Research Paper: The Effects of 3,4-methylenedioxy-methamphetamine on Neurogenesis in the Hippocampus of Male Rats

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ABSTRACT

Introduction: The administration of 3,4-methylenedioxymethamphetamine (MDMA) or ecstasy causes memory impairment, whereas neurogenesis improves memory and learning. Hence, this study evaluated the effects of MDMA on neurogenesis in the hippocampus of male rats.

Methods: Adult male Wistar rats received Intraperitoneal (IP) injections of MDMA (10 mg/kg). We assessed nestin, sex-determining region Y-box 2 (Sox2), and NeuroD expressions according to the immunohistochemistry analyses.

Results: MDMA reduced the expressions of nestin, Sox2, and NeuroD compared with the control groups. The reduction in NeuroD expression was age-related.

Conclusion: MDMA possibly has negative effects on neurogenesis, which specifically results from impaired survival of newborn cells.

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Highlights

- MDMA has negative effects on neurogenesis of the hippocampus.
- There was an age-related decline in the neurogenesis of the control and MDMA-treated groups.

Plain Language Summary

Ecstasy or 3, 4-methylenedioxymethamphetamine (MDMA) binds to 5-HT transporter and inhibits serotonin absorption and subsequently serotonin accumulation in the synaptic cleft, which leads to an early release of serotonin. Following the acute release of serotonin or 5-hydroxytryptamine (5-HT), serotonin levels are reversibly depleted 3-4 h after ecstasy administration. This depletion persists for at least one week and causes neuronal damage and memory impairment. There is a correlation between neurogenesis in the hippocampus and memory. It seems that serotonin alterations in the hippocampus after MDMA administration may play a critical role in the neurogenesis deficits in the hippocampus, which should be investigated further.

1. Introduction

Ecstasy or 3, 4-methylenedioxymethamphetamine (MDMA) has been used recreationally since the 1980s. Initially, MDMA was found to cause 5- hydroxytryptamine (5-HT), dopamine (DA), and epinephrine neurotoxicity with increased affinity to 5-HT receptors (Costa, Morelli, & Simola, 2017; Roberts, Jones, & Montgomery, 2016). Pharmacologic studies have shown that MDMA binds to 5-HT transporter and inhibits serotonin absorption and subsequently 5-HT accumulation in the synaptic cleft, which leads to the early release of 5-HT (Simantov, 2004). Following the acute release of 5-HT, serotonin levels are reversibly depleted 3-4 h after MDMA administration. This depletion persists for at least one week (Colado & Green, 1994). It is well established that MDMA-induced serotonin neurotoxicity is area-specific, with the major changes in the prefrontal cortex, hippocampus, and striatum (Green, Mechan, Elliott, Oshea, & Colado, 2003) as important structures involved in spatial memory (Euston, Gruber, & McNaughton, 2012; McDonald & White, 1994). MDMA causes neuronal damage and memory impairment (Asi et al., 2011; Asl, Saifi, Sakhaie, Zargooshnia, & Mehdizadeh, 2015). Given the fundamental role of the hippocampus in learning and memory (Barzegar et al., 2015), it has been speculated that neurogenesis in the hippocampus (newborn granule cells) contribute to memory formation (Ortega-Martínez, 2017).

Jessberger et al. reported that blockage of neurogenesis in the Dentate Gyrus (DG) by a lentivirus-based strategy impaired spatial and objective recognition memory (Jessberger et al., 2009). We previously demonstrated that MDMA treatment could lead to neuronal degeneration and cell death in the hippocampus, with subsequent memory impairment (Shariati et al., 2014).

Although the toxic effects of MDMA on the brain have been widely studied, little is known about the effects of MDMA on different phases of neurogenesis in the hippocampus. MDMA administration leads to memory impairment (Shariati et al., 2014) and researchers have established a correlation between neurogenesis in the hippocampus and memory; therefore, the aim of this study was to investigate the effects of MDMA on neurogenesis in the DG of the rat hippocampus.

2. Materials & Methods

2.1. Chemicals

We obtained MDMA through the Presidency Drug Control Headquarters (Hamadan, Iran). Antibodies (nestin, Sox2, and NeuroD) and other chemicals were purchased from Abcam (Cambridge, UK).

2.2. Experimental design and animal grouping

Male Wistar rats (200-250 g) were housed in the animal house with the standard condition (a 12-h light/12-h dark cycle, the temperature of 22±2°C, and relative humidity of 55±5%) and ad libitum access to food and water.

The Ethical Committee of the Iran University of Medical Sciences approved the experiments (IR.IUMS.REC.1393.24111). According to our previous study and using the formula 1, we obtained the sample size and randomly divided the rats into two groups (n=16 per group): control saline group that received the Intraperitoneal (IP)

injections of normal saline (1 ml/kg) and MDMA group that received a single dose of MDMA (IP, 10 mg/kg) (Shariati et al., 2014). Based on Schmidt et al. study, administration of the single dose of MDMA (10 mg/kg) inactivates tryptophan hydroxylase and causes poor expression of the brain 5-HT and serotonin transporter (Schmidt, Levin, & Lovenberg, 1987). Subsequently, we divided the control and MDMA groups into four subgroups (n=4 per group) according to the time that the rats were killed: 7, 14, 28, or 60 days after treatment (Zhao, Deng, & Gage, 2008).

$$1.\ n=\frac{(z_a+z_\beta)^{2\times}(s^2{}_1+s^2{}_2)}{(\bar{x}_1-\bar{x}_2)^2}$$

2.3. Tissue preparation and Immunohistochemistry (IHC) protocol

We used our previously reported protocol for the IHC analyses (Alipanahzadeh et al., 2014). In brief, after anesthesia, the rats were transcardially perfused with 4% Para-Formaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4). After tissue processing and paraffin embedding and based on the Paxinos and Watson atlas (Paxinos, Watson, Pennisi, & Topple, 1985), 10 µm coronal sections (-3.3 and -3.8 to the Bregma) were prepared from the brain. The slides were deparaffinized, rehydrated in ascending alcohol, and were then retrieved in sodium citrate buffer and incubated in 10% normal serum with 1% BSA in TBS for 2 h. Then, the slides were incubated with antibodies against NeuroD, sSox^2, and nestin (1:1000, Abcam, Cambridge, UK) at 4°C, and also HRP-conjugated anti-rabbit secondary antibody (1:10000, Abcam, Cambridge, UK). All sections were incubated with DAB solution (Abcam, Cambridge, UK) for 20 min and counterstained using hematoxylin. We assessed the DG region of the hippocampus by a light microscope with an attached digital camera.

2.4. Statistical analysis

Data were presented as Means±SEM. We used SPSS 16 software and One-way Analysis of Variance (ANOVA) and Tukey's multiple comparison test to analyze the data. $P<0.05$ was considered statistically significant.

3. Results

We counted five serial sections at 40x magnification to determine the average neuronal counts for each animal.

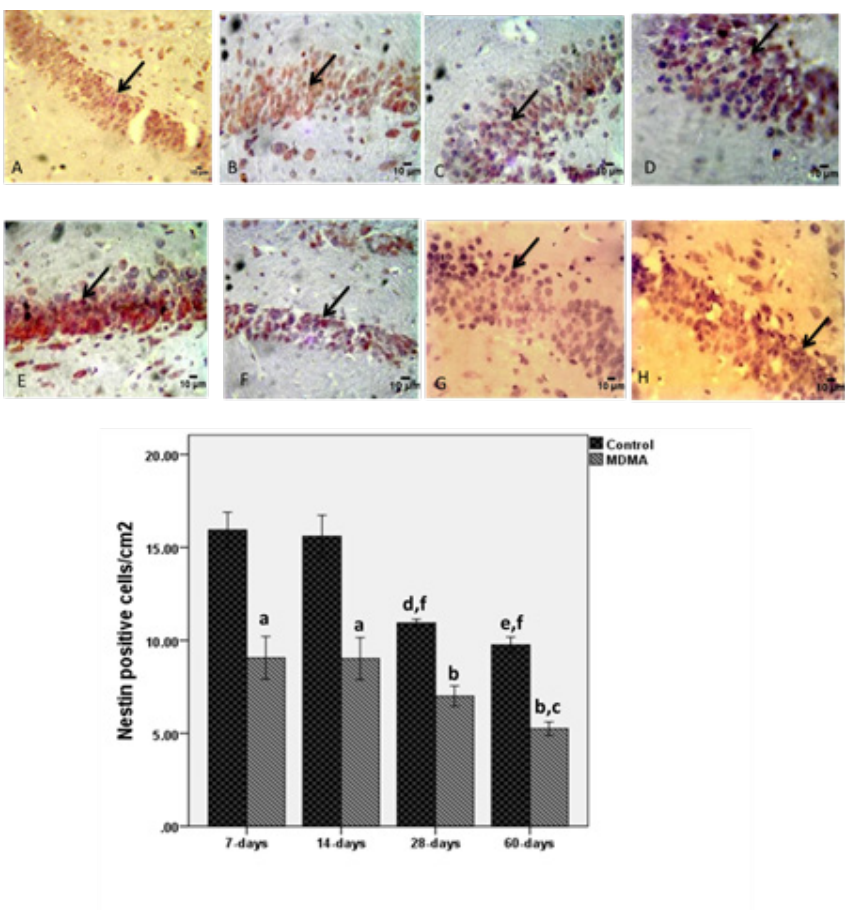

Figure 1. Nestin-positive cells in the hippocampus

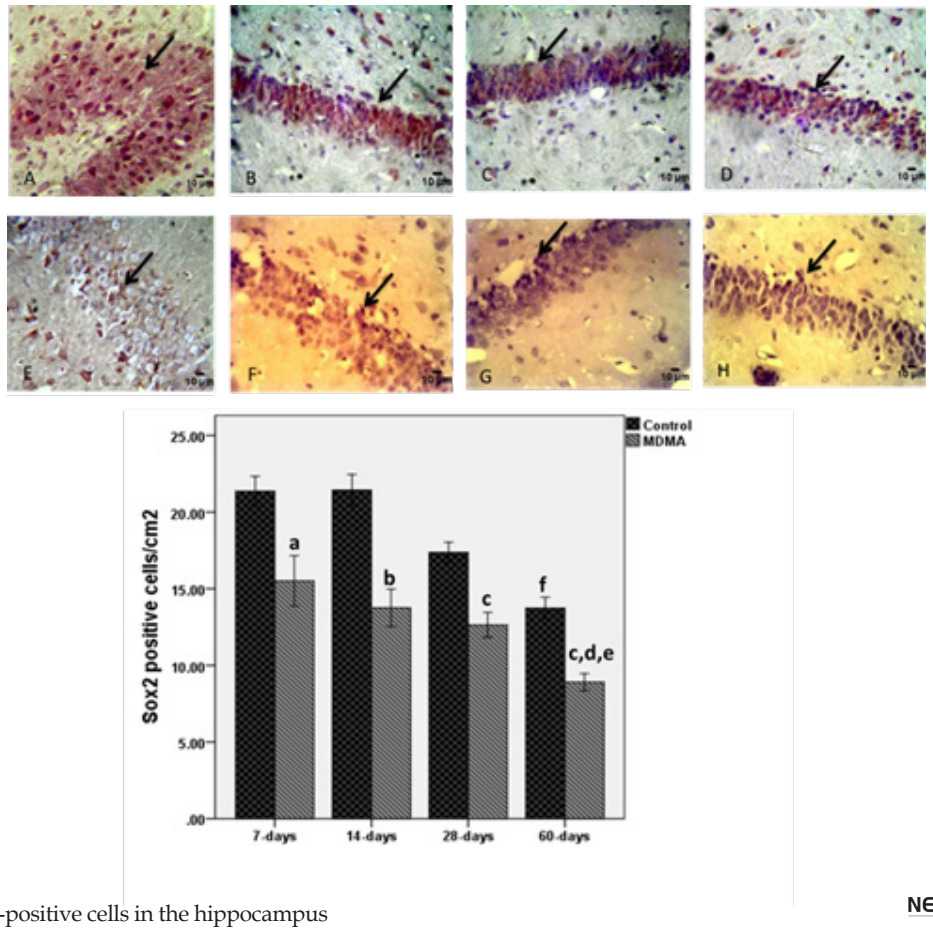

Figure 2. Sox2-positive cells in the hippocampus

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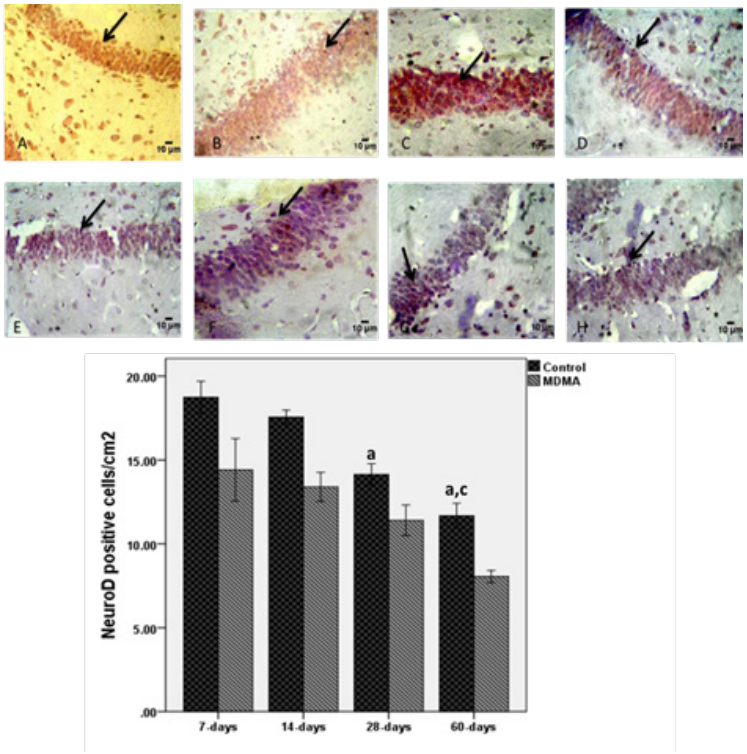

Figure 3. NeuroD-positive cells in the hippocampus

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Nestin-positive cells were counted in the hippocampus DG (Figure 1). Significant differences existed between the control and MDMA groups on days 7 (P<0.001), 14 (P<0.001), 28 (P<0.05), and 60 (P<0.05). The MDMA groups from the day7 to 14 had greater nestin expression levels than the day 60 (P<0.05).

The number of expressed nestin-positive cells was significantly lower on days 28 (P<0.01) and 60 (P<0.001) in the control group compared with day 7. We also observed significantly fewer nestin-positive cells in the control group (days 28 (P<0.01) and 60 (P<0.01)) compared with day 14.

The expression level of nestin in the control (A: 7, B:14, C:28, D:60) and 3,4-methylenedioxymethamphetamine (MDMA)-treated groups (E:7, F:14, G:28, H:60). I: Dentate gyrus at low magnification (x10). The arrow shows nestin-positive cells. A: P<0.001 vs. the control group, B: P<0.05 vs. the related controls, C: P< 0.05 vs. the MDMA groups (days 7 and 14), D: P<0.01 vs. the control group (day 7), E: P<0.001 vs. the control group (day 7), F: P<0.01 vs. the control group (day 14). The results showed that Sox2 expression was higher in the control groups.

Further analysis indicated significant differences between the groups (Figure 2). The Sox2 expression level was significantly lower on the days 7 (P<0.01), 14 (P<0.001), 28 (P<0.05), and 60 (P<0.05) in the MDMA groups compared with their related control groups. Sox2 expression significantly decreased on day 60 compared with the days 7 (P<0.001) and 14 (P<0.05) in the MDMA groups.

There was a difference between the days 7, 14, and 60 in the control groups in the numbers of Sox2-positive cells, and Sox2 expression significantly decreased on day 60 in the control group in comparison with the days 7 and 14 in the control groups (P<0.001).

The expression level of Sox2 in the control (A: 7, B:14, C:28, D:60) and 3,4-methylenedioxymethamphetamine (MDMA)-treated groups (E:7, F:14, G:28, H:60). I: Dentate gyrus at low magnification (x10). The arrow shows Sox2-positive cells. A: P<0.01, B: P<0.001, and C: P<0.05 vs. the related controls; D: P<0.001 vs. the MDMA group (day 7); E: P<0.05 vs. the MDMA group (day 14); F: P<0.001 vs. the control groups (days 7 and 14).

NeuroD expression decreased following MDMA treatment (Figure 3). However, in contrast to nestin and Sox2, no significant differences existed between the control (day 7; 18.74±0.95) and MDMA (day 7) (14.42±1.86) groups; the control (day 14; 17.56±0.41) and MDMA (day 14; 13.4±0.87) groups, the control (day 28; 14.14±0.63) and MDMA (day 28; 11.4±0.91) groups; and the control (day 60; 11.68±0.74) and MDMA (day 60; 8.5±0.36) groups. The NeuroD expression level was significantly higher in the control group (day 7) compared with the days 28 (P<0.05) and 60 (P<0.001). Significant differences were observed between day 14 and day 60 control groups (P<0.001).

The expression level of NeuroD in the control (A:7, B:14, C:28, D:60) and 3,4-methylenedioxymethamphetamine (MDMA)-treated groups (E:7, F:14, G:28, H:60). I: Dentate gyrus at low magnification (x10). The arrow shows NeuroD-positive cells. A: P<0.05 and B: P<0.001 vs. the control group (day 7), C: P<0.001 vs. the control group (day 14).

4. Discussion

In this study, we found a deficit in neurogenesis following MDMA treatment. We observed that the expression levels of nestin, Sox2, and NeuroD decreased in the hippocampus of MDMA- treated rats compared with the control groups. There was an age-related decline in the neurogenesis of the control and MDMA-treated groups.

Neurogenesis normally occurs in adults in the Subgranular Zone (SGZ) of the DG within the hippocampus and Subventricular Zone (SVZ) of the lateral ventricle, which is influenced by physiological and pathological agents at the levels of survival, proliferation, and differentiation (Zhao, Deng, & Gage, 2008). Lineage tracing studies in rodents have identified two types of neural progenitors in the SGZ that express molecular markers, such as nestin, Sox2, and GFAP (Breunig, Silbereis, Vaccarino, Šestan, & Rakic, 2007). The expressions of these markers are affected by toxic agents in the nervous system (Duman, Malberg, & Nakagawa, 2001; Kang et al., 2017).

Increased environmental complexity enhances neurogenesis by increasing cell survival, proliferation, and differentiation in the hippocampus of adult mice (Brown et al., 2003; Zhao, Deng, & Gage, 2008). In contrast, physiological and psychosocial stressors negatively affect adult neurogenesis (Abbink, Naninck, Lucassen, & Korosi, 2017; Levone, Nolan, Cryan, & O'Leary, 2017). Substance abuse reduces neurogenesis and affects cell proliferation and differentiation (Castilla-Ortega et al., 2017; Venkatesan, Nath, Ming, & Song, 2007; Warner□Schmidt & Duman, 2006).

Dominguez□Escriba et al. reported that the chronic administration of cocaine impaired cell proliferation, which supported the results of this study as nestin-positive cells decreased in the DG following MDMA treatment (Domínguez□Escribà et al., 2006).

Our findings supported the results of Hernandez-Rabaza et al. who have shown that administration of MDMA affects neurogenesis in the DG by impairing neural precursor survival in adult rats (Hernandez-Rabaza et al., 2006). Our results showed that MDMA administration decreased NeuroD expression. NeuroD is expressed in intermediate cells in the initial phases of neurogenesis and serves to expand the population of neuroblasts (Kang et al., 2017).

We found significant differences in the expression of neurogenesis-related proteins between day 7 and day 60 groups of the controls. This finding supported the hypothesis that neurogenesis dramatically decreases during aging.

The current study results supported those by Spalding et al. (2013) who reported a moderate decline in neurogenesis by aging. Although a decrease in synaptic density in the DG has been shown in the aged brain, the mechanism of age-related decline in neurogenesis is unclear and needs to be investigated. Previous results have shown that MDMA caused a neuronal degeneration and reduction in the newborn cells in the hippocampus (Renoir et al., 2008; Soleimani Asl, Saifi, Sakhaie, Zargooshnia, & Mehdizadeh, 2015), but the mechanism, by which MDMA decreases the neurogenesis is not fully understood. Lesions in serotonin neurons in the raphe nuclei led to a decrease in newborn cells. In the adult neurogenesis, serotonin might be considered as a positive regulatory agent (Brezun & Daszuta, 2000). The hippocampus is a target of serotonergic nerve cells. MDMA administration results in serotonin depletion in this area (Gurtman, Morley, Li, Hunt, & McGregor, 2002); therefore, it seems that serotonin alterations in the hippocampus after MDMA administration may play a critical role in the neurogenesis deficits in the hippocampus, which should be investigated further.

Ethical Considerations

Compliance with ethical guidelines

The Ethical Committee of the Iran University of Medical Sciences approved the experiments. (IR.IUMS.REC.1393.24111)

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Authors' contributions

Conceptualization: Sara Soleimani Asl, Mehdi Mehdizadeh; Methodology: Sara Soleimani Asl, Mohammad Hassan Farhadi; Investigation: Fahimeh Ghasemi Moravej, Sara Soleimani Asl; Writing – original draft: Sara Soleimani Asl, Bagher Pourhaydar; Writing – review & editing: Hatef Ghasemi Hamidabadi; Funding acquisition: Mehdi Mehdizadeh; Resources, Sara Soleimani Asl, Mehdi Mehdizadeh; Supervision: Mehdi Mehdizadeh.

Conflict of interest

The authors declared no conflict of interest.

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