Cerrahpaşa Med J 2025; 49: 1-5 ORIGINAL ARTICLE

Potential of Pramipexole in Attenuating Morphine Dependence: Insights from a Conditioned Place Preference Study in Mice

Andleeb Shahzadi[1], Oruç Yunusoğlu[2], Mohamad Alhakim[3], Selim Gökdemir[1], Rüveyda Rümeysa Ateşoğlu[3]

[1]Department of Medical Pharmacology, İstanbul University-Cerrahpaşa Faculty of Medicine, İstanbul, Türkiye
[2]Department of Pharmacology, Bolu Abant İzzet Baysal University Faculty of Medicine, Bolu, Türkiye
[3]İstanbul University-Cerrahpaşa Faculty of Medicine-Cerrahpaşa, İstanbul, Türkiye

Cite this article as: Shahzadi A, Yunusoğlu O, Alhakim M, Gökdemir S, Ateşoğlu RR. Potential of pramipexole in attenuating morphine dependence: Insights from a conditioned place preference study in mice. *Cerrahpaşa Med J* 2025, 49, 0020, doi: 10.5152/cjm.2025.25020.

What is already known on this topic?

- *Morphine withdrawal reduces dopamine activity and contributes to reward-related behaviors.*

What this study adds on this topic?

- *Pramipexole, a D2/D3 agonist, reduces morphine-induced conditioned place preference. These findings support pramipexole's role in opioid dependence.*

Abstract

Objective: Pramipexole, a selective dopamine D2/D3 receptor agonist, has been implicated in modulating dopaminergic transmission in brain reward circuits. Morphine reward-related behaviors are associated with a reduction in dopamine levels in these regions. This study aimed to investigate the effects of pramipexole on morphine dependence in a rodent model, based on the hypothesis that enhancing dopaminergic activity may attenuate opioid dependence.

Method: Swiss albino mice with established morphine dependence were assessed using the conditioned place preference (CPP) test. Morphine conditioning was performed with 10 mg/kg morphine (IP) for 8 days. On the ninth day, animals received either 1 mg/kg or 4 mg/kg pramipexole (IP), and CPP expression was evaluated 30 minutes post-administration.

Results: Pramipexole (1-4 mg/kg) significantly reduced morphine-induced CPP expression by 9%-14% ($P <$ .05 to .01). No significant dose-dependent differences were observed, indicating a potential ceiling effect.

Conclusion: These findings indicate that pramipexole, a dopamine D2/D3 receptor agonist, attenuates morphine dependence-related behaviors. Pramipexole may represent a potential pharmacotherapeutic candidate for opioid dependence, warranting further investigation in preclinical and clinical settings.

Keywords: Conditioned place preference, dopamine, morphine, pramipexole

Introduction

Addiction is characterized by the persistent use of a substance despite its detrimental physical, psychological, and social consequences.[1] It involves an uncontrollable craving and the development of withdrawal symptoms when access to the substance is restricted.[2] As a significant and growing medical and social issue worldwide, substance addiction requires urgent attention and research.

Morphine and other opioids are highly addictive substances associated with increased mortality rates due to their potential for misuse.[3] Consequently, extensive research is being conducted to understand the anatomical regions implicated in opioid dependence, the neurobiological changes occurring during addiction and withdrawal, and the neurotransmitter systems involved in these mechanisms.

Morphine, an analgesic agent, is particularly used chronically in the management of cancer pain. Its effects are mediated through mu (μ), delta, and kappa opioid receptors. These receptors are found throughout the brain, including the ventral tegmental area (VTA) and the nucleus accumbens (NAcc). These regions are the anatomical localizations associated with morphine dependence.[4]

Dopamine is a neurotransmitter widely found in the central nervous system. It plays crucial roles in locomotor activity, cognitive functions, and reward mechanisms. The projections of dopaminergic

Received: March 22, 2025 **Revision Requested:** June 2, 2025 **Last Revision Received:** June 19, 2025 **Accepted:** June 28, 2025 **Publication Date:** October 24, 2025.
Corresponding author: Andleeb Shahzadi, Department of Medical Pharmacology, Faculty of Medicine-Cerrahpaşa, Istanbul University-Cerrahpaşa, İstanbul, Turkey **e-mail:** shahzadiandleeb@yahoo.com, andleeb.shahzadi@iuc.edu.tr
DOI: 10.5152/cjm.2025.25020

neurons, especially those in the VTA, are significantly involved in addiction. Opioids suppress inhibitory inputs coming to the VTA, thereby affecting the dopaminergic system and increasing dopamine release.[5] In opioid addiction, withdrawal from the drug acts as a significant stimulus for drug-seeking behavior. However, during the withdrawal period, dopamine levels are low. During this period, D2 dopamine agonist injections into the NAcc can alleviate withdrawal symptoms of opioids and induce strong locomotor activity.[6]

Pramipexole is a selective dopamine D2/D3 receptor agonist.[7] It is currently used in the treatment of Parkinson's Disease[8,9] and motor symptoms of Restless Leg Syndrome.[10] It also shows effects on non-motor symptoms by stimulating D3 receptors in the mesolimbic area. It has been demonstrated to be effective in bipolar depression[11] and also exhibits weak agonistic activity on μ opioid receptors.[12]

An emerging area of research explores pramipexole's potential in the treatment of substance addiction. Recent studies have shown its effectiveness in addressing methamphetamine addiction.[13] To evaluate the reinforcing effects of addictive substances and screen for susceptibility to substance abuse, the conditioned place preference (CPP) test is widely used.[14] This test relies on classical conditioning principles, requiring the association of rewards with specific brain regions.[15,16]

Using this test, the addiction potential of various substances such as morphine, methamphetamine, alcohol, nicotine, and cocaine has been demonstrated in rodents. In CPP test, it is possible to study the potential for developing addiction to a substance[17] as well as the effects of another drug on the addiction symptoms induced by a dependency-causing substance.[18]

In this study, the effects of pramipexole on psychological dependence in mice conditioned with morphine using the CPP test were investigated. Given the limited number of effective pharmacological treatments for morphine addiction, the research aimed to assess whether pramipexole, a selective dopamine D2/D3 receptor agonist, could modulate addiction-related behaviors in morphine-dependent rodents.

Methods

Experiment Animals

In the study, male Swiss albino mice with a weight range of 25–30 grams were selected. (Approval No: 2021/37 from T.C. Bolu Abant İzzet Baysal University Experimental Animals Application and Research Center). The experiments were conducted at T.C. Bolu Abant İzzet Baysal University Experimental Animals Application and Research Center. The experimental animals were housed in separate cages in groups of 4, subjected to twelve-hour light/dark cycles with ventilation and humidity with *ad libitum* access to food and water. Experimental procedures were carried out in compliance to the guidelines of T.C. Bolu Abant İzzet Baysal University Local Ethics Committee for Animal Experiments.

Treatment groups: Groups 1 and 2 are the control and positive control groups. The dose-dependent effects of pramipexole on the psychic addiction induced by morphine were investigated in groups 3 and 4 (Table 1). The number of animals and the morphine dose are determined on the basis of the previous studies.[19,20] For all treatment groups, the total injection volume was adjusted to 1 mL per animal.

Conditioned Place Preference

To assess CPP, a 2-compartment setup was used, featuring surfaces with different tactile and visual stimuli. The apparatus, measuring 30 × 60 × 30 cm, was made of black plexiglass, including the walls and movable partition that separated the compartments. One compartment was white with a perforated floor, while the other was black with a striped floor pattern. The experimental protocol consisted of habitation, preliminary test (pretest), conditioning and post-test, which is accordance with literature and our previous studies.[19,21,22]

Habitation: The habitation phase was carried out to ensure that the animals could adapt to the researcher handling and laboratory environment. During this phase, the partition separating the compartments was removed, and the animals were allowed to freely explore the apparatus for 5 minutes to adapt to the environment.

Preliminary test: After habitation phase the animals were placed in the apparatus with the partition removed, and the time spent in each compartment was recorded for 15 minutes, that helped us in determining their natural place preference.

Conditioning: During this phase, the apparatus was divided into two compartments using a movable partition. In compartments where animals spent less time during the pretest was designated as the drug-paired side and was associated with morphine administration, while the saline-paired side corresponded to the compartment they naturally preferred. This created a conditioned association between the rewarding effects of morphine and the stimuli in that compartment. During the conditioning phase, animals received intraperitoneal (IP) injections of either saline (1 mL/kg) or morphine (10 mg/kg), followed by placement in the apparatus for 40 minutes to undergo conditioning. This procedure was repeated over an 8-day period, with morphine injections on days 1, 3, 5, and 7, and saline injections on days 2, 4, 6, and 8. The control group received saline every day.

Post-test phase: Twenty-four hours after the final conditioning trial (9th day), animals were placed in the apparatus with the partition removed, and the time spent in the compartment paired with morphine (white compartment) was recorded. To examine the effect of pramipexole on morphine-conditioned animals, pramipexole (1 or 4 mg/kg) was administered 30 minutes before the test.[13] On the test day, a researcher who was blinded to the treatment groups collected the data from the animals.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism software version 8.0; (GraphPad Software; San Diego, CA, USA).

Table 1. Experimental Groups

Group	Treatment	Dose	Number of Animals (n)
G1	Physiologic saline (control)	–	7-8
G2	Morphine + saline (positive control)	Morphine 10 mg/kg + saline	7-8
G3	Morphine + pramipexole (low dose)	Morphine 10 mg/kg + pramipexole 1 mg/kg	7-8
G4	Morphine + pramipexole (high dose)	Morphine 10 mg/kg + pramipexole 4 mg/kg	7-8

Data are expressed as mean ± SD. Prior to analysis, data were assessed for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test.

The time spent in the black and white compartments during the pretest phase and the effects of morphine on CPP were compared using a Student's *t*-test. To assess the effects of pramipexole on morphine-induced CPP, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to compare treatment groups. Statistical significance was set at $P < .05$ for all comparisons.

Results

Comparing the time spent in black and white compartments by all groups of animals during the pretest, the average time spent in the black compartment was significantly higher than the time spent in the white compartment ($P < .001$; Figure 1).

By the test phase (ninth day), animals in the morphine-treated group exhibited a pronounced preference for the white compartment, indicating the establishment of CPP. In contrast, control animals administered saline spent significantly more time in the black compartment compared to the morphine group ($P < .01$; Figure 2), confirming that 10 mg/kg morphine effectively induces place preference, which is an indicative of morphine dependence.

A one-way ANOVA revealed a significant effect of treatment on the time spent in the white compartment during the test phase ($F(3,26) = 150.8$, $P < .0001$). Post hoc (Tukey's) comparisons showed that morphine administration (10 mg/kg) significantly increased the time spent in the white compartment, confirming the establishment of morphine-induced CPP and indicating a strong preference for the drug-associated environment. In contrast, mice treated with pramipexole (1 mg/kg and 4 mg/kg) significantly reduced CPP expression compared to the morphine-only group ($P < .05$ and $P < .01$, respectively). However, no significant dose-dependent differences were observed between the 2 pramipexole-treated groups (Figure 3).

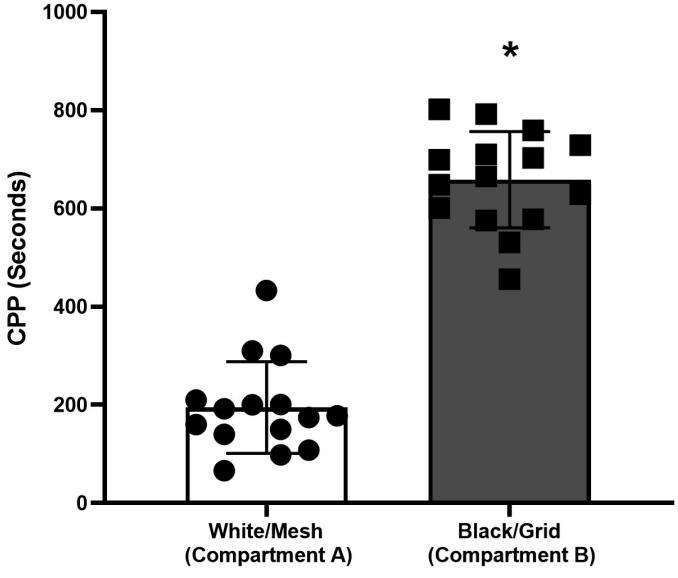

Figure 1. Time spent by the rats in the black and white compartments during the pretest phase of conditioned place preference. During this phase, mice had free access to both compartments for 15 minutes. Data are presented as mean ± SD (n = 7-8 per group). Statistical comparisons were performed using Student's t-test. *$P < .001$.

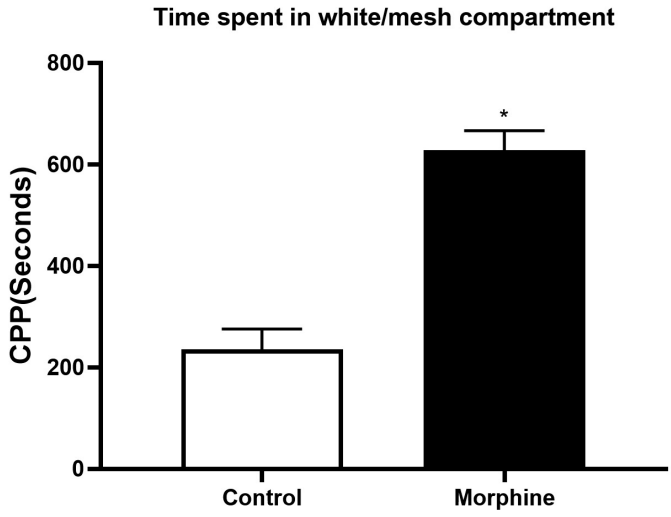

Figure 2. Effect of morphine on conditioned place preference. Morphine treatment (10 mg/kg, IP) induced a significant increase in time spent in the white compartment compared to control (saline-treated) animals, indicating the establishment of CPP. Data are presented as mean ± SD (n = 7-8 per group). Statistical comparisons were performed using Student's *t*-test. *$P < .001$.

Discussion

In this study, it was demonstrated that pramipexole administration significantly attenuated the expression of morphine-induced CPP in mice. Morphine treatment produced a robust CPP, indicating the rewarding properties of morphine, while pramipexole (administered prior to the post-test session) reduced this preference.

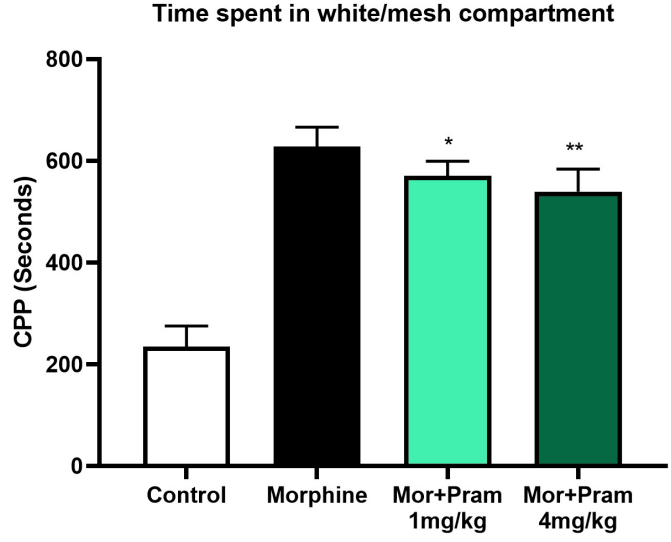

Figure 3. Effect of pramipexole on the expression of morphine-induced conditioned place preference. Thirty minutes prior to testing, pramipexole (1 or 4 mg/kg, IP) or saline was administered to the control, morphine groups. During the post-conditioning test session, the guillotine door was removed to allow free access to both compartments, and the time spent in the white (drug-paired) compartment was recorded over a 15-minute session. Data are presented as mean ± SD (n = 7-8 per group). Compared to the morphine group, *$P < .05$; **$P < .01$; Mor, Morphine; Pram, Pramipexole.

These findings suggest that pramipexole may modulate dopamine-related mechanisms involved in opioid reward behavior.

The potential for addiction is high for both morphine and other opioid analgesics thus, there is a significant need for the investigation of new drugs to treat morphine and other opioid dependence. Morphine addiction is known to alter dopamine receptor signaling, contribute to decreased receptor activation during withdrawal, and reduce dopamine levels in brain reward centers.

Morphine/opioid μ receptors are predominantly located in the VTA, a key brain reward center. Activation of these receptors activates the mesocorticolimbic dopaminergic system and significantly increases dopamine levels.[23-25] In the CPP paradigm, D1 and D2 receptors play significant roles in reward-related learning. The present findings strongly support the D2 receptors, although the role of D1 receptors appears to be less clearly defined and may warrant further investigation. When a D1 receptor agonist is administered to the nucleus accumbens, CPP is established.[26] However, when administered systemically, aversive (CPA) behavior develops.

It is known that dopamine plays a significant role in the development of morphine-induced place preference.[27] When a D3 receptor agonist is administered, it reduces morphine-induced place preference.[22] On the other hand, a D1 receptor agonist has been shown to prevent the development of morphine addiction.[28] In a similar study, the D3 receptor agonist 7-OH-DPAT prevented the development and expression of morphine addiction.[29]

In this study, we evaluated the effects of pramipexole in combination with morphine on CPP. The literature indicates that in the CPP paradigm, animals typically spend more time in environments previously associated with rewarding drugs; however, when a compound possesses aversive properties, they exhibit avoidance behavior toward those environments, reflecting CPA.[14,29] These observations are in accordance with our findings. Studies have shown that arrangements with different wall and floor stimuli (Rezayof et al,[30] 2006), as well as variations in floor[31,32] or wall characteristics[33] are sufficient to reveal place preference. In this study, a box with different wall and floor characteristics was used to evaluate CPP. Since conditioning protocols, like apparatus, can vary, studies utilizing boxes with similar characteristics were taken into consideration, and a protocol suitable for biased designs was selected.[21,31] To ensure that the animals associated the rewarding effects of morphine with specific environmental cues, 4 days of saline conditioning were followed by 4 days of morphine conditioning. Place preference assessments were conducted 1 day after the final conditioning. Various approaches exist for place preference assessments: in biased designs, the time spent in the drug-paired compartment is compared with that of control animals[34] whereas in unbiased designs, either the difference between time spent in the drug- and saline-paired compartments or the change in preference (i.e., test day versus pretest) is evaluated[6,27,34] In the present study, we used a biased CPP design, in which the drug was paired with the non-preferred compartment to evaluate the shift in preference induced by morphine and its modulation by pramipexole.

In conclusion, this study demonstrated that the selective dopamine D2/D3 receptor agonist pramipexole attenuates the expression of morphine-induced CPP. The findings suggest that pramipexole may have therapeutic potential in the treatment of morphine and other opioid addictions. However, before drawing clinical implications, it is important to acknowledge the study's limitations. These results are preliminary and require further validation through more extensive preclinical and clinical investigations.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of T.C. Bolu Abant İzzet Baysal University Experimental Animals Application and Research Center (Approval no: 2021/37; Date: December 8, 2021).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.S., O.Y.; Design – A.S., O.Y.; Supervision – A.S.; Resources – A.S.; R.R.A.; Materials – M.A., S.G.; Data Collection and/or Processing – O.Y., R.R.A.; Analysis and/or Interpretation – A.S., O.Y.; Literature Search – M.A., S.G., R.R.A.; Writing Manuscript – A.S., O.Y., G.O., M.A., R.R.A.; Critical Review – A.S.; O.Y.

Declaration of Interests: The authors declare that they have no competing interests.

Funding: This study was supported by TÜBİTAK 2209-A. Project No: 2209-A 2021/2.

References

1. National Institute On Drug Abuse. Understanding drug use and addiction DrugFacts. *National Institute on Drug Abuse Website*. 2025. Available at: https://nida.nih.gov/publications/drugfacts/understanding-drug-use-addiction.
2. (US) NIoH. The essence of drug addiction. NIH Curriculum Supplement Series [Internet]. 2007.
3. Volkow ND, McLellan AT. Opioid abuse in chronic pain — misconceptions and mitigation strategies. *N Engl J Med*. 2016;374(13):1253-1263. [CrossRef]
4. Wise RA, Morales M. A ventral tegmental CRF-glutamate-dopamine interaction in addiction. *Brain Res*. 2010;1314:38-43. [CrossRef]
5. Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci*. 1992;12(2):483-488. [CrossRef]
6. Harris GC, Aston-Jones G. Involvement of D2 dopamine receptors in the nucleus accumbens in the opiate withdrawal syndrome. *Nature*. 1994;371(6493):155-157. [CrossRef]
7. Evans AH, Strafella AP, Weintraub D, Stacy M. Impulsive and compulsive behaviors in Parkinson's disease. *Mov Disord*. 2009;24(11):1561-1570. [CrossRef]
8. Lam YW. Clinical Pharmacology of dopamine agonists. *Pharmacotherapy*. 2000;20(1 Pt 2):17S-25S. [CrossRef]
9. Jost WH. Ropinirole: current status of the studies. *J Neurol*. 2004;251(6)(suppl 6):VI/13-VI/18. [CrossRef]
10. Hansen RA, Song L, Moore CG, et al. Effect of ropinirole on sleep outcomes in patients with restless legs syndrome: meta-analysis of pooled individual patient data from randomized controlled trials. *Pharmacotherapy*. 2009;29(3):255-262. [CrossRef]
11. Perugi G, Toni C, Ruffolo G, Frare F, Akiskal H. Adjunctive dopamine agonists in treatment-resistant bipolar II depression: an open case series. *Pharmacopsychiatry*. 2001;34(4):137-141. [CrossRef]
12. Tulloch IF. Pharmacologic profile of ropinirole: a nonergoline dopamine agonist. *Neurology*. 1997;49(1)(suppl 1):S58-S62. [CrossRef]
13. Riddle JL, Rokosik SL, Napier TC. Pramipexole- and methamphetamine-induced reward-mediated behavior in a rodent model of Parkinson's disease and controls. *Behav Brain Res*. 2012;233(1):15-23. [CrossRef]
14. Tzschentke TM, Schmidt WJ. N-methyl-d-aspartic acid-receptor antagonists block morphine-induced conditioned place preference in rats. *Neurosci Lett*. 1995;193(1):37-40. [CrossRef]
15. Ferbinteanu J, McDonald RJ. Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus*. 2001;11(2):187-200. [CrossRef]
16. Kawasaki Y, Jin C, Suemaru K, et al. Effect of glutamate receptor antagonists on place aversion induced by naloxone in single-dose

morphine-treated rats. *Br J Pharmacol*. 2005;145(6):751-757. [CrossRef]
17. Allahverdiyev O, Nurten A, Enginar N. Assessment of rewarding and reinforcing properties of biperiden in conditioned place preference in rats. *Behav Brain Res*. 2011;225(2):642-645. [CrossRef]
18. Li SM, Yin LL, Ren YH, Pan LS, Zheng JW. GABA(B) receptor agonist baclofen attenuates the development and expression of d-methamphetamine-induced place preference in rats. *Life Sci*. 2001;70(3):349-356. [CrossRef]
19. Shahzadi A, Yunusoglu O, Karabulut E, Sonmez H, Yazici Z. Influence of selective dopamine agonist ropinirole on conditioned place preference and somatic signs of morphine withdrawal in rats. *Front Behav Neurosci*. 2022;16:855241. [CrossRef]
20. Yunusoğlu O. Resveratrol impairs acquisition, reinstatement and precipitates extinction of alcohol-induced place preference in mice. *Neurological Research*. 2021;43(12):985-994. [CrossRef].
21. Mattioli L, Titomanlio F, Perfumi M. Effects of a Rhodiola rosea L. extract on the acquisition, expression, extinction, and reinstatement of morphine-induced conditioned place preference in mice. *Psychopharmacology*. 2012;221(2):183-193. [CrossRef]
22. Shahzadi A, Ozyazgan S. Docosahexaenoic acid attenuates the rewarding property of nicotine-induced conditioned place preference in male rats. *Int J Med Biochem*. 2022;5(3):166-170. [CrossRef]
23. Kalivas PW. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Brain Res Rev*. 1993;18(1):75-113. [CrossRef]
24. Olmstead MC, Franklin KBJ. The development of a conditioned place preference to morphine: effects of microinjections into various CNS sites. *Behav Neurosci*. 1997;111(6):1324-1334. [CrossRef]
25. van Ree JM, Gerrits MA, Vanderschuren LJ. Opioids, reward and addiction: an encounter of biology, psychology, and medicine. *Pharmacol Rev*. 1999;51(2):341-396. [CrossRef]
26. White NM, Packard MG, Hiroi N. Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. *Psychopharmacology*. 1991;103(2):271-276. [CrossRef]
27. Beninger RJ. Dopamine D1-like receptors and reward-related incentive learning. *Neurosci Biobehav Rev*. 1998;22(2):335-345. [CrossRef]
28. Zarrindast MR, Rezayof A, Sahraei H, Haeri-Rohani A, Rassouli Y. Involvement of dopamine D1 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat. *Brain Res*. 2003;965(1-2):212-221. [CrossRef]
29. De Fonseca FR, Rubio P, Martín-Calderón JL, Caine SB, Koob GF, Navarro M. The dopamine receptor agonist 7-OH-DPAT modulates the acquisition and expression of morphine-induced place preference. *Eur J Pharmacol*. 1995;274(1-3):47-55. [CrossRef]
30. Rezayof A, Zatali H, Haeri-Rohani A, Zarrindast MR. Dorsal hippocampal muscarinic and nicotinic receptors are involved in mediating morphine reward. *Behav Brain Res*. 2006;166(2):281-290. [CrossRef]
31. Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nat Protoc*. 2006;1(4):1662-1670. [CrossRef]
32. Davis CM, Roma PG, Dominguez JM, Riley AL. Morphine-induced place conditioning in Fischer and Lewis rats: acquisition and dose-response in a fully biased procedure. *Pharmacol Biochem Behav*. 2007;86(3):516-523. [CrossRef]
33. Kotlinska J, Pachuta A, Dylag T, Silberring J. Neuropeptide FF (NPFF) reduces the expression of morphine- but not of ethanol-induced conditioned place preference in rats. *Peptides*. 2007;28(11):2235-2242. [CrossRef]
34. Masukawa Y, Suzuki T, Misawa M. Differential modification of the rewarding effects of methamphetamine and cocaine by opioids and antihistamines. *Psychopharmacol (Berl)*. 1993;111(2):139-143. [CrossRef]