

Acute Effects of Ecstasy on Memory Are more Extensive than Chronic Effects

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ABSTRACT

Introduction: Exposure to 3, 4- methylenedioxymethamphetamine (MDMA) could lead to serotonergic system toxicity in the brain. This system is responsible for learning and memory functions. Studies show that MDMA causes memory impairment dose-dependently and acutely. The present study was designed to evaluate the chronic and acute effects of MDMD on spatial memory and acquisition of passive avoidance.

Methods: Adult male Wistar rats (200-250 g) were given single or multiple injections of MDMA (10 mg/kg, IP). Using passive avoidance and Morris Water Maze (MWM) tasks, learning and spatial memory functions were assessed. The data were analyzed by SPSS 16 software and one- way analysis of variance (ANOVA) test.

Results: Our results showed that there were significant differences in latency to enter the dark compartment (STL) between sham and MDMA- treated groups. Acute group significantly showed more STL in comparison with chronic group. Furthermore, MDMA groups spent more time in dark compartment (TDS) than the sham group. Administration of single dose of MDMA significantly caused an increase in TDS compared with the chronic group. In the MWM, MDMA treatment significantly increased the traveled distance and escaped latency compared to the sham group. Like to passive avoidance task, percentage of time spent in the target quadrant in MDMA- treated animals impaired in MWM compared with sham group.

Discussion: These data suggest that MDMA treatment impairs learning and memory functions that are more extensive in acute- treated rats.

Key Words:

Ecstasy,
Spatial Memory,
Learning.

1. Introduction

Ecstasy or 3, 4- methylenedioxymethamphetamine (MDMA) is a synthetic amphetamine analog used as a recreational drug. MDMA causes heightened sense of empathy, feeling of closeness toward others, and elevated mood (Farre et al., 2004). It can produce a set of behaviors referred to serotonin behavioral syndrome (Lyles & Cadet, 2003). It has been reported that MDMA

has neurotoxic effects on serotonergic, dopaminergic, and adrenergic endings with highest affinity to serotonergic transporter (SERT), and 5- hydroxytryptamine 2 (5-HT₂) receptor (Sarkar & Schmued, 2010). MDMA causes acute release of serotonin from nerve endings, binds to the SERT, and inhibits serotonin reuptake (Simantov, 2004) . MDMA administration was found to decrease serotonin in the prefrontal cortex, neostriatum, and hippocampus, which are important structures in learning and spatial memory functions (Able, Gudelsky, Vorhees, & Williams, 2006; Kalechstein, De La Garza II,

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Mahoney III, Fantegrossi, & Newton, 2007). Several evidences show that central executive and decision-making skills alter in persistent MDMA users (Bolla, McCann, & Ricaurte, 1998; J. E. Sprague, Preston, Leifheit, & Woodside, 2003; Zakzanis & Campbell, 2006).

It has been shown that MDMA treatment causes production of hydroxyl radicals and lipid peroxidation and induces serotonergic neurotoxicity (J. Sprague, Everman, & Nichols, 1998). Serotonin has a modulatory effect on long-term potentiation (LTP) in the hippocampus (Slivka, Mytilineou, & Cohen, 1987).

Previous studies reported that MDMA treatment lead to decrease of novel object recognition and anxiety in the elevated plus maze (Piper, Fraiman, & Meyer, 2005). Furthermore, different doses of MDMA impaired locomotor activity and allocentric learning dose-dependently and acutely in rats (Vorhees et al., 2009). Several studies reported acute effect of single or multiple doses of MDMA on the learning and spatial memory functions (Asi et al., 2011; Soleimani Asl et al., 2011; Vorhees, Reed, Skelton, & Williams, 2004), but no study have examined the chronic effects of MDMA on the memory.

There are some tasks to evaluate learning and memory functions. The Passive avoidance learning is believed to be based on the contextual memory, which is associated with the place and the event of "being given the electric shock in the dark box". The hippocampus plays an important role in the contextual memory; injuries of the hippocampus decrease the performance of passive avoidance learning (Hirsh, 1974).

The Morris water navigation task, also known as the Morris water maze, is a behavioral procedure widely used to study learning and spatial memory function. The key brain regions involved in navigation in the Morris water maze task include the striatum, the frontal cortex, and especially the hippocampus (R. G. M. Morris, P. Garrud, J. N. P. Rawlins, & J. O'Keefe, 1982).

Because MDMA treatment lead to toxicity of hippocampus and it involves in navigation of the MWM and passive avoidance tasks, the aims of this study were to evaluate the acute and chronic effects of MDMA on learning and spatial memory in the passive avoidance and MWM tasks.

2. Methods

MDMA was obtained from the Presidency Drug Control Headquarters (Tehran, Iran), and the solutions were made in sterile saline at a specific concentration.

2.1. Animals

Twenty eight male Wistar rats, aged 8-11 months, weighting 200-250 g, were obtained from the Iranian Pasteur Institute, Tehran, Iran. Before any treatment, the rats were allowed to acclimatize to the colony room for 1 week. The rats were kept in colony room at a temperature of $21 \pm 1^\circ\text{C}$ ($50 \pm 10\%$ humidity) at a 12-hour light-dark cycle with access to water and food ad libitum. Then, the rats were randomly classified into four MDMA-treated and sham groups ($n=7$ per group) as followed:

1. Acute sham saline group received intraperitoneal (IP) injection of 1 mls normal saline for once.
2. Chronic sham saline group received IP injections of 1 mls normal saline in the weekend for three weeks (1, 2, 8, 9, 15, 16 days).
3. Acute MDMA group received IP injection of 10 mg/kg MDMA for once.
4. Chronic sham saline group received IP injections of 10mg/kg MDMA in the weekend for three weeks (1, 2, 8, 9, 15, 16 days). The day after last administration, memory was assessed using shuttle box and MWM.

2.2. Inhibitory Avoidance Apparatus (Shuttle box)

Step-through inhibitory avoidance apparatus consisted of two boxes of the same size ($20 \times 20 \times 30$ cm). There was a guillotine door in the middle of a dividing wall. The walls and floor of one compartment consisted of white opaque resin and the walls of the other one was dark. Intermittent electric shocks (50 Hz, 3 s, 1.5 mA intensity) were delivered to the grid floor of the dark compartment by an isolate stimulator.

All animals were allowed to habituate in the experimental room for at least 30 min before the experiments. Then, each animal was gently placed in the white compartment and after 5 s the guillotine door was opened and the animal was allowed to enter the dark module (Azami et al., 2010).

Animals that waited more than 300 s to enter the dark chamber were excluded from the experiment. Once the animal entered with all four paws to the next chamber, the guillotine door was closed and the rat was immediately withdrawn from the compartment. This trial was repeated after 30 min. As in the acquisition trial, when the animal entered the dark (shock) compartment, the door was closed; and a foot shock (50 Hz, 1 mA and 3 s) was immediately delivered to the grid floor of the dark room. After 20 s, the rat was removed from the apparatus

and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials; if the rat did not enter the dark compartment during 300 s, a successful acquisition of inhibitory avoidance response was recorded. Otherwise, when the rat entered the dark compartment (before 300 s) a second time, the door was closed and the animal received the shock again. After retesting, if the rat learned inhibitory avoidance response successfully, it was moved to the cage and received MDMA or saline. On the retention trail (24 h after drug administration), each animal was gently placed in the light compartment and after 5 s the door was opened and the latency which the animal entered the dark chamber (STL) and the total time spent in dark compartment (TDS) were recorded in the absence of electric foot shocks, as indicator of inhibitory avoidance behavior.

2.3. Morris Water Maze Performance

A Morris water maze apparatus, consisted of a circular pool (180 cm in diameter, 60 cm in height), painted black, filled to a depth of 25 cm with water $22 \pm 1^\circ\text{C}$, was used for assessing spatial memory (Asi et al., 2011).

The pool was divided into four quadrants with four starting locations, referred to as north (N), east (E), south (S) and west (W) and an invisible Plexiglass platform (10 cm diameter) was located 1 cm below the water in the center of northern quadrant. The animals were trained for 3 days at approximately the same time (10:00-12:00 a.m.) each day that included two block-swim with four trials (90 seconds). Between two trials, the animals spent 30 seconds on the platform. The rats were allowed to rest for 5 minutes between two consecutive blocks. A video camera (Nikon, Melville, New York, USA) linked to a computer was mounted directly above the water maze pool to record the time taken to reach

the hidden platform (the escape latency), the length of swim path (the traveled distances) and the percentage of spent time in target quadrant for each rat. The day after the last learning trial, each rat was given a single 60-second probe trial (retention trail) and visible test. In probe trials, no platform was present. In the visible trials, the platform was covered with aluminum foil.

2.4. Statistical Analysis

The data were expressed as mean \pm S.E.M and analyzed by SPSS 16 software. The statistical analyses were performed using one way analysis of variance (ANOVA) and Post-hoc comparison of means was carried out with the Tukey test (t-test) for multiple comparisons, when appropriate. The value of $p < 0.05$ was considered significant.

3. Results

As there were no differences between acute and chronic sham saline groups, herein, we reported only one sham group.

3.1. Passive Avoidance Task

In the acquisition trial, we found no difference between the sham and MDMA groups in the STL and TDC (data not shown). However, the injection of MDMA reduced the STL in the retention trial compared to the sham groups ($p < 0.001$, Figure 1A).

Furthermore, MDMA-treated rats spent more time in the dark compartment (TDC) compared to the sham group ($p < 0.001$, Figure 1B). Furthermore, there was significant difference between chronic and acute groups in the TDS ($p < 0.001$, Figure 1B).

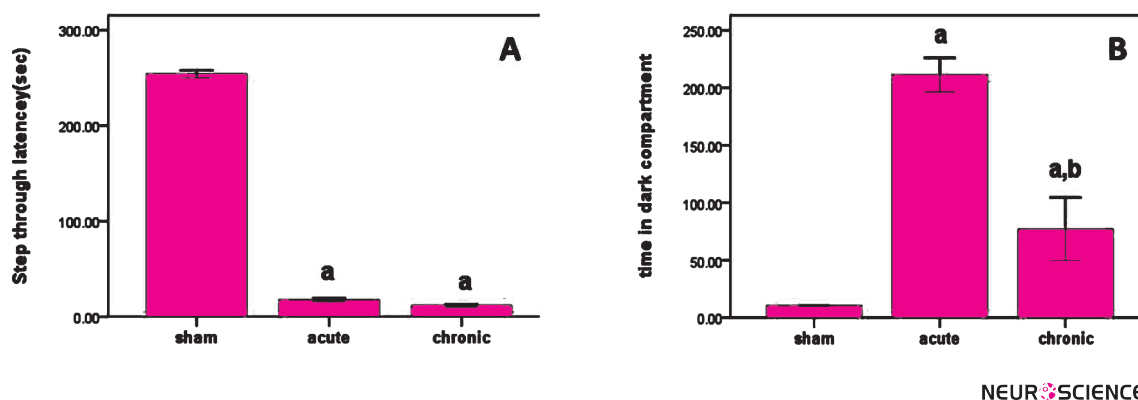
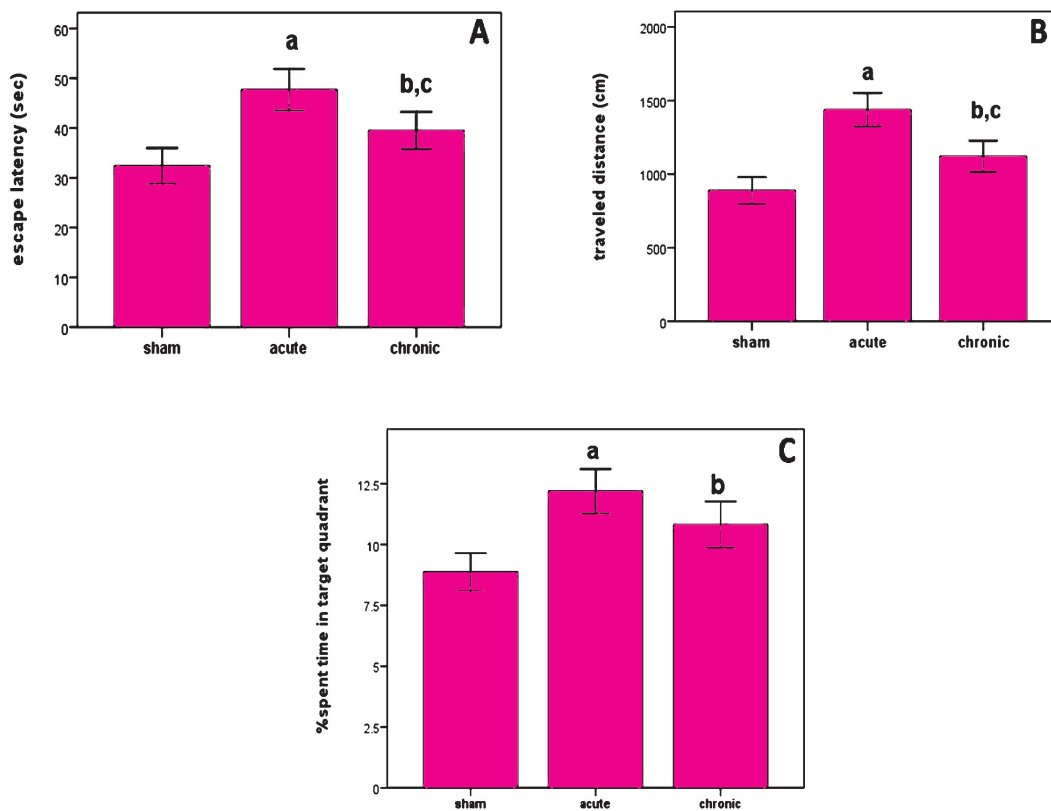


Figure 1. Effects of MDMA treatment on passive avoidance performance. The mean of the step- through latency to enter to the dark compartment (A), (a $p < 0.001$ vs. sham group) and the time spent in dark compartment (B), (a $p < 0.001$ vs. sham group, b $p < 0.001$ vs. acute group). The values were presented as mean \pm S.E.M.



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Figure 2. Effects of MDMA on spatial memory in MWM. MDMA increased the escape latency (a $p < 0.001$ vs. sham group, b $p < 0.05$ vs. sham group, C $p < 0.01$ vs. acute group, A) and traveled distance (a $p < 0.001$ vs. sham group, b $p < 0.01$ vs. sham group, C $p < 0.01$ vs. acute group, B). MDMA reduced the spent time in target quarter (a $p < 0.001$ vs. sham group, b $p < 0.01$ vs. sham group).

3.2. Morris Water Maze performance

The results from MWM showed that MDMA increases the escape of latency to find hidden platform.

Analysis of variance of three training days showed that MDMA treatment significantly increased the escape of latency compared with sham group ($p < 0.001$ for acute group, $p < 0.05$ for chronic group, Figure 2A). Furthermore, there was significant difference between acute and chronic MDMA groups ($p < 0.01$, Figure 2A) and MDMA acutely showed exaggerated response.

As shown in figure 2B, MDMA- treated rats spent more distance to reach to hidden platform that were significant compared with sham group ($p < 0.001$ for acute group, $p < 0.01$ for chronic group). According to the results, acute administration of MDMA significantly increased the traveled distance in comparison with the chronic- treated MDMA group ($p < 0.001$, Figure 2A).

The analysis of variance of our results showed that sham group spent more time in target quadrant that was significant compared with MDMA groups ($p < 0.001$ for acute group, $p < 0.01$ for chronic group, Figure 2C). Acutely- treated rats insignificantly spent less time in target quadrant in comparison with chronic- treated rats.

4. Discussion

The results of this study showed that MDMA led to memory impairment in MWM and passive avoidance tasks. The other finding was that the MDMA- induced memory impairment in the acute- treated rats was more exaggerated than the chronic- treated rats. It was reported that MDMA treatment in rats on postnatal day 11 induced spatial and references memory impairment (Vorhees et al., 2004). In another study, non- acute exposure to MDMA caused memory impairment dose-dependently (Asi et al., 2011). Consistent to our results, Sprague et al. showed that MDMA treatment resulted in reduction of preference for the target quadrant in the

MWM (J. E. Sprague et al., 2003). Similarly, Fukami et al. reported that administration of methamphetamine as another amphetamine derivative caused behavioral change in the rats (Fukami et al., 2004). Furthermore, it was reported that acute MDMA administration impaired passive avoidance retention in the rats (Moyano, Frechilla, & Del Rio, 2004). It was found that acute administration of MDMA before the acquisition trial of a passive avoidance task impaired retention 24 h later (Barrionuevo, Aguirre, Del Ro, & Lasheras, 2000) that confirms our results. Gurtman et al. 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 (Gurtman, Morley, Li, Hunt, & McGregor, 2002). The key brain regions involved in learning and memorizing include the striatum, the frontal cortex, and the hippocampus (Mogensen, Pedersen, Holm, & Bang, 1995; R. Morris, P. Garrud, J. Rawlins, & J. O'Keefe, 1982). These regions are susceptible to 5-HT neurotoxicity that reported following MDMA treatment (J. Sprague et al., 1998). Because the brain has low antioxidant and cell membrane lipids, it is sensitive to oxidative stress (Viegas et al., 2012). Oxidative stress plays a role in MDMA-induced neurotoxicity in the brain (Yamamoto & Raudensky, 2008). Oxidative stress results from an imbalance between ROS and intracellular antioxidant such as glutathione (GSH). GSH has excitatory effects on serotonergic system and serotonin has a modulatory effects on long term potentiation (LTP) in the hippocampus that is a key structure in the memory (Slivka et al., 1987). It has been reported that glutathione depletion causes spatial memory impairment (Choy, Dean, Berk, Bush, & van den Buuse, 2010). Therefore, it seems that MDMA treatment causes glutathione depletion and serotonin neurotoxicity that lead to memory impairment.

In conclusion, our results showed that the acute and chronic administration of MDMA induced deficits in passive avoidance and Morris water maze tasks that were more exaggerated in acute-treated rats. Therefore, it is not possible for the brain to improve the MDMA-induced toxicity in the acute administration.

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Conflict of Interest

None of the authors of this paper reported financial interest.

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