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

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Background: *±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 x 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: *±3,4-Methylenedioxymethamphetamine-treated rats committed more CWM errors than did SAL-treated rats. ±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. ±3,4-Methylenedioxymethamphetamine depleted serotonin in all brain regions and dopamine in the striatum.*

Conclusions: *±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.*

Key Words: MDMA, Morris water maze, Cincinnati water maze, sequential learning, spatial learning

The compound ±3,4-methylenedioxymethamphetamine (MDMA) is a drug of abuse that is widely used throughout the world and is frequently used in the context of nightclubs 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). ±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 at (2001) administered 4 x 5 mg/kg over 2 days in Wistar rats as opposed to our dose of 4 x 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.

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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 x 23.8 x 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 x 16.5 x 12.1 cm polycarbonate cages in a room outside of the home suite at an ambient temperature of 22°C ± 1°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

±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/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°C ± 1°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°C ± 1°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

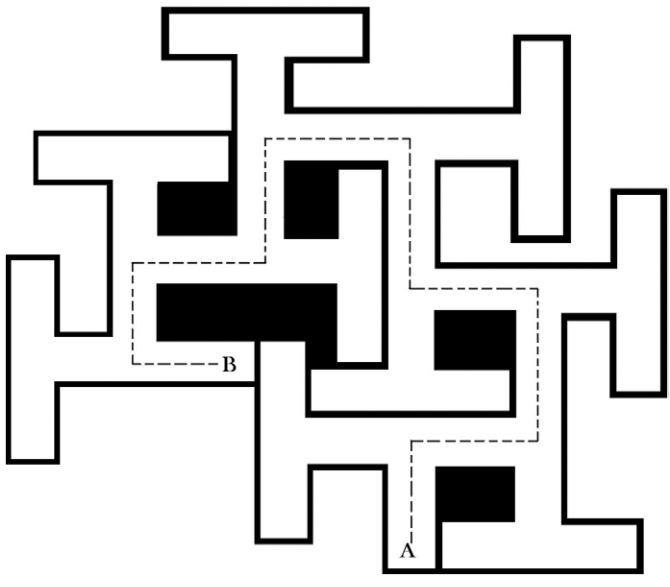

Figure 1. A diagram of the CWM with the "correct" path noted by the line from B to A. Any perseverative swimming within the short arms of the T's was counted as an error. Latency to swim from B to A was also measured. CWM, Cincinnati water maze.