



## Research Paper

# Effect of Sulpiride on Dopaminergic Synapse of Dorsal Hippocampus of Morphine-Treated Rats



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## ABSTRACT

**Background:** As previous studies show, several effects of morphine are induced by the dopaminergic system. Sulpiride is a dopamine D2 receptor (DAD2) antagonist widely used in clinics to treat DA-related disorders. DAD2 receptors are abundant at hippocampal cornu ammonis (CA1).

**Objectives:** This study aimed to investigate the possible interaction of morphine and sulpiride on DA synapses in CA1.

**Materials & Methods:** In this study, 48 Wistar rats weighing 220 to 250 g were used. These animals were classified into eight groups (6 rats per group): saline control group (1 mL/kg), morphine group (5 mg/kg), sulpiride groups alone (1, 2, and 4 mg/kg) and sulpiride groups (1, 2, and 4 mg/kg)+morphine (5 mg/kg). Saline or substances were injected once intraperitoneally. After 48 h, the animals' brains were removed under anesthesia and placed in 10% formalin for fixation. Then, 3- to 4- $\mu$ m slices were cut from these tissues, and the DA synapse was examined by histochemistry and immunohistochemistry techniques. The data were statistically analyzed by the analysis of variance.

**Results:** The control group had DA synapses and healthy neurons. A relative increase in DA synapses compared to the control group was observed in the morphine and single sulpiride groups. However, in sulpiride+morphine groups, DA synapses were reduced compared to morphine or sulpiride alone, but neurons were not destroyed.

**Conclusion:** The interaction effect of sulpiride and morphine in the CA1 region may decrease DA synapses.

**Keywords:** Morphine, CA1 region, Hippocampus, Sulpiride, Dopamine, Synapses

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## Highlights

- Sulpiride is a dopamine D2 (DAD2) receptor antagonist.
- Its intraperitoneal injection (1, 2, and 4 mg/kg) simultaneous with morphine (5 mg/kg) decreases DA synapses in the dorsal hippocampus without neuronal destruction.
- The interaction of sulpiride with morphine in that region may depend on reducing DA synapses.

## Introduction

All synapses are characterized by the ability to change, that is, synaptic flexibility [1]. Over the past few decades, most studies have focused on the excitatory synaptic flexibility of long-term potentiation (LTP) and long-term depression (LTD) models, although it has now been shown that inhibitory synapses can also change. The study of synaptic flexibility using electrophysiological methods in brain sections is not sufficient to recognize these changes, so today cellular mechanisms are used that have the best results [2, 3]. Nevertheless, one of the regions of the brain that is closely related to the subject of synapse flexibility is the hippocampus [4]. Since information is constantly passing through the hippocampus, most of the information is not stored, but some prominent events are labeled with a step signal which allows changing synaptic flexibility and memory. Meanwhile, due to the reinforcement of the specific event with a dopamine (DA) signal, the animal structure or internal memory is updated [5]. Different classes of drug abuse release DA in the nucleus accumbens (NAc), and this result, together with convincing evidence from behavioral studies, suggests that the mesolimbic DA system is required to induce drug effects. Pharmacological studies have also shown that DA plays an important role in modulating neural activity, mainly related to various forms of learning and memory [6]. The search for understanding the cellular mechanisms involved in changing synaptic status is a major challenge in neuroscience. By blocking these receptors using sulpiride, a selective antagonist of dopamine D2 (DAD2) receptors, we may demonstrate the effect of eliminating DA signaling in the dorsal hippocampus of morphine-treated rats, which is rich in these receptors.

## Materials and Methods

### Animal model

In this study, 48 male Wistar rats weighing 220-250 g were provided from Pasteur institute, Tehran, Iran, and kept in autoclaved cages under standard conditions (two animals per cage). The cages were cleaned once every two days and provided with water and food *ad libitum*. After one week of adaptation to the laboratory conditions, the rats were weighed, and the study drugs were injected intraperitoneally.

Based on random sampling, the rats were grouped as follows: group 1 (control group), saline injection at a rate of 1 mL/kg; group 2, 5 mg/kg morphine injection; group 3, 1 mg/kg sulpiride injection; group 4, 2 mg/kg sulpiride injection; group 5, 4 mg/kg sulpiride injection; group 6, injection of sulpiride at a rate of 1 mg/kg and after 20 min, injection of morphine at a rate of 5 mg/kg; group 7, injection of sulpiride at a rate of 2 mg/kg and after 20 min, injection of morphine at a rate of 5 mg/kg; group 8, injection of sulpiride at a rate of 4 mg/kg and after 20 min, injection of morphine at a rate of 5 mg/kg.

In the end, the rats were euthanized with CO<sub>2</sub> gas, and their brains were removed from the skull. Coronal sections of brain samples were prepared in the hippocampus region and fixed in 10% formalin. Then, paraffin slices with a thickness of 3-4 μm were provided with a microtome (Leica, Germany).

### Hematoxylin eosin staining

Tissue samples with a thickness of 3-4 μm were placed in xylol solution in two steps (15 min in each step) for paraffin removal. After placing in 96%, 80%, 70%, and 50% alcohol concentrations (5 min each), the slices were put in hematoxylin dye for 30 min. The slides were washed with water and put in eosin for 20 min, and then placed in 50%, 70%, 80%, and 90% alcohol concentrations about 1 min for each. Afterward, they were exposed to xylol and finally glued by entellan (Merck, Germany) and covered by a coverslip.

### Evans blue coloring

Tissues were cut in slices with a thickness of 3-4  $\mu\text{m}$ . The slides were placed in xylol solution in two steps (15 min in each step) for deparaffinization and put in 96%, 80%, 70%, and 50% alcohol concentrations for 5 min each. Then the specimens were immersed in 0.25 g of Evans blue dye in 100 mL of calcium chloride solution with a pH of 5.7. After about 2 h and 30 min, the slides were washed with water. Then, dehydration in 50%, 70%, 80%, and 90% alcohol concentrations was followed (1 min in each solution), and the slices were finally exposed to xylol in two steps for 2 min per step and then glued and covered.

### Immunohistochemical marking

The tissue specimens were cut into slices with a thickness of 3-4  $\mu\text{m}$ . The slides were placed in xylol solution for 5 min for paraffin removal. The other steps were done as follows.

The slides were put in 100%, 96%, 90%, and 80% alcohol concentrations, in that order, rinsed with distilled water, placed in the citrate buffer, put in Tween 20 and autoclaved for 20 min, washed several times in 0.025% tris-buffered saline (TBS) solution at pH 7, placed in the blocking buffer solution for 2 h, rinsed once in 0.025% TBS solution at pH 7, put in 3%  $\text{H}_2\text{O}_2$  for 10 min, rinsed once in 0.025% TBS solution at pH 7, placed in the primary antibody (specific for dopamine D2 receptor: D2DR) for 24 h, rinsed once in 0.025% TBS solution at pH 7, placed in the secondary antibody (m-IgG $\kappa$  BP-HRP) for 2 h, put in the DAB for 10 min, rinsed once in 0.025% TBS solution at pH 7, put in hematoxylin for 2 min, rinsed with distilled water, and then immersed in

80%, 90%, 96%, 100% alcohol concentrations, in that order, and finally placed in xylol in two stages (5 min in each stage), and mounted by entellan.

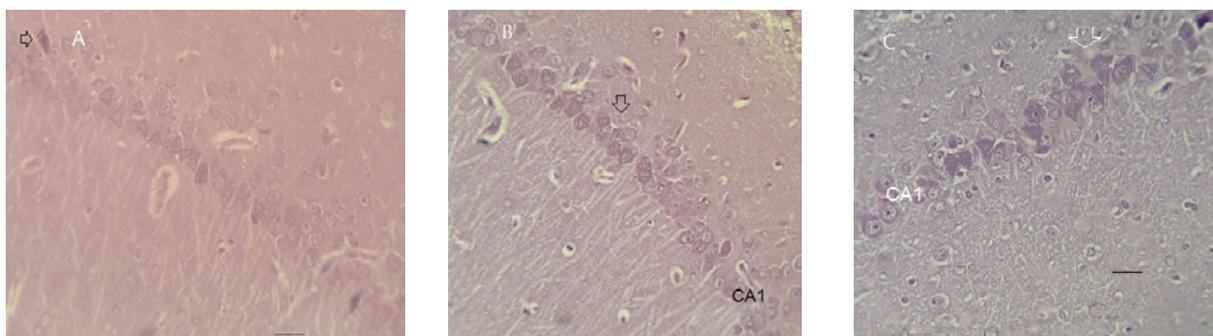
### Statistical analyses

Data were analyzed in SPSS software v. 21. One-way analysis of variance (ANOVA) and Tukey *post hoc* were used to analyze the data after the normality test. The Tukey test was performed to further analyze the data to examine differences between groups. If necessary, two-way ANOVA was used to show drug interactions (Figure 4). Also, all qualitative findings were changed into quantitative data with the help of Image J software v. 1.41.

### Results

#### Finding of hematoxylin eosin staining in the control group

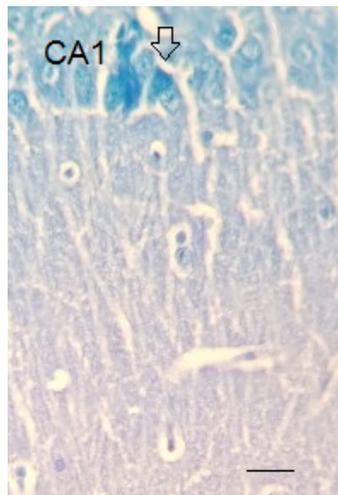
According to the histochemical study conducted in certain dimensions (100  $\mu\text{m}$ ) of the dorsal hippocampus (dH) with the help of hematoxylin eosin (H&E) staining in the control group (saline group), the tissue is seen as normal, and no density change at a significant level or destruction is seen in the pyramidal neurons ( $F=0.745$ ,  $P>0.05$ ) and the dH shows its natural structure (Figure 1A). The density of pyramidal neurons increased by approximately 50% in morphine treated group compared to the control sample ( $F=2.828$ ,  $P<0.05$ ). The H&E staining on rats' brains receiving sulpiride at different doses (1, 2, and 4 mg/kg, IP) showed a dose-dependent increase in cell density. The highest pyramidal cell density is seen at a dose of 4 mg/kg (an increase of 40% compared to the saline group) (Figure 1B-1C).



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**Figure 1.** Four- $\mu\text{m}$  slices of dorsal hippocampus tissue cornu ammonis (CA1) in the brain of male rats that received intraperitoneally saline (1 mL/kg) (A), morphine (5 mg/kg) (B), and sulpiride (4 mg/kg) (C) (40x magnification)

Using H&E staining, the normal density and feature of pyramidal neurons in the control group are shown. In the group treated with morphine, an increase in the density of pyramidal neurons is observed. After sulpiride injection, there is a 40% increase compared to the saline group. The scale bar is 50  $\mu\text{m}$ .



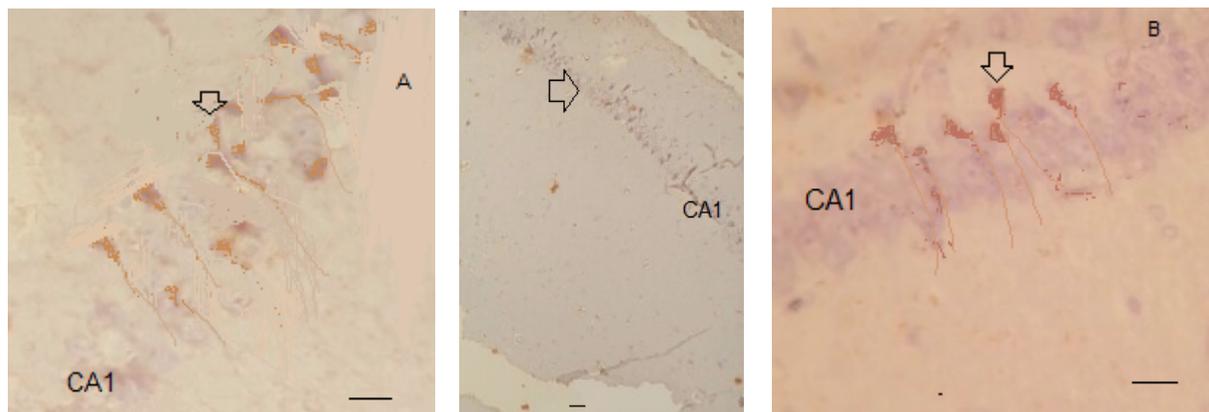
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**Figure 2.** Four- $\mu\text{m}$  section of the dorsal hippocampus tissue cornu ammonis (CA1) of the male rat brain receiving sulpiride (4 mg/kg) and morphine

With the help of evans blue staining, a significant decrease in cell densities is seen compared to single morphine. The scale bar is 50  $\mu\text{m}$ , with 40x magnification.

### Results of histochemical study with evans blue staining

In the histochemical study with Evans blue staining on brain sections of male rats receiving sulpiride (1, 2, and 4 mg/kg) and morphine (5 mg/kg) simultaneously, a decrease in density of pyramidal neurons (dependent on sulpiride dose) is seen compared with single morphine with no damage in the neurons features ( $F=2.648$ ,  $P<0.05$ ) (Figure 2).



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**Figure 3.** Four- $\mu\text{m}$  section of dorsal hippocampus (dH) cornu ammonis (CA1) of the Male Rat Brain Receiving Sulpiride (1 mg/kg) (A: at two magnifications), and Sulpiride+Morphine (B) IP

With the help of immunohistochemical staining, reduction in DA synapses is seen in sulpiride+morphine (40x magnification). The scale bar is 50  $\mu\text{m}$ .

### Results of immunohistochemical staining

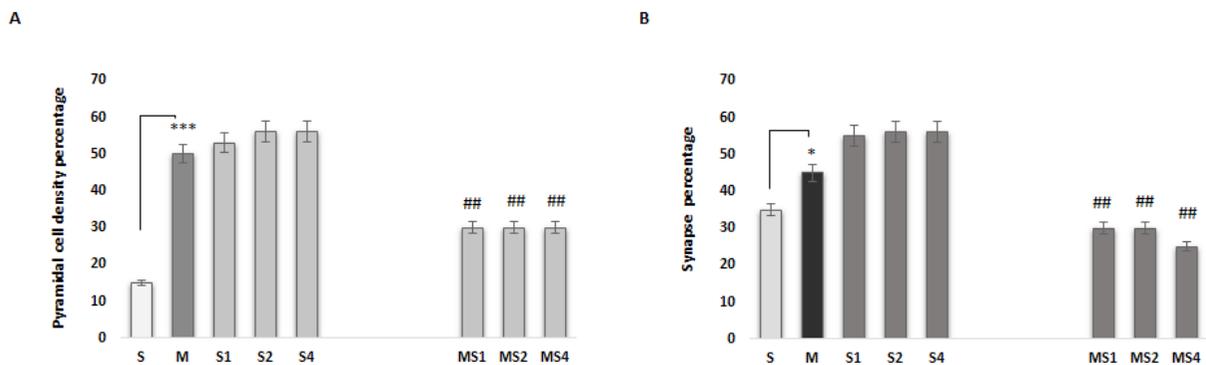
Specific dopamine synapses were observed in this type of staining (Figure 3). A 4  $\mu\text{m}$  section of the dH tissue (CA1) of the brains of rats receiving substances vs. saline (1 mL/kg) IP, using immunohistochemical staining, show significant differences. Reduction in DA synapses is seen in sulpiride+morphine groups vs. morphine and single sulpiride in a manner independent of the dose of sulpiride.

### Quantitative changes in cell and synapse density

The changes in cell density and synapse in all groups were investigated with the help of appropriate analyzes (one-way and two-way ANOVA). Synapses and cell density in the morphine+sulpiride groups showed a decrease due to the interaction between sulpiride with morphine (Figure 4).

### Discussion

Studies by Edelmann and Lessmann have demonstrated that D1- and D2-like receptors bind to dopamine (DA) at low concentrations, activating presynaptic D2R and decrease the stimulus responses that are transmitted to CA1 neurons in the hippocampus [5]. The authors have shown that dopaminergic neurons that travel to the hippocampus first activate D2R by secreting DA, causing a general inhibition of neurostimulatory postsynaptic currents.



**Figure 4.** Cell (A) and synapse (B) density of cornu ammonis (CA1) region

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The groups receiving saline (1 mL/kg) and morphine alone (5 mg/kg) and sulpiride alone (1-4 mg/kg), and sulpiride+morphine, IP, were compared with one-way and two-way ANOVA. The difference between the groups was obtained by Tukey *post hoc* as follows: \* $P < 0.05$  and \*\*\* $P < 0.001$  comparing M with S, ##  $P < 0.01$  comparing M with MS1-MS4 (cumulative groups).

Note: S, saline; M, morphine; S1-4, sulpiride doses of 1-4 mg/kg; MS1-MS4, morphine+sulpiride different doses.

With these interpretations, the results of that study are consistent with the present work that D2R antagonists reduces basal synaptic transmission in the hippocampus.

In contrast, Smiałowski postulated that high concentrations of D2R agonists cause spontaneous firing of CA1 pyramidal neurons [7]. Other studies have also shown that the dorsal hippocampus (dH) is involved in the processing of emotional information [8].

The involvement of D2R signaling in modulating hippocampal synaptic flexibility has also been described by others [8]. Functionally, in various studies, D2R is involved in hippocampal-dependent learning and memory, so the systemic use of receptor blockade creates a set of learning and memory deficits [9].

Nevertheless, the question is whether DA synapses are related to cellular mechanisms such as metabolic mechanisms other than receptors.

Typically, dopamine is synthesized from tyrosine via Tyrosine Hydroxylase (TH), the rate-limiting step in catecholamine synthesis. Studies have previously shown that quinpirole, a D2 receptor agonist, inhibits TH activity and slightly reduces DA [10, 11]. These findings may help us to establish an association between decreased DA synapses in rats receiving sulpiride+morphine compared with morphine alone. As other authors have demonstrated, activation of the D2 receptor for quantitative reduction of DA is probably associated with a decrease in TH affinity for its cofactor tetrahydrobiopterin (BH4) by blocking the cofactor-dependent pathway that mediates TH phosphorylation [10, 11]. The relative increase in DA synapses in the morphine group may only be considered a result of TH activation.

Over the past few years, several researchers have documented that repeated exposure (though not just once) to an abusive drug causes structural changes in certain neurons. For example, repeated exposure to opioids reduces the size of dendritic projections in dopaminergic VTA neurons [12]. Most importantly, these mechanisms have been shown to persist for at least one month after the last drug exposure. The studies also have shown that chronic exposure to opioids also reduces the birth of new neurons in the adult hippocampus [12]. However, the functional importance of such neurogenesis is still debated.

Studies have indicated that acute use of nicotine increases pyramidal cells and that nicotine alters the dendritic morphology of specific subsets of pyramidal neurons and subpopulations, which depends on the age of exposure to the drug [13]. Therefore, the authors have suggested that the use of nicotine provides a unique opportunity to further investigate the mechanisms and functions of synaptic plasticity in brain regions that play an important role in controlling motivated behavior. We found that acute use of morphine did not alter dH cells, which may support the acute use of this substance in the clinic. On the contrary, a previous study has shown that repeated use of this substance decreases the density of pyramidal cells in the CA1 region, highlighting the negative effects of repeated drug use [13]. Also, a single dose of morphine was used in this study. While other studies have shown that low and high doses of morphine have different effects on the seizure threshold caused by pentylenetetrazol. A low dose of 0.5 mg/kg makes animals more susceptible to seizures, while a higher dose of 30 mg/kg shows a protective effect against seizures [14]. In addition, acute and chronic administration of methadone leads to a decrease and an increase in the seizure

threshold, respectively [15]. Sulpiride is involved in a specific group of DA receptors. There are two types of DA receptors in the cell, while D2 receptors are abundant in the pyramidal cell. This may be related to their bioactivity feature. By referring to the previous works, it can also be conferred that different dopamine receptors have different dispersion and interaction effects [16, 17]. This study showed that sulpiride (a DAD2 receptor antagonist) did not reduce cell density in CA1 but did reduce the response to morphine in a manner dependent on DA synapses in that region. From this perspective, the present study has some interesting points, but the mechanisms must be carefully examined.

## Conclusion

According to the present study, the interaction effect of sulpiride and morphine on the surface of the dH probably has a reducing effect on DA synapses; therefore, future study on cellular-molecular interfering mechanisms of sulpiride and morphine is suggested.

## Ethical Considerations

### Compliance with ethical guidelines

All study procedures were in compliance with the ethical guidelines of the Declaration of Helsinki (2013). The study protocol was approved by the Research Committee of [Shahed University](#) (Code: IR.SHAHED.REC. 1399.079).

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### Authors contributions

Conceptualization and methodology; study supervision; writing, review, and editing the paper: Manizheh Karami; Investigation: Marziyeh-Sadat Hashemi and Mahdiyeh Jafarpour Fard; Writing the original draft: Marziyeh-Sadat Hashemi; Resources: Manizheh Karami and Marziyeh-Sadat Hashemi.

### Conflict of interest

The authors declared no conflicts of interest.

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## References

- [1] Kauer JA, Malenka RC. Synaptic plasticity and addiction. *Nat Rev Neurosci*. 2007; 8(11):844-8. [DOI:10.1038/nrn2234] [PMID]
- [2] Lynch MA. Long-term potentiation and memory. *Physiol Rev*. 2004; 84(1):87-136. [DOI:10.1152/physrev.00014.2003] [PMID]
- [3] Thomas MT, Malenka RC, Bonci A. Modulation of long-term depression by dopamine in the mesolimbic system. *J Neurosci*. 2000; 20(15):5581-6. [DOI:10.1523/JNEUROSCI.20-15-05581.2000] [PMID] [PMCID]
- [4] Morris RG. Elements of a neurobiological theory of hippocampal function: The role of synaptic plasticity, synaptic tagging and schemas. *Eur J Neurosci*. 2006; 23(11):2829-46. [DOI:10.1111/j.1460-9568.2006.04888.x] [PMID]
- [5] Edelmann E, Lessmann V. Dopaminergic innervation and modulation of hippocampal networks. *Cell Tissue Res*. 2018; 373(3):711-27. [DOI:10.1007/s00441-018-2800-7] [PMID]
- [6] Schramm NL, Egli RE, Winder DG. LTP in the mouse nucleus accumbens is developmentally regulated. *Synapse*. 2002; 45(40):213-9. [DOI:10.1002/syn.10104] [PMID]
- [7] Smiałowski A, Bijak M. Excitatory and inhibitory action of dopamine on hippocampal neurons in vitro. Involvement of D2 and D1 receptors. *Neurosci*. 1987; 23(1):95-101. [DOI:10.1016/0306-4522(87)90274-0]
- [8] Dubovyk V, Manahan-Vaughan D. Gradient of expression of dopamine D2 receptors along the dorso-ventral axis of the hippocampus. *Front Synaptic Neurosci*. 2019; 11:1-11. [DOI:10.3389/fnsyn.2019.00028] [PMID] [PMCID]
- [9] Rocchetti J, Isingrini E, Dal Bo G, Sagheby S, Menegaux A, Tronche F, et al. Presynaptic D2 dopamine receptors control long-term depression expression and memory processes in the temporal hippocampus. *Biol Psychiatry*. 2015; 77(6):513-25. [DOI:10.1016/j.biopsych.2014.03.013] [PMID]
- [10] Sulzer D, Cragg SJ, Rice ME. Striatal dopamine neurotransmission: Regulation of release and uptake. *Basal Ganglia*. 2016; 6(3):123-48. [DOI:10.1016/j.baga.2016.02.001] [PMID] [PMCID]
- [11] Schultz W. Multiple dopamine functions at different time courses. *Annu Rev Neurosci*. 2007; 30:259-88. [DOI:10.1146/annurev.neuro.28.061604.135722] [PMID]
- [12] Liu QS, Pu L, Poo MM. Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature*. 2005; 437(7061):1027-31. [DOI:10.1038/nature04050] [PMID] [PMCID]
- [13] Stoof JC, Kebabian JW. Two dopamine receptors: Biochemistry, physiology and pharmacology. *Life Sci*. 1984; 35:2281-96. [DOI:10.1016/0024-3205(84)90519-8] [PMID]
- [14] Zamanian G, Shayan M, Rahimi R, Bahremand T, Shafaroodi H, Ejtemaei-Mehr S, et al. Interaction of morphine tolerance with pentylenetetrazole-induced seizure threshold in mice: The role of NMDA-receptor/NO pathway. *Epilepsy Behav*. 2020; 112:107343. [DOI:10.1016/j.yebeh.2020.107343] [PMID]
- [15] Kazemi Roodsari S, Bahramnejad E, Rahimi N, Aghaei I, Dehpour A. Methadone's effects on pentylenetetrazole-induced seizure threshold in mice: NMDA/opioid receptors and nitric oxide signaling. *Ann N Y Acad Sci*. 2019; 1449(1):25-35. [DOI:10.1111/nyas.14043] [PMID]

- [16] Rezayof A, Zarrindast M, Sahraei H, Haeri-Rohani A. Involvement of dopamine receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats. *J Psychopharmacol.* 2003; 17(4):415-23. [DOI:10.1177/0269881103174005] [PMID]
- [17] Haghparast A, Esmaili M-H, Taslimi Z, Kermani M, Yazdi-Ravandi S, Alizadeh A-M. Intrahippocampal administration of D2 but not D1 dopamine receptor antagonist suppresses the expression of conditioned place preference induced by morphine in the ventral tegmental area. *Neurosci Lett.* 2013; 541:138-43. [DOI:10.1016/j.neulet.2013.03.001] [PMID]