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5-HT$_{2A}$ Serotonin Receptor Density in Adult Male Rats' Hippocampus after Morphine-based Conditioned Place Preference

Rabie Mohammadi[1], Mehrdad Jahanshahi[1*], Seyed Behnamedin Jameie[2]

1. Department of Anatomy, Neuroscience Research Center, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
2. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Introduction: A close interaction exists between the brain opioid and serotonin (5-HT) neurotransmitter systems. Brain neurotransmitter 5-HT plays an important role in the regulation of reward-related processing. However, a few studies have investigated the potential role of 5-HT$_{2A}$ receptors in this behavior. Therefore, the aim of the present study was to assess the influence of morphine and Conditioned Place Preference (CPP) on the density of 5-HT$_{2A}$ receptor in neurons of rat hippocampal formation.

Methods: Morphine (10 mg/kg, IP) was injected in male Wistar rats for 7 consecutive days (intervention group), but control rats received just normal saline (1 mL/kg, IP). We used a hotplate test of analgesia to assess induction of tolerance to analgesic effects of morphine on days 1 and 8 of injections. Later, two groups of rats were sacrificed one day after 7 days of injections, their whole brains removed, and the striatum and PFC immediately dissected. Then, the NR1 gene expression was examined with a semi-quantitative RT-PCR method.

Results: Our data showed that the maximum response was obtained with 2.5 mg/kg of morphine. The density of 5-HT$_{2A}$ receptor in different areas of the hippocampus increased significantly at sham-morphine and CPP groups ($P<0.05$). On the other hand, the CPP groups had more 5-HT$_{2A}$ receptors than sham-morphine groups and also the sham-morphine groups had more 5-HT$_{2A}$ receptors than the control groups.

Conclusion: We concluded that the phenomenon of conditioned place preference induced by morphine can cause a significant increase in the number of serotonin 5-HT$_{2A}$ receptors in neurons of all areas of hippocampus.

1. Introduction

Opiates like morphine, and psycho-stimulants such as methamphetamine and cocaine, cause addiction in humans and induce addiction-related behaviors such as behavioral sensitization, drug self-administration, and Conditioned Place Preference (CPP) in rodents. Although these drugs act through different mechanisms, they activate common neural pathways and neurotransmitters that produce their

*** Corresponding Author:**
Mehrdad Jahanshahi, PhD
Address: *Department of Anatomy, School of Anatomy, Neuroscience Research Center, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran.*
Tel: *+98 (17) 32420515*
E-mail: *mejahanshahi@yahoo.com*

rewarding effects (Nakagawa, Fujio, Ozawa, Minami, & Satoh, 2005). Some studies suggest that the hippocampus is important for reward-related learning tasks like CPP (Liu, Jiang, Zhong, Wu, & Luo, 2010), which is used in animal studies to evaluate preferences for environmental stimuli associated with a positive or negative reward (Rezayof, Zarrindast, & Sahraie, 2003).

5-HT_{2A} receptor, one of 14 subtypes of 5-HT (5-hydroxytryptamine) receptors (Hoyer, Hannon, & Martin, 2002), is a G protein-coupled receptor (Berg et al., 1994). This receptor is widely distributed in the CNS; The highest expression is found in the cortex, claustrum, hippocampus, hypothalamus, basal ganglia (Pompeiano, Palacios, & Mengod, 1994; Buhot, 1997; Leysen, 2004), ventral striatum, and amygdala (Millan, 2003). In the hippocampus, 5-HT_{2A} receptor is found in pyramidal cells of CA_{1-3} regions and granular cells of the dentate gyrus (Xu & Pandey, 2000). The functional significance of the 5-HT_{2A} receptor in the hippocampus has received less attention compared to the cortical receptor (Lüttgen, Ögren, & Meister, 2004). There is a close interaction between the brain opioid and serotonin neurotransmitter systems (Bardon & Ruckebusch, 1984; Tao & Auerbach, 1994; Marek, 2003; Zhao et al, 2007). μ-Opioid receptor agonists such as morphine have been reported to increase 5-HT levels in the brain (Tao & Auerbach, 1994).

A rich review of animal literature has shown that the brain neurotransmitter 5-HT plays an important role in the regulation of reward-related processing. For instance, 5-HT is involved in natural reward-related physiology and behavior, from feeding to sexual activity (Pfaus, 2009; Wirtshafter, 2001). An extensive research has implicated serotonergic system in depression (Trivedi et al., 2006), anxiety (Hood et al., 2010), schizophrenia (Emsley, 2009), and addiction (Rothman, Blough, & Baumann, 2008).

Nonetheless, there is an incomplete understanding of the pharmacological mechanisms underlying the role of 5-HT in reward-related processing. Also a few studies have investigated the potential role of 5-HT_{2A} receptors in reward-related behaviors. More research is necessary to investigate the role of this receptor in place conditioning, which may contribute to a better understanding of its potential role in the conditioned effects of drugs abuse (Hayes & Greenshaw, 2011). Since both opioids (like morphine) and 5-HT_{2A} receptors appear to be involved in similar biological processes (Adriaens et al., 2012; Adriaens et al., 2014), the aim of the present study was to assess the influences of morphine and CPP on the density of 5-HT_{2A} receptor in neurons of rat hippocampal formation.

2. Methods

2.1. Animals

Adult male Wistar rats (Obtained from Pasteur Institute, Iran) weighing 200-220 g were used in this study. They were kept in an animal house with a 12:12 h light/dark cycle (light beginning at 7:00 a.m.) and controlled temperature (22±2°C). They were given free access to chow and tap water. All experiments were carried out during the light phase and all the procedures were done in accordance with the institutional guidelines for the care and use of laboratory animals.

2.2. Experimental design

Fifty-six adult male Wistar rats were randomly distributed to one of the following 8 groups:

Control group: without receiving any drugs and CPP test;

Control-saline group: receiving saline (1 mL/kg) on alternate sessions for 3 days (twice daily in the morning and evening sessions) together with CPP test;

Three sham groups: receiving morphine with different doses (2.5, 5, and 10 mg/kg) and saline (1 mL/kg) in alternate sessions for 3 days (twice a day in the morning and evening sessions), without CPP test;

Three experimental groups: receiving morphine with different doses (2.5, 5, and 10 mg/kg) and saline (1 mL/kg) on alternate sessions for 3 days (twice daily in the morning and evening sessions) with CPP test.

Seven animals were used in each group and each animal was used only once. All drugs were injected subcutaneously and morphine sulfate (TEMAD, Iran) was dissolved in sterile saline (NaCl, 0.9%) at final concentrations of 2.5, 5, and 10 mg/kg.

2.3. Conditioning place preference paradigm

2.3.1. Apparatus

The CPP apparatus was prepared based on Carr and White study (1983) with a minor modification and 3 wooden compartments. Compartment A and B were identical in size (30×30×40 cm) but similar in shading and texture. The compartment A had horizontal stripes on the walls with textured floor and the other had vertical wall stripes

with smooth floor (Shaabani, Jahanshahi, Nowrouzian, Sadeghi, & Azami, 2011). Compartment C (15×30×40 cm) was painted red and attached to the rear of compartments A and B; It had a removable wooden partition that separated it from the other compartments. When the partition was removed, the animal could freely move between the two compartments (A and B) via compartment C (Zarrindast, Azami, Rostami, & Rezayof, 2006).

2.3.2. Behavioral testing

CPP was conducted using a biased procedure (Prus, James, & Rosecrans, 2009) that consisted of a 5-day schedule with 3 distinct phases: preconditioning, conditioning, and testing.

2.3.2.1. Preconditioning phase

In pre-conditioning day 1, each animal was placed in the compartment C with the guillotine door removed and rats were allowed to move freely in all compartments for a 15-min period.

2.3.2.2. Conditioning phase

Conditioning phase (from the second to the fourth day) started the day after pre-conditioning test and animals received morphine and saline, twice daily (morning and evening session), according to the model as follows:

On the first day in the morning session (9:00 a.m.), rats received morphine subcutaneously and were placed in the black compartment (B) for 45 minutes. Six hours later (15:00 p.m., evening session), rats received saline subcutaneously and were placed in the white compartment (A) for 45 minutes. On the second day, rats received the saline injections in the morning session and morphine in the evening session. The third day of conditioning had the same schedule as the first day. On each of these days, during these sessions, the animals were confined to one compartment by closing the removable wall.

2.3.2.3. Testing phase

The testing stage was carried out on day 5 (1 day after the last conditioning session). No injection was given on the testing phase. For testing, the rats were placed in compartment C with the guillotine door removed and rats were allowed to move freely in all compartments for 15 minutes. The time spent in each compartment was measured by a stopwatch and the change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the testing day, and the time spent in this compartment in the preconditioning day (Shaabani, Jahanshahi, Nowrouzian, Sadeghi, & Azami, 2011).

2.3.3. Locomotion activity

Locomotion activity was simultaneously recorded throughout preconditioning and testing stages for rats that received place conditioning, using the CPP apparatus. To measure the locomotion activity, the ground areas of the CPP compartments (A and B) were divided into 4 equal sized squares. Locomotion was measured as the number of crossings from one square to another during 15 minutes (Rezayof, Razavi, Haeri-Rohani, Rassouli, & Zarrindast, 2007). The unit of measurement for locomotion activity was counted every 15 minutes.

2.4. Histology

Forty-eight hours after completion of experimental sessions, animals were deeply anesthetized with chloroform and transcardially perfused with 0.9% saline and 4% paraformaldehyde (Merck) solution. After perfusion, the brains were removed carefully and fixed for 2 weeks in 4% paraformaldehyde solution. Different degrees of alcohol were used for dehydration followed by clarification with xylol. After histological processing, tissue was impregnated and then embedded in paraffin wax. Using rotary microtome (MK 1110) 6-µm thick coronal sections were serially collected from the bregma –3.30 mm to –6.04 mm of the hippocampal formation (Paxinos & Watson, 1998). An interval of 20 µm was placed between each two consecutive sections.

The brain slides were stained with antibody against 5-HT_{2A} serotonergic receptor according to our previous report (Moghadami, Jahanshahi, Sepehri, & Amini, 2016). The sections were first incubated for 30 minutes at 37°C, then, the sections deparaffinized in 2 changes of xylene, 5 minutes each. Next, the sections hydrated in graded series of ethanol (absolute, 95%, 80%). Then sections rinsed with distilled water and the sections were placed in epitope retrieval solution for (IHC World) 20 minutes at 90°C-95°C and let it stand at room temperature for 20 minutes and washed two times for 5 minutes in washing buffer (PBS/Tween 20) (Dako) in 0.1% Triton X-100 (Sigma-Aldrich). The sections blocked with a peroxidase blocking solution (3% H_2O_2 in PBS) for 10 minutes at room temperature and then washed two times for 5 minutes in washing buffer. Afterwards, endogenous biotin blocked by Avidin/Biotin blocking solution (IHC World) for 30 minutes at room temperature and washed in PBS for 10 minutes. Next, sections were incubated with 1% bovine serum (Dako)

for 60 minutes at 37°C and followed by incubation with Rabbit polyclonal antibody (1:500, Anti-5-HT_{2A} Receptor antibody, Abcam) overnight at room temperature. After washing with washing buffer, the sections were incubated with secondary antibody (Biotinylated Goat Anti-Rabbit IgG antibody, Abcam) for 60 minutes at 37°C (Figure 1).

2.5. Morphometry

A photograph of each section was taken using a fluorescent microscope (Olympus BX 51, Tokyo, Japan) and a DP 12 digital camera (Olympus) under a magnification of 40X. Using OLYSIA Autobioreport software, the appropriate grids were superimposed on the pictures and the cells were counted manually in 30000 μm^2 for CA_1, CA_3, and DG areas of the hippocampus in the all sections. To perform an unbiased measurement, the examiner was double-blinded and only neurons with significant $5HT_{2A}$ neurons characteristics were counted (Jahanshahi, Sadeghi, & Hosseini, 2006; Emamian et al., 2010; Jahanshahi, Khoshnazar, Azami, & Heidari, 2011; Jahanshahi, Nickmahzar, Seif-hoseini, Babakordi, & Moharreri, 2013).

2.6. Statistical analysis

All data were statistically analyzed using SPSS v.16. Parametric distribution of data was assessed by Kolmogorov-Smirnov test. The means of the parameters measured in the 8 groups compared with 1-way analysis of variance (ANOVA) and post hoc LSD (least significance difference) test. Significance was determined at $P<0.05$.

3. Results

3.1. Behavioral results

Table 1 shows CPP induced by subcutaneous administration of morphine (2.5, 5, and 10 mg/kg). Significant conditioning was observed at low doses of morphine (2.5 and 5 mg/kg). A dose of 2.5 mg/kg of morphine had the greatest preference (175.29 s) and minimum preference was observed by administration of 10 mg/kg of morphine (89.29 s). We found that increasing the dose of morphine does not help with conditioning. Also, different doses of morphine had no significant effect on the locomotion activity compared to the control-saline group (Table 1).

3.2. Histological results

Histological results showed that there was not any significant difference between control-saline and control groups in the number of 5-HT_{2A} receptors in all regions of the hippocampus (Figures 2-4), but we found that the number of 5-HT_{2A} receptors increased after CPP in almost all areas of hippocampus.

Injection of morphine with doses of 5 and 10 mg/kg (without CPP) increased significantly the number of 5-HT_{2A} receptors in the CA_1 area (Figure 2). Also the number of this receptor had significant changes in morphine–induced CPP by administration of doses of 5 and 10 mg/kg of morphine ($P<0.01$). Among experimental groups, morphine with a dose of 5 mg/kg had the maximum number of this receptor.

In the CA_3 area of hippocampus (Figure 3), after injection of morphine ($P<0.05$) and also CPP test ($P<0.001$) the number of 5-HT_{2A} receptors increased significantly in comparison with the control group. The lowest number of this receptor was observed in sham 2.5 mg/kg group.

As we showed in Figure 4, administration of just morphine or in combination with CPP test increased number of 5-HT_{2A} receptors in DG (dentate gyrus) area. The most significant increase in the number of this receptor was observed in the CPP group receiving 5 mg/kg dose of morphine ($P\leq0.01$).

4. Discussion

Our findings in this study indicated that injection of different doses of morphine induces CPP. The maximum preference was observed with 2.5 mg/kg of the morphine. Other studies showed the administration of morphine induced CPP in a dose-dependent manner. For example, Sharifzadeh et al. (2006) showed a dose-response curve for CPP induced by morphine (1, 3, 6, and 9 mg/kg) in rats. The maximum response was obtained with 6 mg/kg of morphine (Sharifzadeh et al., 2006).

Other studies demonstrated that morphine at different doses caused a significant dose-related preference and the maximum response was obtained with 5 mg/kg of morphine (Rezayof, Razavi, Haeri-Rohani, Rassouli, & Zarrindast, 2007; Moaddab, Haghparast, & Hassanpour-Ezatti, 2009; Jahanshahi, Shaabani, Nikmahzar, & Babakordi, 2014).

Likewise, the present study demonstrated that different doses of morphine had no significant effect on the locomotion activity during 15-min test period compared to control-saline group. In agreement with our data, some studies indicated that morphine-induced

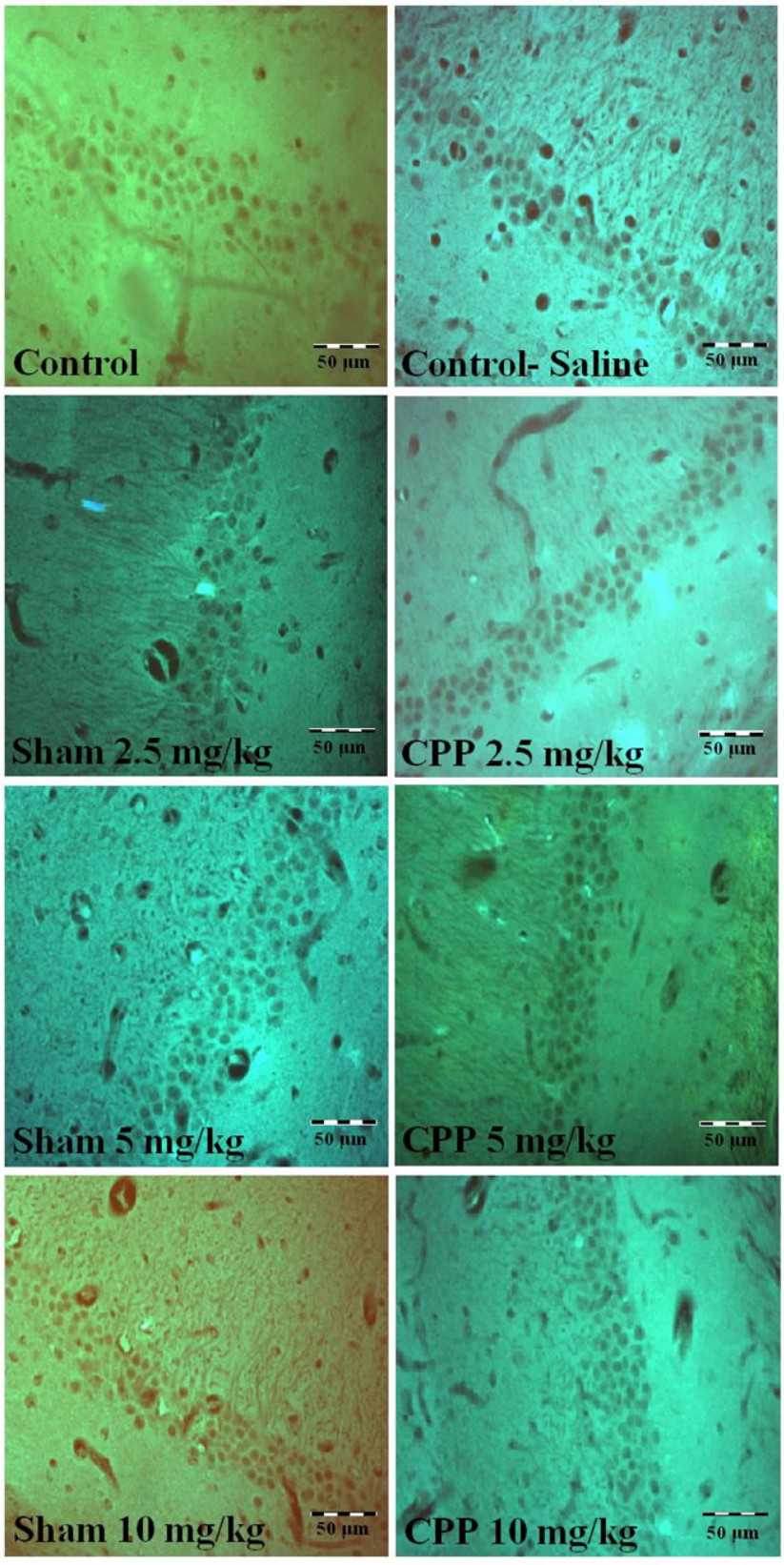

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Figure 1. 5-HT_{2A} positive neurons in the CA_1 area of hippocampus in all groups. Immunostaining image 40X magnification, Scale bar: 50 µm.

Table 1. Preconditioning, Conditioning time, and Conditioning score in black and white chambers.

			Pre-conditioning		Conditioning		Conditioning score	
			Black	White	Black	White	Black	White
Control-saline		Time (s)	88.85	199.57	145.42	189.42	56.57	-10.15
		Locomotion activity	15.14	24	26.28	22.42	26	22
Morphine (mg/kg)	2.5	Time	190.28	172.57	365.57	179.85	175.29	7.28
		Locomotion activity	23.71	22	21	19.42	21	19
	5	Time	166.57	233.71	313.71	106.42	147.14	-127.29
		Locomotion activity	25.85	32.14	23.71	13.58	23	13
	10	Time	163.85	209.42	253.14	196	89.29	-13.42
		Locomotion activity	22.71	26.42	20.57	17.71	20	17

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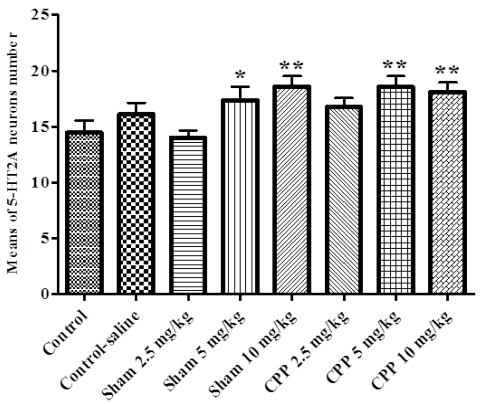

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Figure 2. 5-HT_{2A} neuron number in CA_1 area of hippocampus (in blocks of 30000 µm²).

Data are presented as mean. *P<0.05 and **P<0.01 different from the control group.

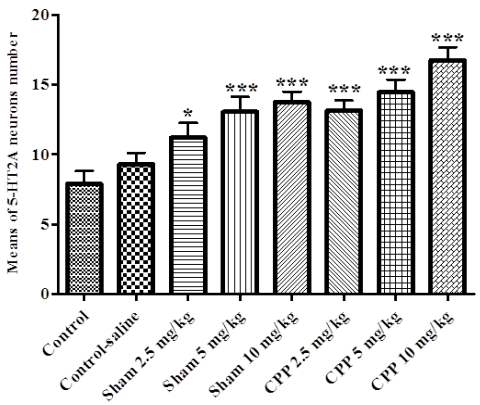

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Figure 3. 5-HT_{2A} neuron number in CA_3 area of the hippocampus (in blocks of 30000 µm²).

Data are presented as mean. *P<0.05 and ***P<0.001 different from the control group.

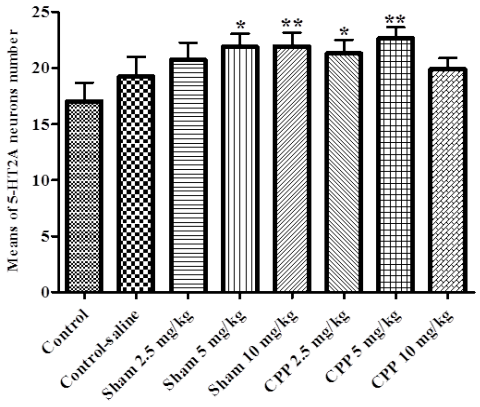

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Figure 4. 5-HT_{2A} neuron number in DG area of the hippocampus (in blocks of 30000 µm²).

Data are presented as mean. *P<0.05 and **P≤0.01 different from the control group.

CPP had no significant effect on the locomotion activity (Sharifzadeh et al., 2006; Moaddab, Haghparast, & Hassanpour-Ezatti, 2009; Jahanshahi, Shaabani, Nikmahzar, & Babakordi, 2014).

Our histological findings demonstrated that the number of 5-HT_{2A} receptors increased due to morphine injection and CPP test in hippocampus. On the other hand, injection of saline cannot change the density of this receptor. Indeed the present study confirmed the relation between morphine-induced CPP and density of 5-HT_{2A} receptor in neurons of rat hippocampal formation.

In addition to, increased reward was seen with CPP following administration of drugs that increase brain 5-HT (Subhan, Deslandes, Pache, & Sewell, 2000). As we have shown in this study, morphine-induced CPP could increase the number of 5-HT_{2A} receptors in the CA_1, CA_3, and DG areas of hippocampus.

Our findings showed that the injection of morphine, alone (in sham groups), increased the number of 5-HT_{2A} receptors in hippocampus in a dose-dependent manner. In agreement with these results, Gulati and Bhargava indicated that implanted morphine pellets were associated with an up-regulation of 5-HT_2 receptors in the cortex of rats and increase in 5-HT_2 receptor binding in the amygdala, midbrain, and brain stem of rats (Gulati & Bhargava, 1988; Gulati & Bhargava, 1989).

Also in our previous studies, we showed that CPP is able to affect the hippocampal cell density. For instance, both injections of morphine and CPP can decrease the density of neurons in the female rat hippocampus (Jahanshahi, Shaabani, Nikmahzar, & Babakordi, 2014), but the number of astrocytes increases after CPP in male (Shaabani, Jahanshahi, Nowrouzian, Sadeghi, & Azami, 2011) and female Wistar rats (Jahanshahi, Shaabani, Nikmahzar, & Babakordi, 2014).

Also in recent years, some researchers have studied the influence of morphine on cerebral 5-HT_{2A} receptor availability in dogs; their results indicated that a single dose of morphine decreased 5-HT_{2A} receptor binding index in the frontal cortex (Adriaens et al., 2012), but prolonged morphine exposure lowered the 5-HT_{2A} receptor binding index in frontal, temporal, and parietal cortex as well as subcortical region (Adriaens et al., 2014). These data provide further evidence for the interaction between the opioid and serotonergic system and encourages further research on the possible beneficial effects of opioids in the treatment of mood disorders (Adriaens et al., 2014). Apparently, the influence of morphine on the availability of 5-HT_{2A} receptor might be different in the presence of acute noxious stimulation (Adriaens et al., 2012).

In conclusion, morphine and morphine based CPP increase the number of 5-HT_{2A} receptors in all areas of rat hippocampal formation in experimental groups compared to controls and this increase is dose-dependent.

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References

Adriaens, A., Polis, I., Vermeire, S., Waelbers, T., Duchateau, L., Sys, S., et al. (2012). The influence of morphine on cerebral 5-HT_{2A} receptor availability in dogs: A SPECT study. *Journal of Nuclear Medicine, 53*(12), 1969–1973.

Adriaens, A., Polis, I., Vermeire, S., Waelbers, T., Croubels, S., Duchateau, L., et al. (2014). The effect of prolonged exposure to morphine on canine cerebral 5-HT_{2A} receptors measured with 123 I-R91150 SPECT. *European Neuropsychopharmacology, 24*(7), 1133-8.

Bardon, T., & Ruckebusch, M. (1984). Changes in 5-HIAA and 5-HT levels in lumbar CSF following morphine administration to conscious dogs. *Neuroscience Letters, 49*(1-2), 147–151.

Berg, K. A., Clarke, W. P., Chen, Y., Ebersole, B. J., McKay, R. D. G., & Maayani, S. (1994). 5-hydroxytryptaminetype$_{2A}$ receptors regulate cyclic AMP accumulation in a neuronal cell line by protein kinase C-dependent and calcium/calmodulin-dependent mechanisms. *Molecular Pharmacology, 45*(5), 826–836.

Buhot, M. C. (1997). Serotonin receptors in cognitive behaviors. *Current Opinion in Neurobiology, 7*(2), 243-54.

Carr, G. D., & White, N. M. (1983). Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sciences, 33*(25), 2551-7.

Emsley, R. (2009). Drugs in development for the treatment of schizophrenia. *Expert Opinion on Investigational Drugs, 18*(8), 1103–1118.

Emamian, S., Naghdi, N., Sepehri, H., Jahanshahi, M., Sadeghi, Y., & Choopani, S. (2010). Learning impairment caused by intra-CA_1 microinjection of testosterone increases the number of astrocytes. *Behavioural Brain Research, 208*(1), 30-37.

Gulati, A., & Bhargava, H. N. (1988). Cerebral cortical 5-HT_1 and 5-HT_2 receptors of morphine tolerant-dependent rats. *Neuropharmacology, 27*(12), 1231–7.

Gulati, A., & Bhargava, H. N. (1989). Brain and spinal-cord 5-HT_2 receptors of morphine-tolerant- dependent and morphine-abstinent rats. *European Journal of Pharmacology, 167*(2), 185–92.

Hoyer, D., Hannon, J. P., & Martin, G. R. (2002). Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacology Biochemistry and Behavior, 71*(4), 533-554.

Hood, S. D., Hince, D. A., Davies, S. J., Argyropoulos, S., Robinson, H., Potokar, J., et al. (2010). Effects of acute tryptophan depletion in serotonin reuptake inhibitor-remitted patients with generalized anxiety disorder. *Psychopharmacology (Berl.), 208*(2), 223–232.

Hayes, D. J., & Greenshaw, A. J. (2011). 5-HT receptors and reward-related behaviour: A review. *Neuroscience and Biobehavioral Reviews, 35*(6), 1419-49.

Jahanshahi, M., Sadeghi, Y., & Hosseini, A. (2006). Estimation of astrocyte number in different subfield of rat hippocampus. *Pakistan Journal of Biological Sciences, 9*(8), 1595-1597.

Jahanshahi, M., Khoshnazar, A. K., Azami, N. S., & Heidari, M. (2011). Radiation-induced lowered neurogenesis associated with shortened latency of inhibitory avoidance memory response. *Folia Neuropathologica, 49*(2), 103-108.

Jahanshahi, M., Nickmahzar, E. G., Seif-hoseini, S., Babakordi, F., & Moharreri, A. (2013). Scopolamine Reduces the Density of M_1 Muscarinic Neurons in Rats' Hippocampus. *International Journal of Morphology, 31*(4), 1227-32.

Jahanshahi, M., Shaabani, R., Nikmahzar, E., & Babakordi, F. (2014). Female Rat Hippocampal Cell Density after Conditioned Place Preference. *Folia Biologica (Praha), 60*(1), 47-51.

Leysen, J. (2004). 5-HT_2 receptors. *Current Drug Targets-CNS & Neurological Disorders, 3*(1), 11-26.

Liu, F., Jiang, H., Zhong, W., Wu, X., & Luo, J. (2010). Changes in ensemble activity of hippocampus CA_1 neurons induced by chronic morphine administration in freely behaving mice. *Neuroscience, 171*(3), 747-759.

Lüttgen, M., Ögren, S. O., & Meister, B. (2004). Chemical identity of 5-HT_{2A} receptor immunoreactive neurons of the rat septal complex and dorsal hippocampus. *Brain Research, 1010*(1-2), 156-165.

Millan, M. J. (2003). The neurobiology and control of anxious states. *Progress in Neurobiology, 70*(2), 83-244.

Marek, G. J. (2003). Behavioral evidence for mu-opioid and 5-HT_{2A} receptor interactions. *European Journal of Pharmacology, 474*(1), 77- 83.

Moaddab, M., Haghparast, A., & Hassanpour-Ezatti, M. (2009). Effects of reversible inactivation of the ventral tegmental area on the acquisition and expression of morphine-induced conditioned place preference in the rat. *Behavioural Brain Research, 198*(2), 466-471.

Moghadami, S., Jahanshahi, M., Sepehri, H., & Amini, H. (2016). Gonadectomy reduces the density of androgen receptor-immunoreactive neurons in male rat's hippocampus: testosterone replacement compensates it. Behavioral and Brain Functions, 12(1), 1-10.

Nakagawa, T., Fujio, M., Ozawa, T., Minami, M., & Satoh, M. (2005). Effect of MS-153, a glutamate transporter activator, on the conditioned rewarding effects of morphine, methamphetamine and cocaine in mice. *Behavioural Brain Research, 156*(2), 233-239.

Pompeiano, M., Palacios, J., & Mengod, G. (1994). Distribution of the serotonin 5-HT_2 receptor family mRNAs: Comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Molecular Brain Research, 23*(1-2), 163-78.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates*. San Diego, Elsevier: Academic Press.

Prus, A. J., James, J. R., & Rosecrans, A. J. (2009). *Methods of Behavioral Analysis in Neuroscience* (2nd ed.). Boca Raton (FL): CRC Press.

Pfaus, J. G. (2009). Pathways of sexual desire. *Journal of Sexual Medicine, 6*(6), 1506–1533.

Rezayof, A., Zarrindast, M. R., & Sahraie, H. (2003). Involvement of dopamin receptors of dorsal hippocampus on the acquisition and expression of morphin-induced place preference in rats. *Jourrnal of Psychopharmacol, 17*(4), 415-423.

Rezayof, A., Razavi, S., Haeri-Rohani, A., Rassouli, Y., & Zarrindast, M. R. (2007). $GABA_A$ receptors of hippocampal CA_1 regions are involved in the acquisition and expression of morphine-induced place preference. *European Neuropsychopharmacology, 17*(1), 24-31.

Rothman, R. B., Blough, B. E., & Baumann, M. H. (2008). Dopamine/serotonin releasers as medications for stimulant addictions. *Progress in Brain Research, 172*, 385–406.

Sharifzadeh, M., Haghighat, A., Tahsili-Fahadan, P., Khalaj, S., Zarrindast, M. R., & Zamanian A. R. (2006). Intra-hippocampal inhibition of protein kinase AII attenuates morphine-induced conditioned place preference. *Pharmacology Biochemistry and Behavior, 85*(4), 705-12.

Shaabani, R., Jahanshahi, M., Nowrozian, M., Sadeghi, Y., & Azami, N. S. (2011). Effect of Morphine Based CPP on the Hippocampal Astrocytes of Male Wistar Rats. *Asian Journal of Cell Biology, 6*(3), 89-96.

Subhan, F., Deslandes, P. N., Pache, D. M., & Sewell, R. D. (2000). Do antidepressants affect motivation in conditioned place preference? *European Journal of Pharmacology, 408*(3), 257–263.

Tao, R., & Auerbach, S. B. (1994). Increased extracellular serotonin in rat brain after systemic or intraraphe administration of morphine. *Journal of Neurochemistry, 63*(2), 517–524.

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. *American Journal of Psychiatry, 163*(1), 28–40.

Wirtshafter, D. (2001). The control of ingestive behavior by the median raphe nucleus. *Appetite, 36*(1), 99–105.

Xu, T., & Pandey, S. C. (2000). Cellular localization of serotonin $_{2A}$ ($5HT_{2A}$) receptors in the rat brain. *Brain Research Bulletin, 51*(6), 499-505.

Zarrindast, M. R., Azami, B. N., Rostami, P., & Rezayof, A. (2006). Repeated administration of dopaminergic agents in

the nucleus accumbens and morphine-induced place preference. *Behavioural Brain Research, 169*(2), 248-255.

Zhao, Z. Q., Gao, Y. J., Sun, Y. G., Zhao, C. S., Gereau, R. W., & Chen, Z. F. (2007). Central serotonergic neurons are differentially required for opioid analgesia but not for morphine tolerance or morphine reward. *Proceedings of the National Academy of Sciences of the United States of America, 104*(36), 14519–14524.