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NeuN Expression Alterations in the Hippocampus Following Ecstasy Treatment

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Abstract

Background: The administration of 3-4-methylenedioxymethamphetamine (MDMA) leads to learning and memory impairment.
Objectives: Due to the effect of neurogenesis on memory and learning, in this study, we investigated the effects of MDMA on NeuN expression (a marker of neurogenesis) in the hippocampus.
Methods: Adult male Wistar rats (weighing 200 - 250 g) received a single intraperitoneal dose of 10 mg/kg of MDMA or were left undisrupted. The expression of NeuN was assessed using the immunohistochemistry method 7, 14, 28, and 60 days following MDMA administration.
Results: Our results showed that MDMA administration caused a decrease in NeuN expression in the experimental group compared with the control group.
Conclusions: These results suggest a negative correlation between MDMA administration and adult hippocampal neurogenesis.

Keywords: Ecstasy, NeuN, Hippocampus, Rat

1. Background

The use of 3,4-methylenedioxymethamphetamine (MDMA) as a recreational drug leads to alterations in serotonin, dopamine, and noradrenaline transmission and a decrease in the activity of their transporters in the brain (1, 2). Our previously published study showed that MDMA neurotoxicity is characterized by spatial and avoidance learning memory impairment (3, 4). Furthermore, MDMA can result in the degeneration of neurons in the hippocampus, striatum, and neocortex that are responsible in memory and learning (5).

In the adult brain, neurogenesis and progenitor cell division only occur in the subventricular zone of the lateral ventricle and in the subgranular zone of the hippocampus dentate gyrus (DG) (6). Neurogenesis includes distinct stages, such as cell proliferation, neuronal differentiation, and survival, that can be influenced by various environmental features. For example, morphine implantation decreases the proliferation of cells in the hippocampus DG (7). In one study, buprenorphine used as an opiate analgesic decreased the number of proliferating cells (8). In another study, the simultaneous abuse of alcohol and MDMA during pregnancy decreased doublecortin (DCX, a neuronal precursor landmark) expression (9).

One of the most important genes in neurogenesis is NeuN (a neuronal nuclear antigen) that that is commonly used as a biomarker for neurons. NeuN is expressed in most neuronal cell types, including cerebellar Purkinje cells, olfactory bulb mitral cells, hippocampus pyramidal cells, and retinal photoreceptor cells (10).

2. Objectives

Since adult hippocampal neurogenesis plays an essential role in learning and memory and since MDMA causes memory impairment, we investigated the effects of MDMA on NeuN in the hippocampus.

3. Methods

A total of 25 adult male Wistar rats (weighing 200 - 250 g) were obtained from our animal house (Hamadan, Iran) and housed with controlled temperature (22 ± 2) and humidity (50 ± 5) on a 12-hour dark and light cycle with ad libitum access to water and food.All experimental protocols were approved by the ethics board of Hamadan University of Medical Sciences.

The animals were divided into control and MDMA groups. The control group (n = 5) was left undisrupted. The MDMA group (n = 20) received a single intraperitoneal injection of 10 mg/kg MDMA (11). The rats were killed 7, 14, 28, and 60 days following MDMA administration.

3.1. Immunohistochemistry

We prepared the sections using our previously published protocol (12). In brief, the rats were transcardially perfused with 4% paraformaldehyde, and their brains were dissected out. The hippocampi were sectioned into 5-μm coronal sections. After antigen retrieval in a sodium citrate buffer (10 mm sodium citrate, 0.05% Tween 20, pH: 6.0) and blocking in 10% normal serum with 1% BSA in TBS for two hours, the sections were incubated overnight with a rabbit anti-NeuN antibody (1:1000, Abcam, Cambridge, UK) at 4°C. Then, the sections were incubated with an HRP-conjugated antirabbit secondary antibody (1:10000, Abcam, Cambridge, UK) for one hour. To visualize the antigen–antibody reaction, the slides were incubated with DAB (Abcam, Cambridge, UK) for 20 minutes.

Mounted sections were photographed with a digital camera attached to a light microscope. The number of the brown dark cells that expressed the NeuN was counted.

3.2. Data Analysis

Statistical analyses were performed with SPSS 20 software and a one- way ANOVA followed by a Tukey's multiple comparison tests. Data was expressed as mean ± SEM, and a value of $P < 0.05$ was considered to be statistically significant.

4. Results

Figure 1A-E show the coronal sections of the cells in the hippocampus. The brown dark cells express the NeuN. Our results showed a significant time-dependent reduction in NeuN expression in the hippocampus by MDMA (Figure 2) when compared with the control group ($P < 0.01$ for 14 and 28 days and $P < 0.001$ for 60 days). Additionally, there was a significant difference between the 7- and 60-day MDMA groups ($P < 0.01$).

5. Discussion

The major finding of this study was that MDMA administration caused a decrease in NeuN expression in the hippocampus. In a study that was consistent with our results, Canales et al. showed that MDMA treatment produced a decrease in the Brdu+ cells in the adult DG (9). Moreover, they found that DCX expression as a nerve cell marker was significantly reduced in the DG of subjects in an MDMA group in comparison to a control group.

Another study suggested that morphine affects the transition of neural progenitor/stem cells to immature neurons via a mechanism involving NeuroD1 (13). Many intrinsic factors, such as neurotropic factors, neurotransmitters, and steroids, have been reported to influence hippocampal neurogenesis (14). In contrast to the enhancement of neurogenesis due to neurotropic factors, morphogenesis, physical activity, and learning (see the Lieberwirth et al. review), the activation of the inhibitory GABA neurotransmitter system downregulates cell proliferation and accelerates the synaptic integration of adult-generated hippocampal neurons (15). On the contrary, serotonin upregulates hippocampal neurogenesis. Studies have shown the downregulation of hippocampus neurogenesis following the inhibition of serotonin synthesis and the lessening of the raphe nuclei (16).

Taken together, MDMA may inhibit the serotonin system, may increase the GABA neurotransmitter, and might be implicated in the downregulation of NeuN as a biomarker for neurons in the hippocampus. Our results suggest a negative correlation between MDMA administration and adult hippocampal neurogenesis.

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Footnotes

Authors' Contribution: Fahimeh Ghasemi Moravej and Sara Soleimani Asl contributed to study design and conducted the experiments. Iraj Amiri and Siamak Shahidi contributed to manuscript writing and editing. Mehdi Mehdizadeh contributed to the study design. Asrin Rahimi conducted the experiments. Banafsheh Esmaeilzadeh contributed to data analysis.

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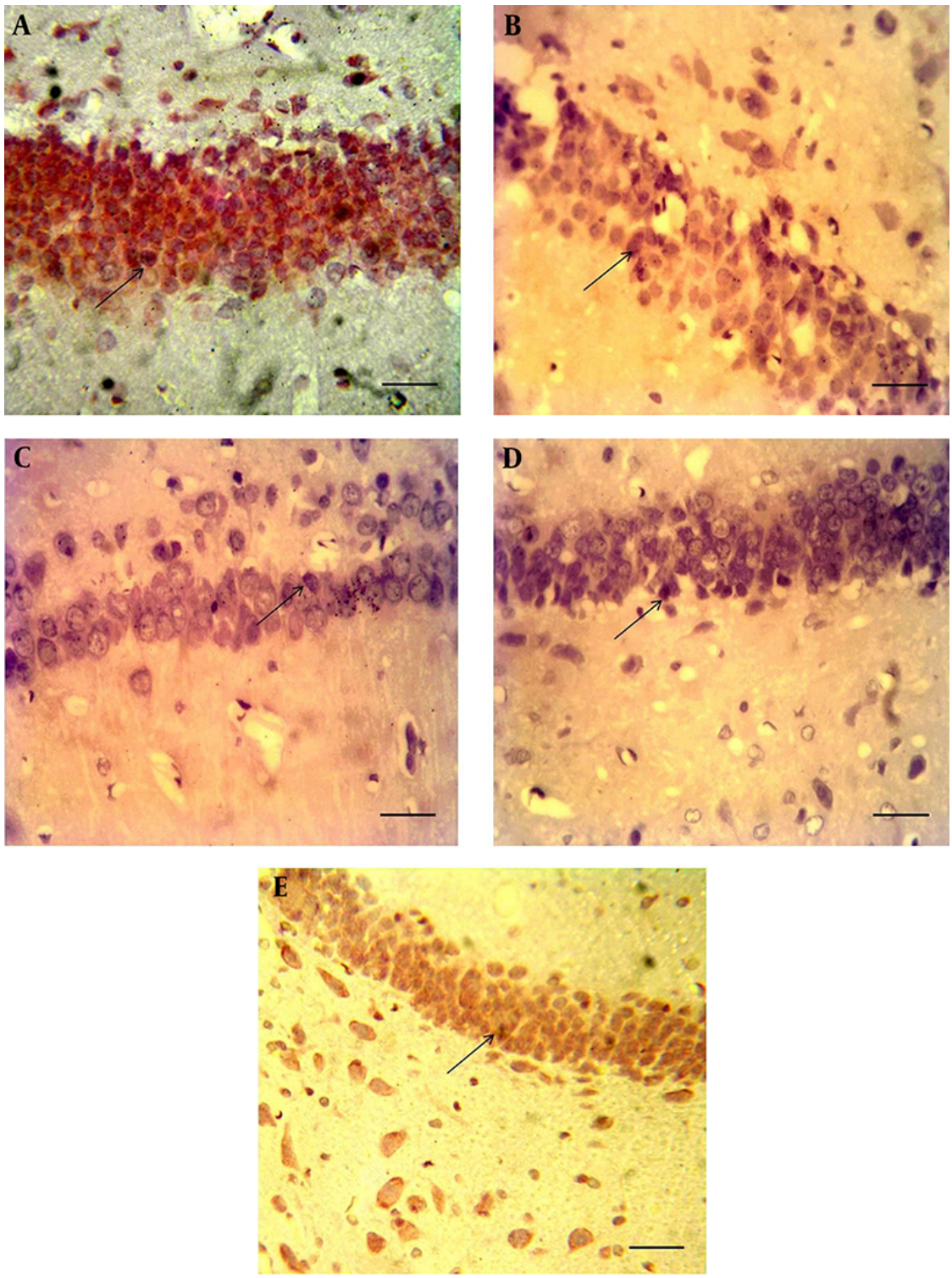

Figure 1. Photomicrograph of the Coronal Section of the Hippocampus in the Control (A), 7-day (B), 14-day (C), 28-day (D), and 60-day (E) groups.

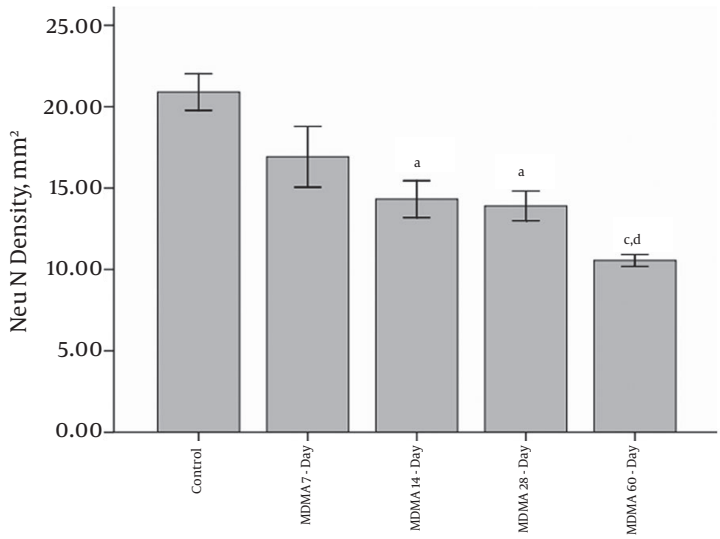

Figure 2. Mean Value (± SEM) of NeuN-Positive Cell Density in the Hippocampus (a: P < 0.01 and b: P < 0.001 vs. control, c: P < 0.01 vs. the 7-day group).

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References

1. Baumann MH, Wang X, Rothman RB. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. *Psychopharmacology (Berl).* 2007;**189**(4):407–24. doi: 10.1007/s00213-006-0322-6. [PubMed: 16541247].
2. Grob CS, Poland RE, Chang L, Ernst T. Psychobiologic effects of 3,4-methylenedioxymethamphetamine in humans: methodological considerations and preliminary observations. *Behav Brain Res.* 1996;**73**(1-2):103–7. [PubMed: 8788485].
3. Asl SS, Pourheydar B, Dabaghian F, Nezhadi A, Roointan A, Mehdizadeh M. Ecstasy-induced caspase expression alters following ginger treatment. *Basic Clin Neurosci.* 2013;**4**(4):329–33. [PubMed: 25337365].
4. Asi S, Farhadi H, Naghdi N, Choopani S, Samzadeh-Kermani A, Mehdizadeh M. Non-acute effects of different doses of 3, 4-methylenedioxymethamphetamine on spatial memory in the Morris water maze in Sprague–Dawley male rats. *Neural Regen Res.* 2011;**6**(22):1715–9.
5. Lyles J, Cadet JL. Methylenedioxymethamphetamine (MDMA, Ecstasy) neurotoxicity: cellular and molecular mechanisms. *Brain Res Brain Res Rev.* 2003;**42**(2):155–68. [PubMed: 12738056].
6. Couillard-Despres S, Iglseder B, Aigner L. Neurogenesis, cellular plasticity and cognition: the impact of stem cells in the adult and aging brain–a mini-review. *Gerontology.* 2011;**57**(6):559–64. doi: 10.1159/000323481. [PubMed: 21311170].
7. Arguello AA, Harburg GC, Schonborn JR, Mandyam CD, Yamaguchi M, Eisch AJ. Time course of morphine's effects on adult hippocampal subgranular zone reveals preferential inhibition of cells in S phase of the cell cycle and a subpopulation of immature neurons. *Neuroscience.* 2008;**157**(1):70–9. doi: 10.1016/j.neuroscience.2008.08.064. [PubMed: 18832014].
8. Pettit AS, Desroches R, Bennett SA. The opiate analgesic buprenorphine decreases proliferation of adult hippocampal neuroblasts and increases survival of their progeny. *Neuroscience.* 2012;**200**:211–22. doi: 10.1016/j.neuroscience.2011.10.039. [PubMed: 22079577].
9. Canales JJ, Ferrer-Donato A. Prenatal exposure to alcohol and 3,4-methylenedioxymethamphetamine (ecstasy) alters adult hippocampal neurogenesis and causes enduring memory deficits. *Dev Neurosci.* 2014;**36**(1):10–7. doi: 10.1159/000356820. [PubMed: 24457993].
10. Mullen RJ, Buck CR, Smith AM. NeuN, a neuronal specific nuclear protein in vertebrates. *Development.* 1992;**116**(1):201–11. [PubMed: 1483388].
11. Shariati MB, Sohrabi M, Shahidi S, Nikkhah A, Mirzaei F, Medizadeh M, et al. Acute Effects of Ecstasy on Memory Are more Extensive than Chronic Effects. *Basic Clin Neurosci.* 2014;**5**(3):225–30. [PubMed: 25337384].
12. Alipanahzadeh H, Soleimani M, Soleimani Asl S, Mehdizadeh M. The Effect of TGF-alpha on Neurogenesis in Subventricular Zone of Rat Brain after Ischemia-Reperfusion. *Basic Clin Neurosci.* 2012;**3**(2):12–5.
13. Zhang Y, Xu C, Zheng H, Loh HH, Law PY. Morphine Modulates Adult Neurogenesis and Contextual Memory by Impeding the Maturation of Neural Progenitors. *PLoS One.* 2016;**11**(4):0153628. doi: 10.1371/journal.pone.0153628. [PubMed: 27078155].
14. Cameron HA, Hazel TG, McKay RD. Regulation of neurogenesis by growth factors and neurotransmitters. *J Neurobiol.* 1998;**36**(2):287–306. [PubMed: 9712310].
15. Lieberwirth C, Pan Y, Liu Y, Zhang Z, Wang Z. Hippocampal adult neurogenesis: Its regulation and potential role in spatial learning and memory. *Brain Res.* 2016;**1644**:127–40. doi: 10.1016/j.brainres.2016.05.015. [PubMed: 27174001].
16. Radley JJ, Jacobs BL. 5-HT1A receptor antagonist administration decreases cell proliferation in the dentate gyrus. *Brain Res.* 2002;**955**(1-2):264–7. [PubMed: 12419546].