrated a similar pattern of results with a significant day effect, $p < .0001$, and a trend for the treatment $\times$ day interaction, $p < .07$ (not shown). The cooled MDMA-treated animals did not differ significantly from the noncooled MDMA-treated animals in errors or latency.

Morris Water Maze

For the MWM testing, the SAL- and MDMA-treated groups did not perform differently during the acquisition phase on first bearing, path length, or cumulative distance to the platform. Latency to the target showed a trend ($p < .08$). Both the MDMA and SAL animals learned the task as demonstrated by a significant day effect for latency, $F(1,144) = 57.71$, $p < .0001$ (Figure 4A).

During the reference memory (probe) phase of testing, the MDMA-treated animals showed a trend toward a less direct route (i.e., first bearing) to the former platform position than the SAL-treated animals, $p < .07$ (Figure 4B). $\pm 3,4$ -Methylenedioxymethamphetamine-treated animals were further from the former platform position than SAL-treated animals, as demonstrated by a significant main effect for average distance from the platform, $F(1,36) = 7.10$, $p < .01$ (Figure 4C).

In the reversal phase of MWM testing, the animals had to find a smaller platform in the opposite quadrant from acquisition training. As with the acquisition phase, no differences in learning were seen between the treatment groups in terms of the main effect of treatment or the interaction of treatment and day. Both groups learned the task, as demonstrated by the significant effect of day (latency data shown), $F(4,144) = 40.75$, $p < .0001$ (Figure 5A). During the probe trial, no difference was noted for first bearing (Figure 5B); however, for average distance from the platform, the MDMA-treated animals were further away than the SAL-treated animals, $F(1,36) = 4.64$, $p < .04$ (Figure 5C). The six cooled MDMA-treated animals did not differ from the rest of their treatment group in any of the MWM test phases.

Novel Object Testing

There was no significant effect of treatment on NOR testing. After the 1 hour intertrial interval, both groups explored the new object for a similar amount of time. The means $\pm$ SEM for new object exploration out of a possible 30 seconds were 18.34 ± 1.88 seconds for the SAL-treated animals and 17.90 ± 1.46 seconds for the MDMA-treated animals.

Neurotransmitters

Both 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus were significantly reduced in the MDMA-treated animals relative to SAL-treated animals, $t(34) = 6.67$, $p < .0001$, and $t(34) = 7.93$, $p < .0001$ (Figure 6A). Prefrontal 5-HT and 5-HIAA were reduced in the MDMA-treated animals compared with SAL-treated animals, $t_{36} = 6.91$, $p < .0001$ and $t_{36} = 4.98$, $p < .0001$, respectively (Figure 6B). There was a trend seen in prefrontal dopamine (DA) levels, with the MDMA-treated group having higher concentrations than control subjects, $p < .10$. In the striatum, 5-HT and 5-HIAA were both depleted as a result of MDMA treatment, $t_{36} = 5.49$, $P < .0001$ and $t_{36} = 3.25$, $p < .0025$, respectively. Interestingly, DA levels in the striatum were reduced in the MDMA-treated animals compared with the SAL-treated animals, $t(36) = 2.3$, $p < .03$, although no differences in 3,4-dihydroxyphenylacetic acid (DOPAC) were noted (Figure 6C). Correlations were calculated to determine the relationship between neurotransmitter depletion and behavioral deficits in the MDMA-treated animals, and no significant correlations were found. As in other measures, the six cooled MDMA-treated animals did not differ significantly from the other MDMA-treated animals in neurotransmitter levels.

Discussion

The major new finding within this set of experiments was that MDMA-treated animals committed more errors in the CWM than control subjects. Cincinnati water maze requires a path integration strategy for finding the goal, as opposed to the MWM, where an animal relies on the use of spatial cues to navigate through its environment (Morris et al 1982). Path integration involves the use of both allothetic (external) and idiothetic (internal) cues (Whishaw et al 2001). Idiothetic cues may be visual, vestibular, or proprioceptive, and they can be used by an animal for guidance during movement (Etienne and Jeffery 2004). In the CWM paradigm, we eliminated as many allothetic cues as possible by testing the animals under red light to differentiate the path integration learning strategy from that used in MWM that relies on distal cues. Brain regions used in path integration both overlap and are distinct from those used in spatial navigation. The prefrontal cortex is implicated in controlling behaviors that need to be performed sequentially, as would be the case in the CWM (Dalley et al 2004). Additionally, the fimbria-fornix has been shown to be important for exploration and foraging behavior, where an animal must remember a path to a food source then get home again (Whishaw et al 2001). The posterior parietal and retrosplenial cortices are other areas that have been shown to be involved in path integration behavior (Cooper and Mizumori 2001). The hippocampus may play a part in integrating the spatial components of an idiothetic task (Etienne and Jeffery 2004). However, at least one study has shown that hippocampally lesioned rats can perform a homing-type path integration task, indicating that the hippocampus may not be essential for this form of learning (Alyan and McNaughton 1999). Hence, most of the evidence points to cortical areas as primarily mediating path integration.

In previous experiments, we demonstrated that a 1-day, four-dose regimen of fenfluramine produces a significant increase in errors committed in the last 3 days of CWM testing, while MWM performance is spared (Williams et al 2002; Skelton et al 2004). Importantly, this deficit in the CWM was apparent regardless of whether MWM testing preceded or followed CWM testing, suggesting that times from the dose and test order were not the important factors in the CWM deficits. The current set of experiments with MDMA resulted in the same learning decrements in the CWM; however, unlike FEN, MDMA produced long-term deficits in reference memory in the MWM on probe trials. Fenfluramine is an amphetamine derivative that causes 5-HT release and inhibits reuptake, as does MDMA. It is unlikely the decreases in 5-HT alone account for the learning deficits produced by FEN, since corticosterone (CORT) and perhaps dopamine are also affected and may be important in the learning deficits (Skelton et al 2004). In our current study, it was not feasible to measure CORT in such a way that we could understand the interplay between hormone release and learning deficits caused by MDMA without disrupting the learning tests. Certainly, both drugs deplete 5-HT, but how that depletion along with CORT release might impair learning requires further investigation. A role for CORT in the present study seems possible, however, and might be a key to understanding our similar results in CWM testing with both FEN and MDMA. This is apparent since others have found that MDMA increases CORT levels for at least 18 hours after drug administration in adult animals (Nash et al 1988) and FEN also produced lasting increases in CORT. Moreover, blockade of the CORT increase in FEN-treated animals alleviated the CWM deficits; this should be examined for the MDMA effects in future experiments.

The MWM testing performed here did not bring to light any spatial deficits during acquisition or reversal learning trials between MDMA- and saline-treated rats, much like the previously mentioned FEN studies (Skelton et al 2004; Williams et al 2002). We were, however, able to replicate the MDMA-induced probe trial deficits seen by Sprague et al (2003), despite the use of a different MWM procedure. This was found even 2 and 3 weeks after drug administration. Sprague et al (2003) trained their animals over a period of 3 days, with two blocks of four trials each day. After training on the third day, the animals were given a 60-second probe trial with

the platform removed. During the probe trial, the time that each animal spent in each quadrant of the maze was measured and then a proximity score was generated. The MDMA-treated animals had a higher proximity score, indicating a greater distance from the original platform position, similar to our average distance parameter used in this study. In contrast, our training was done over 5 days, with one four-trial block administered each day. On the sixth day, the animals were given a 30-second probe trial with the platform removed. For both the acquisition and reversal phases of testing, MDMA animals had a less accurate first bearing and maintained a further distance from the platform location on probe trials. Our results verify the Sprague et al (2003) finding and extend the period of time after drug administration that the deficits are apparent.

In our NOR testing, no effect of treatment was evident, which is a result similar to what has been obtained by others. In a previous study, male Wistar rats that were given MDMA and tested in NOR showed no group differences at a 1-hour delay interval (Morley et al 2001); however, they did see a transient effect using a 15-minute delay interval. The differences between the Morley et al (2003) testing and ours include dose (4×5 mg/kg over 2 days as opposed to 4×15 mg/kg on 1 day), rat strain (Wistar as opposed to Sprague-Dawley), and method of testing NOR. Also, our testing was performed on the fourth week after the drug was administered, whereas Morley et al (2003) tested their animals 3½ months after drug treatment. Despite the variation between our two experiments, MDMA-treated animals have consistently shown no difference in recognition memory at a 1-hour delay compared with control subjects.

Interestingly, we observed a decrease in DA in the striatum. A decrease in striatal DA has been seen before in male Wistar rats that were administered MDMA (4×5 mg/kg intraperitoneal [IP] over 4 hours, over 2 consecutive days) and then subjected to behavioral testing (McGregor et al 2003). This decrease in DA in the striatum is a finding our laboratory has also seen before in animals given MDMA as neonates or adults (Cohen et al 2005). In both cases, behavioral tests were performed to assess learning and memory, and neurotransmitter levels were not assessed until the end of testing (Cohen et al 2005). Recently, however, there has been work done examining cerebral DA and its role in characteristic MDMA-induced behaviors (Colado et al 2004). Throughout most of the literature, the 5-HT depletion caused by MDMA is extensively documented, but DA is usually described as not being affected (Green 2004). Many of these studies used in vivo microdialysis to measure DA and did so in animals only hours to a few days after MDMA administration. In our experiment, the animals were given drug on one day followed by 4 weeks of behavioral testing before DA concentrations were determined. It may, therefore, be that DA shows a delayed reduction after MDMA treatment that was not seen in previous experiments.

In modeling the effects of MDMA, there is a recurring concern regarding how the dose administered to rats translates to a biologically relevant dose for humans. This issue has been addressed in a review (Green et al 2003) detailing a method of scaling dose from humans to animals (from Mordenti and Chappell 1989). The equation is $D_{\text{human}} = D_{\text{animal}} (W_{\text{human}}/W_{\text{animal}})^{-7}$, where D = dose and W = weight. Therefore, if a rat weighs approximately one third of a kilogram and a 15 mg/kg dose is administered, the actual dose is 5 mg. Using a human weight of 70 kg, the equation would be as follows, $D_{\text{human}} = 5 (70/.333)^{-7}$ or 211 mg for a 70 kg human. This is 3.02 mg/kg, slightly lower than the dose used in this study per injection. However, the amount of MDMA a human user would take is difficult to measure but is likely higher. For example, people have reported taking anywhere from one-half tablet to 10 to 25 tablets in one instance of use (Scholey et al 2004), and one tablet of ecstasy can contain anywhere from 80 to 150 mg (Green et al 2003). Pharmacokinetic differences between species are also an important consideration, since the half-life of MDMA in humans is 5 to 10 hours and in the rat about 90 minutes. To make the same amount of drug available in the system of a rat compared with a human, it would require dosing the rat more frequently with the same

level of drug taken in a single “hit.” As demonstrated for methamphetamine, which has a half-life of 70 minutes in rats opposed to 12 hours in a human, a rat would require drug administration every 15 minutes relative to human administration every 3 hours to obtain similar plasmatic levels (Cho et al 2001). Also, while humans are most likely to take the drug orally, it is known that people also utilize other routes of administration. Therefore, the dosing regimen in this study is likely within the range of human use.

±3,4-Methylenedioxymethamphetamine is reported to cause learning disturbances in human users. Human users report difficulties with memory, but it is not yet clear how memory deficits between humans and rats relate. Human users often take more than one dose of ecstasy in an episode of use and may do this on multiple occasions (Farre et al 2004). There is some indication of a memory deficit occurring in young adult rats repeatedly administered MDMA, but how this model would relate to human use remains unclear (Piper and Meyer 2004). More investigation into these effects is required. Although rodent memory tasks do not directly mimic tasks used to test human memory, the CWM has proved useful in uncovering MDMA-induced learning deficits in rats and may be analogous to path-integration tasks used in humans, such as landmark- or map-based navigation tasks (Foo et al 2005).

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Figure 1.

A diagram of the CWM with the “correct” path noted by the line from B to A. Any perseverative swimming within the short arms of the T’s was counted as an error. Latency to swim from B to A was also measured. CWM, Cincinnati water maze.

Figure 2.

Body temperature measurement during MDMA administration. MDMA increased body temperatures starting at the 2-hour time point ($p < .02$) and the temperatures remained elevated throughout the dosing period ($p < .003$). Arrows indicate MDMA administration. $*p < .01$. MDMA, $\pm$ 3,4-methylenedioxymethamphetamine.

Figure 3.

Cinnati water maze errors. MDMA-treated animals made significantly more errors on days 4 and 5 with a trend toward significance on day 6. Latency data (not shown), while similar, only showed a trend toward significance between groups. * $p < .05$, † $p < .1$. MDMA, $\pm$ 3,4-methylenedioxymethamphetamine.

Figure 4.

Morris water maze testing in the acquisition phase. **(A)** Latencies in the acquisition phase of MWM testing approached significance ($p < .1$). The probe trial data for first bearing **(B)** and average distance **(C)** are represented. $*p < .02$, $†p < .1$. MWM, Morris water maze.

Figure 5. Morris water maze testing in the reversal phase. **(A)** Latency during the reversal phase of MWM testing. In the probe trial, first bearing **(B)** and average distance from the target **(C)** are shown. Average distance was significant in the probe phase as indicated. $*p < .04$. MWM, Morris water maze.

Figure 6.

Neurotransmitter assessment. (A) In the hippocampus, the MDMA-treated group had depletions in 5-HT and 5-HIAA. $*p < .0001$. (B) In prefrontal cortex, the MDMA-treated animals had depleted 5-HT and 5-HIAA. $*p < .0001$, $†p < .1$. (C) Striatal 5-HT and 5-HIAA were depleted in the MDMA group. However, DA was also significantly depleted. $*p < .03$, $**p < .003$. Correlations were calculated to determine the relationship between neurotransmitter depletion and behavioral deficits in the MDMA-treated animals, and none were found. MDMA, $\pm$ 3,4-methylenedioxymethamphetamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.