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Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning

Jon E. Sprague[a,*], Amanda S. Preston[a], Michael Leifheit[a], Brian Woodside[b]

[a]*Department of Pharmaceutical and Biomedical Sciences, Raabe College of Pharmacy, Ohio Northern University, Ada, OH 45810, USA*
[b]*Psychology Department, Bowling Green State University, Bowling Green, OH 43403, USA*

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA) use has been associated with a decline in various aspects of mnemonic function in humans. We therefore postulated that MDMA-induced damage of serotonergic nerve terminals would alter hippocampal processing. Seven days following treatment with MDMA (2 × 20 mg/kg sc, given 12 h apart), rat spatial learning and memory were tested utilizing the Morris water maze (MWM). No statistical differences were found in MWM platform aquisition latency or pathlength between controls and MDMA-treated animals. Probe trials revealed significantly higher proximity score averages and significantly reduced preference for the target quadrant in the MDMA-treated animals. MDMA treatment resulted in significant reduction (34%) in hippocampal serotonin (5-HT) levels 14 days after initial treatment. The findings of this study demonstrate that hippocampal serotonergic lesions induced by MDMA may be ostensibly linked to a reference memory deficit in rats tested with the MWM.

Keywords: Morris water maze; MDMA; Ecstasy; Spatial learning; Memory

1. Introduction

MDMA has become a highly abused ‘‘recreational’’ drug among the current generation of teenagers and young adults in the United States and Europe. The Monitoring the Future Study has shown that 11% of U.S. high school seniors and 7.3% of 10th graders have used MDMA. An even more staggering statistic is that 4.3% of U.S. 8th graders have experimented with MDMA [1]. Although these numbers are still less than alcohol, nicotine or cannabis use, widespread concern has developed due to the psychiatric changes that often occur upon discontinuation of MDMA use which primarily includes memory loss [2].

Several human studies have shown that MDMA impairs episodic memory such as immediate and delayed verbal recall [2–4]. Recently, basic level cognitive function aberrations have been associated with MDMA use. Wareing et al. [5] reported that MDMA users were able to process information as quickly as nonusers but less accurately. Prospective long-term memory deficits have also been reported in MDMA users and appear to be associated with storage and retrieval difficulties [6,7]. These memory effects have also been ostensibly linked to MDMA-induced serotonergic neurotoxicity [4,8,9]. However, the ability to ascertain the contributions of the MDMA-induced serotonergic neurotoxicity to the memory deficiency seen is difficult in human studies. Differences in dose, polydrug use and the subjective nature of these human memory tests confound the correlation of MDMA-induced neurotoxicity to its memory effects.

Nonhuman primate and rodent studies have yielded a vast array of responses. Rhesus monkeys treated with neurotoxic doses of MDMA have been shown to have persistent alterations in late peak latencies of brainstem auditory evoked potentials [10]. Other nonhuman primate studies suggest no change in rate or error in repeated acquisition tasks [11,12] or a battery of operant tasks [13] following varying dosing regimens of MDMA. Rodent studies have also generated mixed results. Lever-press responding for a water reward was shown to be reduced after high-dose MDMA suggesting impairment of acquisition learning [14]. Prenatal exposure results in spatial learning and memory impairment that is only associated

* Corresponding author. Tel.: +1-419-772-2296; fax: +1-419-772-1917.
E-mail address: j-sprague@onu.edu (J.E. Sprague).

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with the gestational timing of the drug administration [15]. Parallel to the nonhuman primate studies, other rodent studies would suggest no effect on learning or memory [16]. Again, ascertaining the contribution of MDMA-induced serotonergic neurotoxicity to these memory effects or lack thereof has proven to be difficult.

Spatial learning and memory can be tested utilizing a Morris water maze (MWM), an instrument that has been developed over the past 21 years specifically to investigate this type of mnemonic function in laboratory animals [17]. Various brain regions, including the striatum [18], basal forebrain [19–21], cerebellum [22], and frontal cortex [23] are required to navigate through the MWM; however, the MWM is especially sensitive to lesions of the hippocampus, the primary brain region believed to be involved in spatial learning and memory processing [17,24].

The purpose of the current study was to examine the effects of hippocampal serotonergic neurotoxicity induced by MDMA on spatial learning. The findings from this spatial learning and memory study would surely assist in determining the mediators of the functional consequences induced by MDMA.

2. Materials and methods

The present study was carried out in accordance with protocols approved by the Ohio Northern University Animal Care and Use Committee.

2.1. Animals

Twenty-three adult, male, Sprague–Dawley (SD) rats (Harlan Sprague–Dawley, Indianapolis) weighing 200–250 g were used. All animals were group housed (three per cage) and given ad libitum access to food and drinking water. Housing conditions were maintained at a constant temperature of 23 °C and a 12:12 light–dark cycle.

2.2. Drugs and chemicals

(±)-MDMA hydrochloride was generously provided by Dr. David E. Nichols (Purdue University, West Lafayette, IN). All other reagents were purchased from Sigma (St. Louis, MO).

2.3. Treatment groups and drug administration

MDMA (2×20 mg/kg sc, given 12 h apart—$n = 11$) or saline ($n = 12$) was administered 7 days prior to memory testing. The MDMA treatment group only contained 11 animals because 1 animal expired immediately after the first injection. The 7-day delay between MDMA administration and MWM testing ensured that serotonergic neurotoxicity would be present while the acute effects of MDMA would not be a factor [25].

2.4. Morris water maze

The behavioral training and testing was conducted in a circular pool (diameter 180 cm, depth 60 cm) painted white and filled with water that was rendered opaque with condensed milk and maintained at a temperature of 27 ± 2 °C. Numerous visual cues were present around the room and remained constant during the length of the experiment. A circular, clear escape platform (diameter 12 cm) was placed 2 cm below the water surface and centered in a quadrant arbitrarily defined as ‘‘Northeast’’. The position of the escape platform remained consistent for all animals across all training trials.

The rats were trained in four groups with each group consisting of a balanced number of animals from the saline and MDMA treatment groups. During training, each animal was started from one of four equally spaced compass-point positions (north, south, east, or west) at the inside perimeter of the tank facing out toward the wall of the tank. Subjects were allowed to swim until they located the hidden platform, or until 90 s had elapsed, at which time data collection ended and the animals, if necessary, were placed on the hidden platform. The animals were left on the platform for 15 s after the end of each trial, then removed from the pool, towel dried and returned to their cage. Once every animal had run through the maze from each of the four starting positions, one trial block was complete. Training consisted of two trial blocks per day. All groups were trained between 6 a.m. and 12 noon for 3 days, for a total of six trial blocks over three training days.

Installed above the pool was a video camera connected to a tracking system (Videomex-V Monitoring System with Watermaze software; Columbus Instruments, Columbus, OH). The system creates a map of the pool in which it is divided into three concentric rings labeled A, B, and C from the center to the edge, respectively, and further divided into four equal quadrants. The escape platform was located in quadrant 1 (our ‘‘Northeast’’); ring B (see Fig. 3). The tracking system then calculates a number of parameters including swimming time in seconds to the platform (latency), swimming distance in centimeters prior to reaching the platform (pathlength), and a proximity score. The proximity score is a composite score computed by the software program from several different escape measures. It is designed to compensate for the discrepancies found between latency and pathlength when an animal swims slowly but straight to the platform, or swims rapidly but on an indirect path. It does this by calculating a correction factor based on an ideal straight-line path to the target at a steady swim speed, and then subtracting this factor from the animal’s actual path. When examining this measure, it should be noted that it is a computed number ‘‘score’’ and not directly tied to a particular physical measurement. A lower proximity score represents better performance, and negative scores are possible (and better) scores (for a more detailed explanation, see Ref. [26]).

At the end of the third day of training, a probe trial was preformed in which the escape platform was removed from the pool. Each animal was placed in the pool, facing the outside wall, approximately opposite to the quadrant that had contained the escape platform during the training trials, and was allowed to swim for 60 s. For these probe trials, swimming time spent in each quadrant was recorded, and a proximity score was computed for each animal.

2.5. Neurotoxicity analysis

Fourteen days after initial treatment, animals were sacrificed and the brains dissected out on ice. The hippocampus was removed from both hemispheres and immediately frozen in liquid nitrogen. The samples were stored at − 80 °C until the samples could be analyzed using HPLC technology.

2.6. Tissue preparation

Tissue for analysis was placed into a microcentrifuge tube and was homogenized for 15 s in 100 μl of the aqueous portion of the mobile phase using a Fisher Scientific Sonic Desmembranator on a setting of four. The homogenate was then centrifuged for 12 min at 14,000 × g at 4 °C. The supernatant (50 μl) was injected into the HPLC column.

2.7. HPLC assessment of 5-HT

A modified procedure of Chapin et al. [27] was used to determine 5-HT levels in 50 μl samples. The aqueous portion of the mobile phase consisted of 0.05 M sodium phosphate, 0.03 M citric acid buffer, 0.1 mM disodium ethylenediamine-tetraacetate (EDTA), and 0.042% sodium octyl sulfate (SOS) dissolved in HPLC grade water. The pH of the aqueous portion of the mobile phase was in the range of 2.7 to 2.9. The mobile phase consisted of 80% aqueous phase and 20% methanol. A Waters HPLC (Model 600) with electrochemical detection (Waters 464) was used. The column was a C_{18} reverse phase analytical column (4 μm spheres; 3.9 × 300 mm; Waters). The electrode was set at +750 mV, and the flow rate was set at 0.7 ml/min. Millennium software was used to integrate and analyze the raw data for the determination of 5-HT levels.

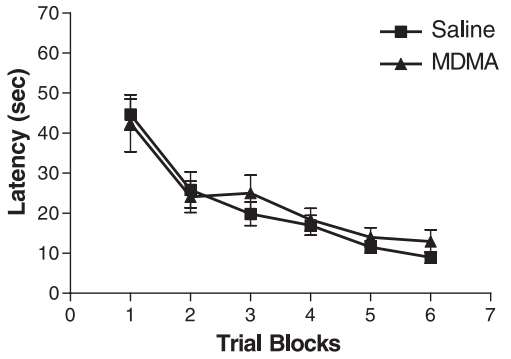

Fig. 1. MWM average latency (s) to escape platform 7 days after saline or MDMA (2 × 20 mg/kg sc, given 12 h apart). Each value is the mean ± S.E.M. (n = 11 or 12).

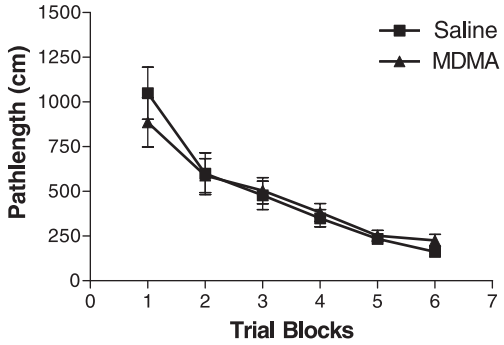

Fig. 2. MWM average distance (cm) swam to escape platform 7 days after saline or MDMA (2 × 20 mg/kg sc, given 12 h apart). Each value is the mean ± S.E.M. (n = 11 or 12).

2.8. Statistics

The results are presented as the mean of the latency or pathlength, respectively, for each of the treatment groups ± S.E.M in the MWM data. For the neurotoxicity data, tissue

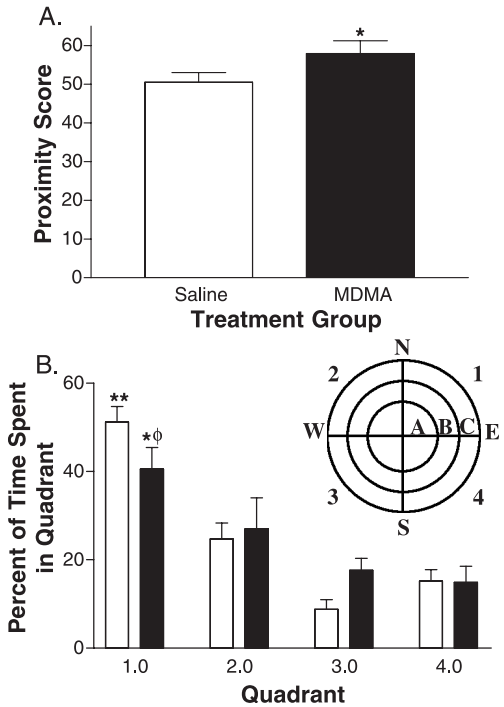

Fig. 3. MWM Probe trial proximity score averages (A) and percent of total time spent in quadrant (B) where the platform was previously located. Each value is the mean ± S.E.M. (n = 11 or 12). The platform's original location was quadrant 1; ring B. * Significantly different from corresponding saline control (P < .05). * * Saline (open bars) quadrant 1 is significantly different from saline treatment time spent in all other quadrants (P < .05). $^{\Phi}$MDMA (solid bars) quadrant 1 is significantly different from MDMA quadrants 3 and 4 (P < .05).

contents of the treatment groups are also presented as the mean ± S.E.M. Latency (s) and pathlength (cm) were analyzed using a repeated-measures ANOVA to determine statistical significance among treatment groups for the training trials. For the probe trial, a one-way ANOVA for quadrant was used to determine significance for quadrant preference of each treatment group. A Student–Newman–Keuls (SNK) post hoc test was then performed if statistical significance was determined. Swimming time in the B ring and proximity score data for the probe trial, and neurotoxicity data were analyzed using Student's *t* test. Significance was reported if $P < .05$.

3. Results

3.1. Effect of MDMA on latency in the MWM

An overall decrease in latency was seen from block 1 to block 6 in both treatment groups, however, no significant differences were found between treatment groups. No significant interaction of MDMA treatment and MWM latency occurred (Fig. 1).

3.2. Effect of MDMA on pathlength in the MWM

Both treatment groups demonstrated a decrease in swimming distance to the escape platform across blocks 1–6 (Fig. 2). In accordance with the latency data, no significant differences in pathlength were seen between those animals treated with MDMA and those treated with saline.

3.3. Effect of MDMA on probe trial performance

MDMA treatment resulted in a slight, albeit significant ($P < .05$) increase in proximity score averages (Fig. 3A). MDMA-treated animals also demonstrated no significant preference for the original escape quadrant, while saline animals spent significantly ($P < .05$) more time in the target quadrant than the other quadrants during their probe trial (Fig. 3B). There was no significant difference between groups for the time spent swimming in the B ring (data not shown).

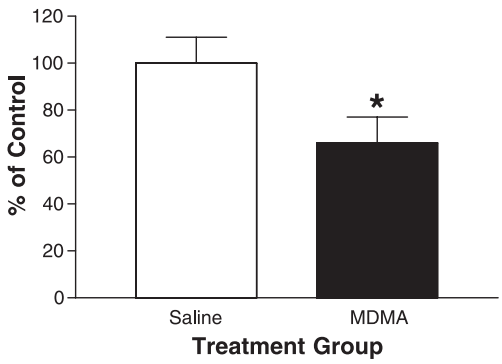

Fig. 4. Effects of MDMA (2 × 20 mg/kg sc, given 12 h apart) on hippocampal 5-HT levels 14 days after initial treatment. Each value is the mean ± S.E.M. ($n = 11$ or 12). * Significance from saline control ($P < .02$).

3.4. Effects of MDMA on hippocampal 5-HT levels

MDMA treatment resulted in a significant ($P < .05$) reduction (34%) in hippocampal 5-HT levels 14 days after initial treatment (Fig. 4).

4. Discussion

The results of the present study suggest that hippocampal serotonergic neurotoxicity induced by MDMA may contribute to a reference memory deficit in the MWM. MDMA treatment resulted in a 34% reduction in hippocampal 5-HT levels (Fig. 4) that corresponded to a significant impairment in recall of spatial information in the MWM probe trials (Fig. 3).

The training data alone (Figs. 1 and 2) would suggest that MDMA did not impair acquisition of the MWM task. In the probe trials, however, where there was no platform, a significant difference in retention of spatial information was revealed by quadrant preference analysis [17] and proximity scores [26], which are more sensitive measures of spatial learning. Saline animals demonstrated a significant preference for the target quadrant, while MDMA animals did not (Fig. 3). Control animals also demonstrated significantly lower proximity scores than the MDMA group, indicating better retention of the former spatial location of the escape platform.

A number of previous studies have demonstrated that spatial or nonspatial pretraining can ameliorate the effects of drugs that block spatial learning in the MWM [28–32]. Specifically, deficits in behaviors such as swimming to the platform, climbing on the platform, and also incidences of swimming over the platform, were correlated with acquisition impairments caused by NMDA or cholinergic receptor antagonists. Nonspatial pretraining, that is training in a tank with spatial cues blocked by a curtain, or spatial pretraining in a different environment, were shown to partially or completely block spatial learning deficits in subsequent acquisition trials, depending on the specific training paradigm [28–32].

In this study, animals from both groups were observed to demonstrate no difficulty in climbing on and remaining on the hidden platform. Also, there were no significant acquisition differences between groups as demonstrated by path length and latency scores (Figs. 1 and 2). Therefore, no apparent sensorimotor impairments contributed to the spatial memory deficits present in the probe trials; however, future research employing nonspatial and spatial pretraining must be performed to draw a definitive conclusion about possible effects of MDMA exposure on sensorimotor and nonspatial behaviors.

Training in this task allows animals to find the escape platform using a taxon strategy without necessarily acquiring spatial information [17,30,33,34]. That is, with the platform in place, animals can learn to escape by swimming in a circle a specific distance from the wall until they bump into the platform. Thus, the animals have learned a procedural process based on a taxon strategy that allows them to find the platform without utilizing spatial cues. This difference in strategy is evident in our probe trial results (Fig. 3). Animals treated with MDMA showed no significant difference from controls in time spent swimming in the B ring, in fact, MDMA animals actually spent more of their time swimming in the B ring (39%) than controls (35%). In contrast to this, though, control animals demonstrated a significant preference for the target quadrant and significantly lower proximity scores than MDMA animals. These data suggest that control animals either displayed better retention for, or relied more on, spatial information to search for the escape platform than did the MDMA group. Use of spatial information has been shown to depend on a functional hippocampus [17,24,35], and the results from this study that showed a 34% reduction in 5-HT levels in the hippocampus of the MDMA group suggest that this neurotoxicity could be responsible for the differences in probe trial performance.

Consistent with our findings, neonatal rats treated with neurotoxic doses of MDMA show differential learning and memory responses in the MWM depending on the stage of brain development [15]. MDMA exposure on Days 11–20 (analogous to late human third trimester brain development) resulted in impairment of spatial learning and memory, whereas exposure on Days 1–10 (analogous to early human third trimester brain development) had no effect on spatial learning and memory [15]. However, the finding of Broening et al. [15] did not correspond to any neurochemical changes. This lack of a neurochemical effect may be the result of an undeveloped dopaminergic system [36]. Rat pups less than 28 days old with undeveloped dopaminergic systems do not exhibit MDMA-induced neurotoxicity; at 35 days, however, the dopaminergic system is developed and the neurotoxicity occurs [37]. The results of the present study are in contrast to those of Ricaurte et al. [16] who showed no change in choice accuracy of a spatial alternation T-maze task after neurotoxic doses of MDMA. The T-maze differs from the MWM in that T-maze is more a test of procedural learning [38] whereas the MWM is more a test of spatial learning [17]. Thus, our results suggest that neurotoxic doses of MDMA can indeed impair recall of spatial information in the MWM.

Another possible explanation for the probe trial differences in our MDMA-treated animals is that neurotoxic doses of MDMA have been shown to induce anxiety [39,40]. Gurtman et al. [39] showed that MDMA induced a reduction in 5-HT levels in the amygdala, hippocampus, and striatum that corresponded to increased anxiety on the elevated plus-maze. Thus, in our probe trial, the 5-HT damage seen following treatment with MDMA may have resulted from reduced attention span, impulsivity, or frustration in the testing procedure. How the serotonergic neurotoxicity in the hippocampus relates to this increase in anxiety and possible memory impairment is not known and warrants further investigations.

The brain regions required to navigate the MWM include the striatum, [18], frontal cortex [23], and especially the hippocampus [17,24]. These brain regions are also susceptible to the serotonergic neurotoxicity seen following MDMA treatment in rats (for a review, see Ref. [36]). The animals in this study also had reduction in 5-HT levels in the striatum (data not shown). The key role of the hippocampus in navigating the MWM was demonstrated by a study conducted by Morris et al. [24]. That particular study employed female Lister rats which were subjected to total hippocampal lesions via aspiration prior to being tested in the MWM. In addition to spatial discrimination impairment, total hippocampal lesions also induced a profound and lasting place navigational impairment in the MWM [24]. In the present study, we saw a reduction in hippocampal 5-HT levels that may have contributed to the functional impairment seen in the MWM task.

The neurochemistry involved in solving the MWM can be very complex and is often debated. While acetylcholine is known to be one of the primary neurochemical systems involved in certain forms of learning and memory in animal and human models [19,20] other pathways such as the serotonergic system have also been implicated to play a minor role [16,41]. Little is known about the modulatory effects of these neurotransmitters on hippocampal function. However, a number of studies have demonstrated that 5-HT has a modulatory effect on hippocampal long-term potentiation, a cellular mechanism of synaptic plasticity believed to underlie learning and memory [42,43]. Although 5-HT's role in the memory deficits induced by MDMA has been the topic of several experiments in human models [2,4], its role in learning and memory in animal models has been studied to a lesser degree. Recently, the selective serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) was shown to drastically reduce hippocampal 5-HT levels without altering spatial working and spatial reference memory performances in the radial or the MWM [44]. This would suggest that more than the serotonergic neurotoxicity may be involved in our present results. Indeed, MDMA has been shown to alter acute acetylcholine function [45]. Yet, to the best of our knowledge there are no studies that have examined or suggest any long-term consequences of MDMA on cholinergic function.

Several experimental studies have produced results which indicate that MDMA use is associated with a decline in various aspects of mnemonic function in humans [2–4,46]. The results of the present study suggest that the MWM may be used as a model to mimic the learning deficits induced in humans. However, finding an animal model that directly corresponds to the verbal recall tasks

used to produce previously reported results in human models [4,44] proves difficult. The findings of this study demonstrate that hippocampal serotonergic lesions induced by MDMA may be ostensibly linked to a reference memory deficit in rats tested with the MWM.

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