

# Chronic and Sub-acute Effects of 3,4-methylenedioxy methamphetamine (MDMA) on Spatial Memory and Passive Avoidance Learning in Wistar Rats

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## ABSTRACT

**Introduction:** In the recent years, the use of several drugs such as cocaine and morphine has reduced, but the use of "club drugs" has increased in many countries. 3, 4-methylenedioxyamphetamine (MDMA) which has known as 'ecstasy' among people has many harmful effects on serotonergic system in the brain. This system is responsible for learning and memory. In this study, we tested sub-acute and chronic effects of MDMA on spatial memory in the Morris water maze (MWM) task and passive avoidance tasks.

**Methods:** 18 Adult male wistar rats (200-250 g) were given single or multiple injections of MDMA (10 mg/kg, IP). Learning and spatial memory functions were assessed using passive avoidance and Morris water maze (MWM) tasks, respectively. Data was analyzed by SPSS 16 software and one-way analysis of variance (ANOVA) test.

**Results:** The results showed that sub-acute and chronic administration of MDMA have adverse effects on escape latency, traveled distance and avoidance learning in the rats. The changes were more significant in sub-acute group.

**Conclusion:** These data suggest that MDMA treatment impairs learning and memory performances that are more extensive in sub-acute group.

## 1. Introduction

(±) -3, 4-methylenedioxyamphetamine which known as "Ecstasy" is a psychoactive entertaining mood-altering agent and one of the main sort of drug

abuse in the world. Many studies have been shown that ecstasy can induce neurotoxicity in both animals and humans [1]. Many researchers surveyed adverse effects of ecstasy in human and reported that this drug lead to vomiting, nausea, clenching of jaw, grinding of teeth,

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high pressure, headaches, hyperreflexia, palpitations, walking problems, urinary urgency, pains and tension of muscle, dry mouth and vision problems [2].

Carvalho et al. have shown that ecstasy causes hyperthermia in the rats. The natural mechanisms of production and development of hyperthermia in ecstasy users are multifactorial and almost combination of the dopaminergic, serotonergic, and adrenergic function lead to heat production [1].

It has been shown that MDMA administration decreases serotonin in the prefrontal cortex, neostriatum, and hippocampus, which are important structures in learning and memory. Vorhees CV, et al. [3] reported that use of different doses of MDMA in rats at postnatal days 11-12 impaired locomotor activity and allocentric learning in the Morris water maze, dose-dependently and acutely. Moreover, Sprague JE, et al. [4] reported the effects of MDMA treatment (20 mg/kg, twice daily) on escape latency, swim distance in Morris water maze task. However, that study used a 7-day delay between MDMA administration and Morris water maze testing, and did not examine the sub-acute and chronic effects of MDMA. Several studies have shown the acute effects of single or multiple doses of MDMA on learning and memory [4-5], but in our knowledge, no studies have examined the difference between sub-acute and chronic effects of doses of MDMA on learning and memory in the Morris water maze task and passive avoidance learning.

The key regions of brain involved in navigation in the Morris water maze task include the striatum, the frontal cortex, and especially the hippocampus [6-7]. These regions are also susceptible to the serotonergic (5-HT) neurotoxicity that has been reported following MDMA administration in rats [8]. Furthermore, the hippocampus plays an important role in contextual memory; injuries of the hippocampus decrease the performance of passive avoidance learning [9].

As MDMA treatment lead to toxicity of hippocampus and it involves in navigation of the MWM and passive avoidance tasks, the aim of this study was to compare the sub- acute and sub- chronic effects of MDMA on learning and spatial memory in the passive avoidance and MWM tasks.

## 2. Materials and Methods

### Animals

18 male Wistar rats, weighting 200-250 g, were obtained from the Pasteur Institute (Karaj- Iran) . The rats were allowed to acclimatize to the colony room for one week prior to any treatment. All rats were kept in colony room at a temperature of  $21 \pm 2^\circ\text{C}$  ( $50 \pm 10\%$  humidity) on a 12-hour light-dark cycle with free access to water and food ad libitum. The study was approved by the ethics committee of Hamadan University of Medical Sciences. The rats were randomly classified into three groups as follow:

#### 1. Sham Saline Group Received Normal Saline

2. Sub-acute group received IP injection of 10 mg/kg MDMA for once, and three days after MDMA administration, learning and memory were assessed using passive avoidance and Morris water maze tasks.

3. Sub-chronic received IP injection of 10 mg/kg MDMA, twice daily day for 7 days. The day after last administration, learning and memory were assessed using passive avoidance and Morris water maze tasks.

### Morris Water Maze

Morris water maze test was performed for evaluation of spatial memory as our previously described method. It included a circular pool (180 cm in diameter, 60 cm in height) that was painted black and filled to a depth of 25 cm with water at a temperature of  $22 \pm 1^\circ\text{C}$ . The pool was divided into four quarters with four starting locations: north (N), south (S), east (E), and west (W) located at equal distances on the rim. An invisible platform (10 cm in diameter) made of Plexiglass was located 1 cm below the water in the center of the northern quadrant. The animals were trained for three days at approximately the same time (10-12 am) each day. Each training day included two blocks with four trials. The time limit on each animal was 90 seconds and the inter-trial was 30 seconds that was spent on the platform. The rats rested for 5 minutes between two consecutive blocks. A video camera mounted directly above the water maze pool was linked to a computer and recorded the time to reach the hidden platform (escape latency) and the length of swim path (traveled distances). The day after the last learning trial, each rat was given a single 60 second probe trial and visible test. The probe trials were performed without a platform but the visible tests

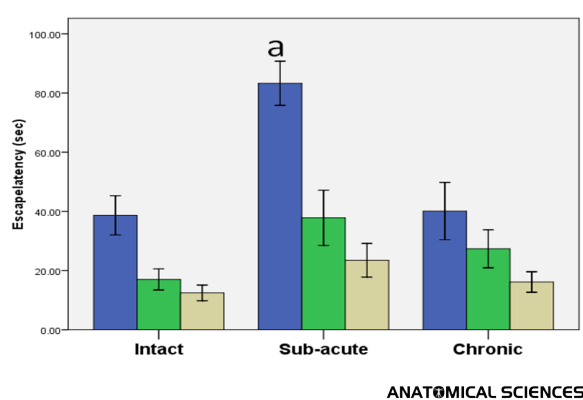
were performed with a platform that was covered with aluminum foil [10].

### Active Avoidance Apparatus (Shuttle box)

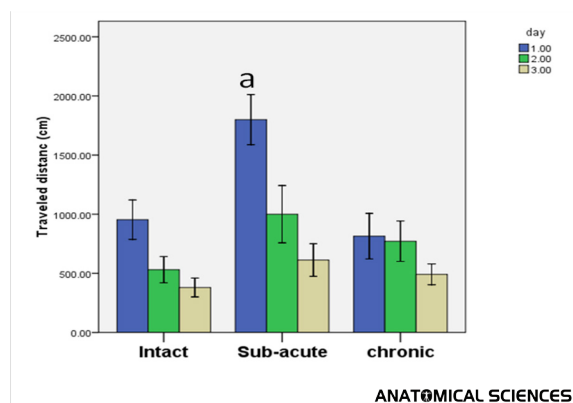
Step-through inhibitory avoidance apparatus was consisted of two boxes of the same size (20 × 20 × 30 cm). There was a guillotine door in the middle of a dividing wall. The walls and floor of one compartment were consisted of white opaque resin and the walls of the other one was dark. Intermit-tent electric 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.

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 sec-



**Figure 1.** Mean of escape latency to find the hidden platform in three training days in Morris water maze. Administration of MDMA increased escape latency in sub-acute group (a  $p < 0.001$  vs. intact and chronic groups).



**Figure 2.** Mean of traveled distance to find the hidden platform in the Morris water maze. Administration of MDMA increased travel distance in sub-acute (a  $p < 0.001$  vs. intact and chronic groups).

ond 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 escape 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 (11).

### Statistical Analysis

The data were presented as the mean  $\pm$  SD and the results were analyzed by SPSS 16 software and one-way ANOVA. Post-hoc comparisons were performed using Tukey's test (t-test). P-values  $\leq 0.05$  were statistically considered significant.

## 3. Results

### Morris Water Maze Performance

Analysis of variance of training days showed that the mean of escape latency markedly increased in sub-acute group compared with the other groups ( $p < 0.001$  for both groups, Figure 1). There was no significant difference in escape latency between sham and sub-chronic groups.

As shown in figure 2, MDMA-treated rats spent more distance to reach to hidden platform that were significant in sub-acute group compared with sham group ( $p < 0.001$ ). According to the results, sub-acute administration of MDMA caused to a significant increase in

traveled distance in comparison with the sub- chronic-treated MDMA group ( $p < 0.001$ , Figure 2).

### Passive Avoidance Learning

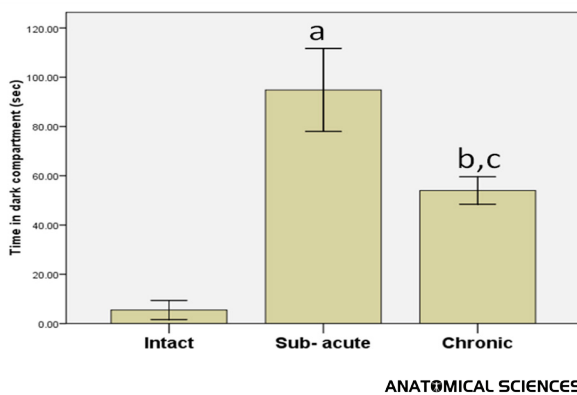
The results of this study showed that the injection of MDMA reduced the STL in the retention trial compared to sham group ( $p < 0.001$  for both groups, Figure 3). Moreover, the STL significantly was increased in sub-acute group compared to chronic group ( $p < 0.01$ ).

Furthermore, MDMA- treated rats spent more time in the dark compartment (TDC) compared with the sham group ( $p < 0.001$  for sub- acute group,  $p < 0.01$  for chronic group, Figure 4). Hence, there was significant difference between chronic and acute groups in the TDS ( $p < 0.05$ ).

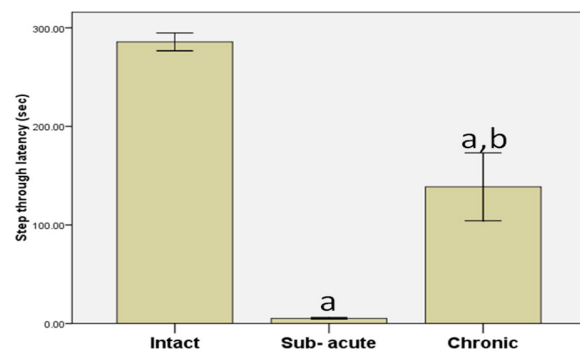
## 4. Discussion

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 sub acute- treated rats was more exaggerated than the chronic- treated rats. Experimental and clinical studies have been shown that MDMA acts by raising the net release of the monoamine neurotransmitters including serotonin, noradrenaline and dopamine. MDMA binds to serotonin transporter and interferes with reuptake of this neurotransmitter [12].

The hippocampus is very susceptible to neurotoxicity of serotonergic (5-HT) subsequent administration of MDMA [13]. Aguirre N, et al. showed that administration of a single dose of MDMA (20 mg/kg,i.p) can decline 40-60% of 5- hydroxytryptamine (5-HT) content



**Figure 4.** Effects of MDMA treatment on passive avoidance performance. The mean of the time spent in dark compartment, (a  $p < 0.001$  and b  $p < 0.01$  vs. sham group, c  $p < 0.05$  vs. sub- acute group). The values were presented as mean  $\pm$  S.E.M.



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**Figure 3.** Effects of MDMA treatment on passive avoidance performance. The mean of the step- through latency to enter to the dark compartment, (a  $p < 0.001$  vs. sham group and b  $p < 0.01$  vs. sub- acute group). The values were presented as mean  $\pm$  S.E.M.

and 5-HT transporter in the hippocampus, frontal cortex and striatum after one week [14]. Also, it was reported that MDMA can raise immunoreactivity of glial fibrillary acidic protein (GFAP) in the hippocampus [14].

Hippocampus is a vital part of brain that involved in learning and memory (Deng et al S-1). Many experiments have reported that many neurotoxic agents lead to damage of neuronal in the hippocampus [15]. Jahan-shahi M, et al. showed that administration of MDMA (2.5 mg/kg, 5 mg/kg, and 10 mg/kg) reduced the number of neurons in comparison with control group. Consistent to our results, Sprague et al have shown that MDMA treatment resulted in reduction in preference for the target quadrant in the MWM (16). It was found that acute administration of MDMA before the acquisition trial of a passive avoidance task impaired retention 24 h later that confirms our study results. The brain is sensitive to oxidative stress due to low antioxidant and cell membrane lipids. The antioxidant levels in brain are lower than other organs, so brain is susceptible to oxidative stress damage and lipid peroxidation [17].

Colado et al. showed that MDMA increased formation of free radicals in the hippocampus of rats [18]. It has been reported that administration of MDMA to rats changed the activities of different antioxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase in some areas of brain, and glutathione depletion caused spatial memory impairment. Taken together it seems that MDMA treatment causes to glutathione depletion and serotonin neurotoxicity that lead to memory impairment [19].

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

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