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Ecstasy, anxiety and rat hippocampal astrocytes

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SUMMARY

Ecstasy (MDMA) is a popular drug a used recreationally with the rave culture and consumed in a high environment temperature. Repeated and prolonged MDMA ingestion is well known to cause depression, anxiety and aggression. The aim of this study was to evaluate the sub-chronic effects of MDMA on anxiety in Wistar rats and to determine astrocytes density in the rat hippocampus after anxiety.

In this study, 28 adult male Wistar rats were used. The animals were distributed randomly in four groups, one sham group (receiving 1 ml/kg 0.9% saline solution) and three experimental groups: Exp. 1 (1.25 mg/kg/day MDMA), Exp. 2 (2.5 mg/kg/day MDMA), and Exp. 3 (5 mg/kg/day MDMA). The animals received Saline or MDMA for a week (sub-chronic period). An Elevated Plus Maze apparatus was used to examine anxiety levels in the rats. 24 h. after the last injection and behavioral test, the rat brains were withdrawn and fixed with 4% paraformaldehyde, and then – after histological processing – the slices of hippocampus were stained with PTAH for astrocytes.

Our results showed that MDMA at 2.5 mg/kg/day for a week was most effective in causing anxiety. We found that the number of astrocytes was increased after this period. The greatest increase in astrocyte numbers was observed in the dentate gyrus of the5 mg/kg MDMA group.

We concluded that the administration of MDMA over 7 days (sub-chronic period) can cause anxiety and can have an effect on the astrocyte density of the rat hippocampus.

Key words: Ecstasy – Anxiety – Hippocampus – Astrocyte – Rat

INTRODUCTION

Ecstasy is one of the most dangerous drugs threatening young people today. Ecstasy or 3,4-methylenedioxymethamphetamine (MDMA) is a drug used recreationally, is often associated with the rave culture and is consumed in a high environment temperature (Green et al., 2003). The neurotoxicity of MDMA has been reported in the rodent hippocampus (Stone et al., 1987; Elayan et al., 1992; Sanchez et al., 2004; Miranda et al., 2007) and cerebral cortex (Sanchez et al., 2004; Colado et al., 1999; O'Shea et al., 2005; Capela et al., 2007).

Our knowledge of the neurobiology of MDMA is growing rapidly. Early MDMA

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administration increases the levels of oxidative species, producing both lipid peroxidation and a depletion of antioxidant systems in specific brain regions, including the neostriatum (Gibb et al., 1990; Jayanthi et al., 1999). An increase in the production of oxidative species within affected neurons is considered a critical step in mediating MDMA neurotoxicity (Gibb et al., 1990). In particular, recent studies have demonstrated that MDMA produces an increase in the levels of free radical, which are involved in the neurotoxic effects (Green et al., 2003).

The effects of MDMA vary according to the dose and the frequency and duration of use (Kalant, 2001). In human post mortem tissue, a distinct immunopositive reaction for MDMA and MDA was observed in the white matter of all cortical regions and in neurons of the basal ganglia, the hypothalamus, the hippocampus, and the cerebellar vermis, but a relatively weak staining of neurons in the brainstem was seen (De Letter et al., 2003). In rodents, repetitive doses of MDMA produce oxidative stress, DNA single- and double-strand breaks, and long-lasting metabolic changes in the hippocampus, associated with an increased susceptibility to limbic seizures (Frenzilli et al., 2007).

MDMA-induced neurotoxicity in rats is usually determined 7 days post-treatment (Battaglia et al., 1988; Colado et al., 2001, Logan et al., 1988, Ricaurte et al., 2000). When neuronal injury is accompanied by reactive microgliosis, this glial response is maximal 2-3 days post-treatment and gradually decreases. Recovery can be achieved about 7 days post-treatment (Pubill et al., 2003).

Although astrocytes play an essential role in the maintenance of BBB, the regulation of glutamatergic neurotransmission, learning and memory, little is known about the alterations of these cells in human drug abusers (Miguel-Hidalgo, 2009; Sorensen and Lawrence, 2009; Jahanshahi et al., 2008).

Some studies indicate that hypertrophy of astrocytes is accompanied by neuronal injury, and can lead to an enhanced expression of GFAP (Ádori et al., 2006; Aguirre et al., 1999), A few studies have reported an increase in GFAP expression in the context of MDMA-induced neurotoxicity (Ádori et al., 2006; Pubill et al., 2003; O'Callaghan and Miller, 1994), whereas others have failed to observe this (Wang et al., 2004; Orio et al., 2004; Straiko et al., 2007).

Therefore, the aim of this study was to examine the long-term effects of MDMA on memory and the rats' hippocampus neurons and astrocyte number.

Materials and Methods

Twenty-eight adult male Wistar rats weighing 200-250 g, (Pasteur institute, Tehran, Iran) were used in this study. They were given free access to normal laboratory chow and water. The temperature of the animal house was 22 ± 3°C.

Rats were randomly distributed in four groups: a sham group received 0.9% saline solution (1 ml/kg) and three experimental groups received: Exp. 1 (7 × 1.25 mg/kg/day MDMA + Plus Maze test), Exp. 2 (7 × 2.5 mg/kg/day MDMA + Plus Maze test), and Exp. 3 (7 × 5 mg/kg/day MDMA + Plus Maze test). In each group at least seven rats were used. All animals received seven intra-peritoneal injections over a week. MDMA (Sigma-Aldrich, Germany) was dissolved in normal saline (0.9%).

An elevated plus maze apparatus was used to examine anxiety in rats. This apparatus consisted of two open arms (50 × 10 × 1 cm) across from each other and perpendicular to two closed arms (50 × 10 × 30 cm). The open arms had a very small (1 cm) wall to decrease the number of falls, whereas the closed arms had a high (30 cm) wall to enclose the arm. The entire apparatus was 50 cm above the floor. Behavioral testing was performed between 09:00 AM and 18:00 PM. All the experimental rats were transferred to the behavior-testing room 60 min prior to beginning the first trial to habituate them to the conditions of the behavior-testing room.

The rats were placed in the center area of the maze with their heads directed toward a closed arm. Rats were allowed to move freely about the maze for 5 min. The number of entries (an entry was defined as the center of mass of the mouse entering the arm) into each arm and the time spent in the open arms were recorded and these measurements served as an index of anxiety-like behavior. The distance traveled, the number of entries into each arm, the time spent in each arm, and the percent of entries into the open arms were calculated.

24 hours after the last injection and after the behavioral test, all rats were anesthetized with chloroform and their brains were dissected out and then fixed for two weeks in 4% paraformaldehyde. Different degrees of alcohol were used for dehydration, followed by clarification in xylol. After histological processing, the tissue was impregnated and then embedded in paraffin wax.

Eight-μm coronal sections were collected serially from Bregma –3.30 mm to –6.04 mm of the hippocampal formation (Paxinos and Watson, 1998). An interval of 20 μm was placed between each two consecutive sections.

The sections were stained with phosphotungstic acid haematoxylin (PTAH) stain (Specific staining for astrocytes) (Bancroft and Gamble, 2004; Jahanshahi et al., 2009, 2011). A photograph of each section was obtained using an Olympus BX 51 microscope and a DP 12 digital camera under a magnification of 400. An area of 30,000 $μm^2$ was selected randomly in all areas of the hippocampal formation. To measure the area density of the astrocytes, the images were transferred onto a computer. Using OLYSIA Autobioreport software, Olympus Co., the appropriate grids were superimposed over the pictures and the cells were counted manually.

The experimental results from this study were evaluated using SPSS v.16 and expressed as means ± SD. To compare the means of the parameters measured in the four groups by analysis of variance test, after confirmation of normality, the means were compared with the ANOVA Post-Hoc Tukey test; $p < 0.05$ was considered significant.

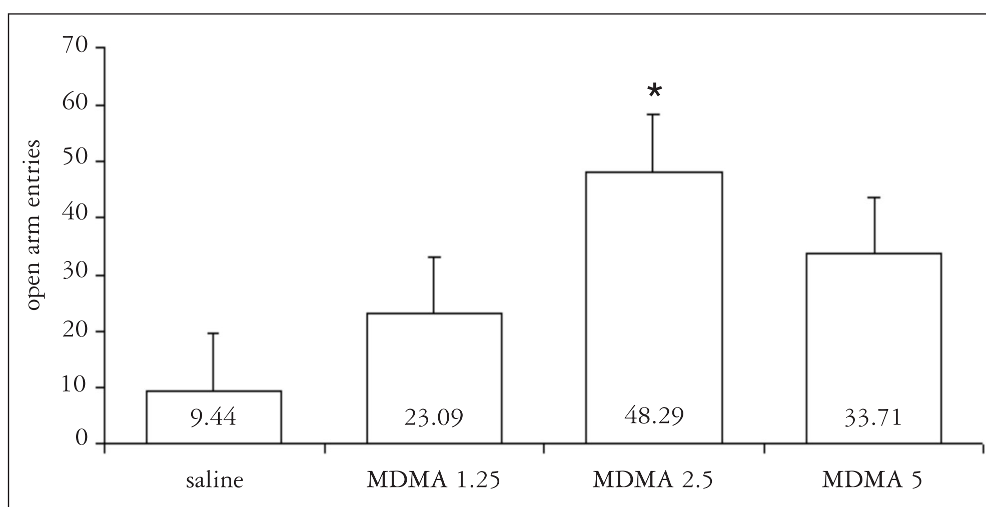

Fig. 1. Average percentage of open arm entry, the most increase of open arm entries as an index for decrease of anxiety showed in 2.5 mg/kg group, the differences was significant (P<0.05).

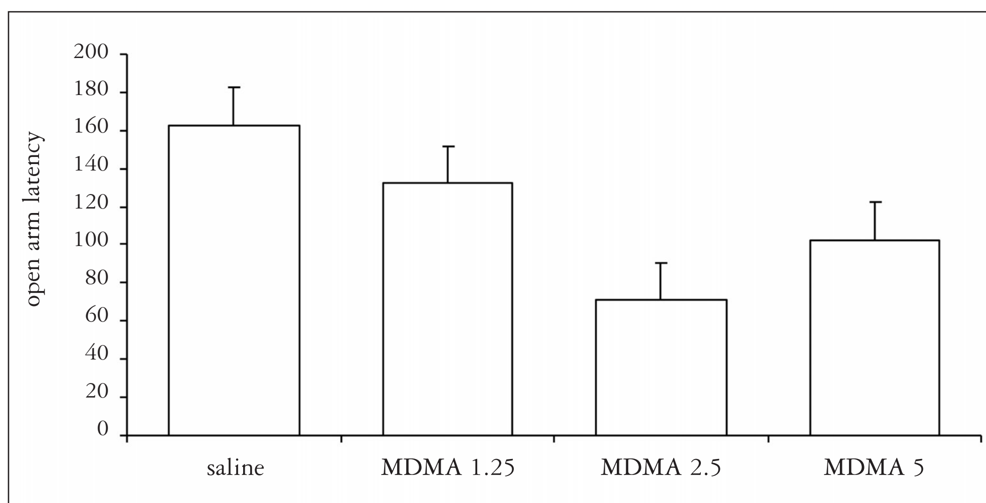

Fig. 2. Delay in entering the open arm. The least delay in open arm entries as an index of a decrease in anxiety was observed in the 2.5 mg/kg group.

Results

Behavioral results

The mean percentage of open arm entry is shown in Fig. 1. As may be seen, MDMA increased the percentage of open arm entries in all the experimental groups as compared to the saline group. The greatest increase in open arm entries was observed in the 2.5 mg/kg group.

Delayed entry into open arms is shown in Fig. 2. As seen, delayed entry into open arms decreased in all experimental groups as compared to the saline group. The least delay on entering the open arm was observed in 2.5 mg/kg group, in comparison with the other groups.

Histological results

It was noted that there were significant differences in the number of astrocytes in all regions of the hippocampus and dentate gyrus between the sham and the three experimental groups (Table 1 and Fig. 3). Fig. 3 and Table 1 also show that the number of astrocytes increased significantly after anxiety in the all regions of the rat hippocampus.

The greatest increase in astrocyte numbers was seen in dentate gyrus of the 5 mg/kg MDMA group (Table 1). Also, this increase was dose-dependent in dentate gyrus.

Table 1. Mean and SD of astrocyte numbers in hippocampus (30,000 μm^2).

Groups	CA1 Mean ± SD	CA3 Mean ± SD	DG Mean ± SD
Saline	5.48 ± 2.29	5.98 ± 2.65	7.77 ± 3.42
MDMA (1.25 mg/kg)	8.52 ± 2.76	9.22 ± 3.01	11.95 ± 5.45
MDMA (2.5 mg/kg)	8.27 ± 3.36	10.45 ± 3.80	13.53 ± 8.89
MDMA (5 mg/kg)	7.75 ± 2.88	9.80 ± 3.36	15.40 ± 9.00

Discussion

In this work intra-peritoneal injection of MDMA (over 7 days: a sub-chronic period of time) was administered to study the changes in the number of astrocytes in the hippocampus and dentate gyrus, following the anxiety caused by ecstasy. Our results showed that the administration of MDMA increased the latency of open arm entry and the percentage of open arm entry as compared with the intact (sham-operated) group in the adult male rats. In present study, the number of astrocytes increased in the ecstasy-administered groups *versus* the sham-operated rats.

Our results are similar to those reported by other authors others. For example, in 2010 and 2011 Granado demonstrated that amphetamine-derivatives like methamphetamine or MDMA induce neurotoxicity accompanied by astrogliosis (Granado et al., 2010, 2011a, b).

Another study showed post-MDMA increases in GFAP immunoreactivity in some areas of the brain such as the hippocampus, cortex, and cerebellum in response to a very high dose (40 mg/kg) of the drug administered i.p. (Sharma and Ali, 2008). This finding is similar to our results, indicating a reaction of astrocytes after exposure to some neurotoxic drugs.

Some studies have reported different effects of MDMA. For example, in 2004 and 2005 Wang et al. demonstrated that two weeks after MDMA administration (7.5 mg/kg i.p., q 2h × 3 doses) to male Sprague-Dawley no significant effect on the expression level of SERT and GFAP was observed in any brain region (Wang et al., 2004; Wang et al., 2005).

In 2006, after treatment with 7.5, 15, 30 mg/kg single intraperitoneal doses of MDMA in Dark Agouti rats, Adori et al. found a significant increase in the GFAP immunostaining density of protoplasmic astrocytes only in the CA1 area of the hippocampus (Ádori et al., 2006).

MDMA caused a dramatic, dose-dependent decrease in stem cells and neurons in embryonic cortical primary cell cultures (Kindlundh-Högberg et al., 2010), accompanied with increase of astrocytes.

Some authors have indicated that the effects of ecstasy on cells may be age-related. For example, in 2010 Dzietko showed that MDMA (60 mg/kg) significantly enhanced neuronal death in the brains of 6- day-old rats, but no significant toxicity was detected at the ages of 14 and 21 days. They found that some brain regions strongly affected were the cortex, septum, thalamus, hypothalamus and the CA1 region of hippocampus. Their data suggest that a single injection of MDMA causes neurodegeneration in the neonatal rat brain (Dzietko et al., 2010).

As discussed above, the reactions to ecstasy are different, which could be related to the dose or duration of use. Therefore, it may be con-

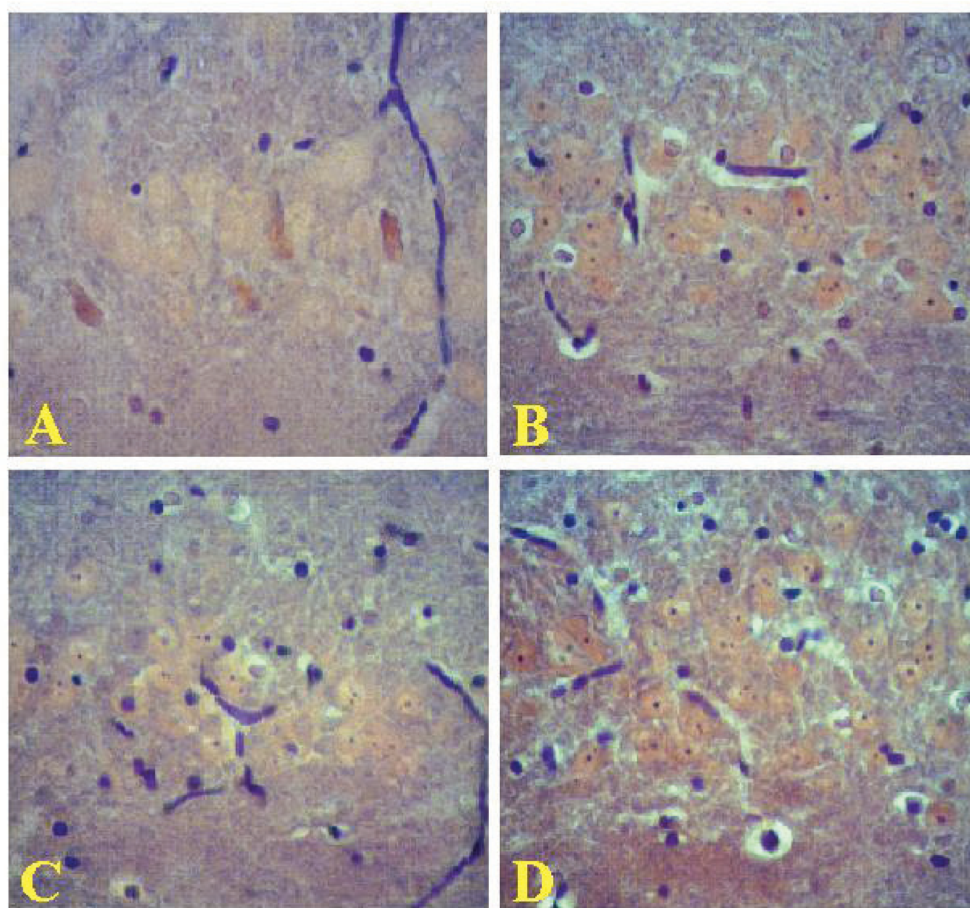

Fig. 3. CA3 area of hippocampal astrocytes in all groups (PTAH staining ×40). **A**: sham, **B**: Exp. 1 (1.25 mg/kg), **C**: Exp. 2 (2.5 mg/kg), **D**: Exp. 3 (5 mg/kg) (purple points are astrocytes).

cluded that sub-chronic (one week) administration of MDMA can cause anxiety and it can increase astrogliosis in the rat hippocampus.

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