Effect of electrical stimulation of central nucleus of the amygdala on morphine conditioned place preference in male rats

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Objective(s): The central nucleus of the amygdala (CeA) is one of the most important areas for the morphine reward system. This study investigated the effect of electrical stimulation of CeA on morphine conditioned place preference (CPP) in male rats.

Materials and Methods: After anesthetizing male Wistar rats, both electrode and cannula were implanted into CeA for stimulating (low intensity: 25 μA, and high intensity: 150 μA) and injecting (lidocaine and dopamine D2 receptor antagonist), respectively. Then, CPP induced by effective (5 mg/kg) and ineffective (0.5 mg/kg) doses of morphine was evaluated for five consecutive days (n = 6 / group).

Results: The low electrical stimulation intensity of 25 μA suppressed both acquisition and expression phases, but the high intensity of 150 μA attenuated only the expression phase. On the other hand, intra-CeA administration of dopamine D2 receptor antagonist, eticlopride (2 μg/rat), with the effective dose of morphine, decreased CPP. In addition, infusion of lidocaine into the CeA inhibited morphine-induced CPP in both acquisition and expression phases with the effective dose of morphine.

Conclusion: Electrical stimulation of the CeA may play an important role in attenuating morphine induced CPP via possible changes in neurotransmitters involved in the reward system such as dopamine (DA) and gamma-aminobutyric acid (GABA).

Introduction

Morphine as an opioid drug is widely used in clinical practice for decreasing sever pain. On the other hand, there is a serious risk of addiction when taking this drug (1). Also, morphine abuse can lead to pain tolerance, hyperalgesia, physical dependence, and other negative effects (2). Morphine addiction induces the reward circuit to become overactive, leading to compulsive substance-seeking (3).

For decreasing morphine-induced addiction, several treatment methods have been suggested, despite high recurrence rates (4). Currently, deep brain stimulation (DBS) is known as one of the neurosurgical procedures (5). DBS has been used to treat Parkinson’s disease (6), depression (7), Tourette syndrome (8), and Obsessive-Compulsive Disorder (OCD) (9). In this method, electrodes are inserted in some specific areas of the brain to induce different electrical pulses into the target sites (5). Additionally, some experimental studies have reported the effects of DBS on treatment of drug addiction, especially in decreasing drug-seeking behavior (10-12). Also, some researchers demonstrated that DBS reduced addictive behavior in the brain nuclei such as nucleus accumbens (Nac) (12), lateral hypothalamus, lateral habenula (11), subthalamic nucleus (STN), and the insula (13, 14).

The amygdaloid complex, especially the central nucleus of the amygdala (CeA) is one of the brain regions associated with drug reward that contain high concentrations of opioid receptors (15). CeA receives abundant DAergic afferents from the ventral tegmental region projected physically to the Nac and ventral tegmental area (VTA) (16). It also contains a higher number of DA terminals than other parts of amygdaloid nucleus. In addition, the CeA as the major output nucleus of the amygdala, plays a significant role in stimulus-reward learning (16-18).

Conditioned place preference (CPP), the most common behavioral model, is frequently used to measure learning and memory in animal models (19). The underlying feature of CPP includes dependence of a certain environment on the medication treatment, followed by association of another environment with the absence of the drug (20).

Previous studies have shown that morphine induces conditioned preference in rats (21, 22). Also, several experimental studies have revealed that the DBS of brain nuclei involved in the reward system reduces morphine-induced CPP (10, 23). In spite important role of CeA in reward system, the effect of electrical stimulation of CeA on morphine-induced CPP was unclear; therefore, this study was planned to investigate the effect of CeA electrical stimulation with high and low intensities on CPP induced by effective and ineffective doses of morphine in male rats. Also, the effects of reversible inactivation by lidocaine injection and antagonist of dopamine D2 receptor, eticlopride, on CeA in CPP were evaluated.

Materials and Methods

Experimental design

In this experiment, animals were randomly divided into the following surgical groups (n = 6): saline (Sal),...
saline + electrical stimulation 25 (Sal + St 25), morphine 0.5 (Mor 0.5), morphine 0.5 + electrical stimulation 25 (Mor 0.5 + St 25), morphine 5 (Mor 5), morphine 5 + electrical stimulation 25 (Mor 5 + St 25), saline + electrical stimulation 150 (Sal + St 150), subcutaneous saline (Sal SC), intracerebral saline (sal i.c.), morphine 0.5 + lidocaine (Mor 0.5 + Lido), morphine 5 + lidocaine (Mor 5 + Lido), expression lidocaen (Exp Lido), and morphine 5 + Eticlopride (Mor 5 + Eticlopride). All above mentioned groups were assigned in both expression and acquisition phases. According to previous studies, the morphine doses were chosen as effective (5 mg/kg) and ineffective (0.5 mg/kg) doses (22, 24). A schematic diagram of experimental design was illustrated in Figure 1.

Animals
This study was performed on male Wistar rats weighing 250–300 g obtained from Isfahan University, Isfahan, Iran. The animals were carried to the animal house to adapt for ten days before surgery. They were maintained under controlled conditions (12 hr light - 12 hr dark and temperature of 22 ± 2 °C) with available food and water. All animal experiments were approved by the Ethics Committee of Kazeroo Azad University (IR.IAU.KAU.REC.1400.061). Also, the experiments were done according to The National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication, 8th edition, 2011).

Drugs
The drugs used in the experiment were ketamine (100 mg/kg; TRITTAU Co, Germany), xylazine (10 mg/kg; Interchemie Co., Holland), Gentamicin (6 mg / kg; Alborz Darou Co Iran), Lidocaine 2% hydrochloride (0.3 µl per site ; Caspian Tamin Pharmaceutical Co. Iran), morphine (0.5 and 5 mg/kg; Pade Co., IRAN), and Eticlopride hydrochloride (2 µg/kg; Sigma-Aldrich Co, Germany). During the experiment, morphine and saline were subcutaneously (SC) administered as well as lidocaine and dopamine D2 receptor antagonist which were injected by intracerebral injection (i.c.).

Surgery
The rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). After shaving, the animal head was fixed in a stereotaxic apparatus (RWD Life science Co, China). Then, an incision along the midline was made to expose the skull. For observing the bregma and lambda areas, the skull surface was entirely cleaned. Next, both unilateral cannula and electrode were implanted into the CeA for stimulating and injecting, respectively. The coordinates of CeA included Anterior-Posterior (AP) = -2.2 mm; Medial-Lateral (ML) = ± 4.2 mm and Dorsal-Ventral (DV) = -8.4 mm (25). Afterward, both cannula and the electrode were fixed to the skull surface with dental acrylic cement (Acropars Co. Tehran Iran). To prevent infection, the animals received gentamicin (6 mg/kg; SC). Finally, they were transferred to Plexiglas cages to recover for 5 to 7 days.

Behavioral procedure
CPP apparatus
The place conditioning apparatus consisted of three metal chambers (A, B, and C). Both chambers A and B were equal-size (30 cm × 30 cm × 40 cm) separated by a guillotine door. The A chamber had black and white walls as well as a rough floor, while the B chamber had white walls and a smooth floor. Chamber C was the smallest (30 cm ×10 cm × 40 cm) linked to chambers A and B by a guillotine door. When the guillotine door was removed, the animal could freely move between the two chambers A and B through chamber C. CPP was performed on five continuous days using a biased procedure that included three phases of pre-conditioning, conditioning, and post-conditioning (26).

Pre-conditioning phase
During this phase (day 1), each rat was placed in chamber C to explore freely the three chambers for 15 min, while all doors of the apparatus had been removed. In addition, a camera placed above the apparatus recorded the time spent by each animal in chambers A and B. Whenever the animal spent 60% of its stopping time in a chamber, the opposite side was considered the morphine injection chamber.

Figure 1. A: Schematic diagram of the experimental protocol for method of electrical stimulation or drug administration in the acquisition phase. B: Method of electrical stimulation or drug administration in the expression phase. Electrical stimulation: drug microinjection and morphine injection.
Conditioning phase
The conditioning phase included days 2–4 of the study. In this phase, all groups received morphine and saline once per day. The phase contained six 30-min sessions (three saline and three morphine administrations). In addition, lidocaine, ethichlopride, and electrical stimulations were accompanied with morphine injection. On the 2nd day morning of the conditioning phase, the rat receiving morphine was immediately placed in the less-preferred chamber of the CPP apparatus. During the conditioning phase, all guillotine doors were closed for 30 min. After six hours, the animal received saline (1 ml/kg) and was immediately placed in the other chamber of the CPP apparatus, similar to the previous session. On the 3rd day, morphine and saline injections were the opposite of the 2nd day. Also, the morphine and saline injections on days 4 and 2 were the same. Moreover, the control group received saline twice per day.

Post-conditioning phase
The post-conditioning phase included the fifth day of the experiment. Each rat had a free choice in the three chambers of apparatus for 15 min while the guillotine doors had been removed. The time spent in the drug-paired chamber was recorded by ANY-Maze software (Stoelting Co, USA) and compared with the pre-conditioning phase. The changes of preference (preference index) were computed as the difference between the times spent in the morphine-paired chambers in post-conditioning and pre-conditioning phases (Preference index = time spend post-conditioning – time spend pre-conditioning).

Electrical stimulation induction
In this study, electrical intensities 25 or 150 μA were induced by a Stimulator Isolator A360, (World Precision Instruments, CO, USA). The electrical intensities were chosen according to the protocol used for stimulation of the prelimbic cortex of medial prefrontal cortex (mPFC) (12). Ten min before administration of morphine, each animal was stimulated by electrical intensities of 25 or 150 μA with a constant frequency of 25 Hz, once every five seconds for 10 min. In addition, the acquisition and expression groups received electrical stimulation during the conditioning and post-conditioning phases, respectively.

Histology
In order to confirm the stimulating electrode or cannula site in the CeA, a histological evaluation was performed. At the end of the experiment, the rats were anesthetized with ketamine and xylazine. Then, they were transcardially perfused with a saline solution of 0.9% followed by a formalin solution of 10%. The brain tissues were removed and kept in the formalin solution for one week. Then, the tissue slices with a thickness of 60 μm were prepared by frize-microtome. The sections were examined by an optical microscope (ERMA, Tokyo, Japan) and compared with the rat brain atlas (Figure 2)(12).

Statistical analysis
All data were expressed as mean ± standard error of the mean. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test by SPSS software version 16. The statistical differences with $P<0.05$ were considered to be significant.

Results
Effect of CeA electrical stimulation with various doses of morphine on the acquisition phase of CPP
In this study, the low current intensity in combination with effective ($P=0.006$) and ineffective ($P=0.03$) doses of morphine reduced the acquisition phase in the CPP when
compared with the Mor group (Figure 3A). In contrast, high current intensity accompanied with effective ($P=0.873$) and ineffective ($P=0.807$) morphine doses did not significantly decrease the acquisition phase compared with morphine alone treated groups (Figure 3B). Also, the effective dose of morphine alone increased CPP compared with Sal ($P=0.03$) and Sal + St 25 ($P=0.03$) groups, significantly (Figure 3A). The high current intensity in combination with effective ($P=0.873$) and ineffective ($P=0.807$) doses of morphine could not attenuate CPP in the acquisition phase of CPP in comparison with morphine alone treated groups, while the effective dose of morphine induced CPP in comparison with the sal group, significantly ($P=0.045$) (Figure 3B).

**Effect of CeA electrical stimulation with various doses of morphine on the expression phase of CPP**

The findings showed that administration of the morphine effective dose alone increased CPP in the expression phase in comparison with sal ($P=0.035$) and Sal + St 25 ($P=0.044$) groups, significantly (Figure 4A). Also, electrical stimulation of CeA with low intensity in combination with the effective dose of morphine significantly suppressed CPP in the expression phase in comparison with the Mor 5 group ($P=0.003$) (Figure 4A). Moreover, the effective dose of morphine alone induced a significant increment in CPP compared with Sal ($P=0.05$) and sal + St 150 ($P=0.05$) groups (Figure 4B). Also, CeA stimulation with high current intensity in combination with the effective dose of morphine could significantly suppress morphine-induced CPP compared with the Mor 5 group ($P=0.006$) (Figure 4B). On the other hand, the ineffective dose of morphine in combination with either low ($P=0.112$) or high ($P=0.158$) current intensity exhibited no significant effects (Figure 4A, B).

**Effect D2 receptor antagonist microinjection into CeA on the acquisition phase of morphine-induced CPP**

The results showed that administration of eticlopride into CeA with the effective dose of morphine could significantly attenuate conditioning score in comparison with Mor 5 SC group ($P=0.03$). In contrast, administration of Mor 5 enhanced conditioning score compared with the Sal SC group ($P=0.05$) (Figure 5).

![Figure 4. Effect of electrical stimulation with low and high current intensities on the central nucleus of the amygdala (CeA) in the expression phase of conditioned place preference (CPP) in male rats](image)

![Figure 5. Effect of eticlopride on the acquisition phase of conditioned place preference (CPP) in male rats. One-way analysis of variance followed by Tukey post hoc test showed that eticlopride in combination with the effective dose of morphine significantly inhibited the acquisition phase of CPP compared with mor 5 SC group. *$P<0.05$ compared with the Mor group; $^*P<0.05$ compared with the Sal SC group](image)

![Figure 6. Effect of lidocaine reversible inactivation on the central nucleus of the amygdala (CeA) in both acquisition and expression phases of conditioned place preference (CPP) in male rats. One-way analysis of variance followed by Tukey post hoc test showed that lidocaine in combination with effective dose of morphine significantly inhibited both expression and acquisition phases of CPP compared with Mor5 SC group, +$P<0.05$ compared with the Mor 5 SC group](image)
**Effect of lidocaine reversible inactivation on the CeA**

Injection of lidocaine into the CeA in combination with the effective dose of morphine in both acquisition ($P=0.02$) and expression ($P=0.03$) phases reduced morphine-induced CPP in comparison with the Mor 5 SC group, significantly. Also, the observation was detected in combination with the ineffective dose of morphine, but was not significant ($P=0.48$) (Figure 6).

**Discussion**

The purpose of this study was to evaluate the effect of electrical stimulation of CeA on morphine-induced CPP in male rats.

The current study results showed that CeA electrical stimulation with current intensity of 25 µA in combination with the ineffective dose of morphine could suppress the acquisition phase of morphine-induced CPP compared with the Mor group (Figure 3A). In contrast, stimulation of other areas of the brain such as the prelimbic cortex of mPFC enhanced the morphine-induced CPP in the rat (12). Several investigations on mPFC and NAc indicated that electrical stimulation in combination with the ineffective dose of morphine induced CPP (24, 27). However, some researchers reported different effects of electrical stimulation on CPP (26, 28). It is important to notice that CPP is a learning model that requires formation of associations between reward and particular location (29). Since the CeA has a role in morphine-dependent memory retrieval (30), absence of morphine-induced CPP in this study may be attributed to a decrease in the reward signal or an insufficient response to rewarding stimuli, which would impair learning and memory formation throughout the conditioning phase (Figure 3B).

Moreover, the present investigation showed that CeA stimulation with current intensity of 150 µA in combination with 5 mg/kg dose of morphine could block CPP in the expression phase; although, the low intensity blocked CPP in both phases (Figure 4 B). In agreement with these findings, previous research demonstrated that peripheral electrical stimulation inhibited both the expression of morphine-induced CPP and reactivation of extinguished CPP (27).

In the present study, it is possible that electrical stimulation of CeA induced the release of gamma-aminobutyric acid (GABA) in CeA. The increment of GABA reduces DA release of CeA induced the release of gamma-aminobutyric acid (GABA) in CeA. The increment of GABA reduces DA release in CeA, which may be implicated in opioid reward (31, 32).

The present study showed that microinjection of dopamine D2 receptor antagonist, eticlopride, into the CeA with the effective dose of morphine decreased morphine conditioning (Figure 4). Rezayof et al. reported that dopamine D2 receptor antagonist decreased morphine-induced CPP dose-dependently. Also, the drug attenuated the potentiation induced by dopamine D2 receptor agonist (37). Thus, inhibition of DA D2 receptors by eticlopride probably blocked the reward-related motivation learning. In line with this possibility, some studies proposed that dopamine receptor agonists could support reward-related motivation learning, while antagonists prevent the typical effects of reward on behavior (38, 39). An evidence also presented NMDA receptor antagonist, impairing learning and memory, could prevent opiate dependence (40). Furthermore, some studies showed that the amygdala may play a critical role in stimulus-reward learning (18, 41). The CeA lesion before conditioning impaired the acquisition of the conditioned responses (42). Therefore, either activation or inhibition of dopamine D2 receptors may influence the CeA-associated memory. In this study, the effect of electrical stimulation on CPP was similar to the dopamine antagonist. The object reveals that electrical stimulation could be an appropriate way to understand and detect the areas and mechanisms involved in addiction (Figure 5).

In addition, the present study decided to investigate the effect of CeA inactivation by lidocaine on morphine-induced CPP. Lidocaine hydrochloride is a transitory inhibitor of steady-state tetrodotoxin-sensitive sodium channels. It reversibly blocks neuronal action, unlike lesion. Lidocaine neuronal inactivation allows study of the role of particular brain areas in learning and memory (43). In accordance with this, the current findings showed that injection of lidocaine into the CeA accompanied with morphine effective dose significantly reduced morphine-induced CPP during both expression and acquisition phases (Figure 6). As mentioned above, CeA has a key role in reward-related memory, therefore, morphine-dependent learning is probably linked to the rewarding effects of morphine (18) proven by the present study.

**Conclusion**

The present study showed that electrical stimulation of CeA with low current intensity in combination with both morphine effective and ineffective doses blocked morphine-induced CPP in both expression and acquisition phases. It is possible that electrical stimulation of CeA disrupted memory and learning in the paradigm by changing concentration of DA resulting from changing the GABA concentration. The possibility was approved by the effects of electrical stimulation and DA antagonist on CPP. Conversely, inactivation of CeA by lidocaine disrupted the morphine-induced CPP indicating the important role of CeA in learning.

**Acknowledgment**

The results presented in this paper were part of a student PhD thesis.

**Authors’ Contributions**

HAA Conceived the study and design; ZJ and HAA Analyzed data and prepared the draft manuscript; ZJ, HA A, SK, and MS Critically revised the paper; ZJ, HA A, SK, and MS Approved the final version to be published.

**Funding**

This research was financially supported by the Kazerun Branch, Islamic Azad University, Kazerun, Iran (IR.IAU. KAU.REC.1400.061). We appreciate all who assisted us in this research.

**Conflicts of Interest**

The authors declare that they do not have any conflicts of interest.
References

1. Hemati K, Pourhanifeh MH, Dehdashtian E, Fatemi I, Mehrzadi S, Reiter RJ, et al. Melatonin and morphine: potential beneficial effects of co-use. Fundam Clin Pharmacol 2021; 35:25-39.

2. Ueda H, Ueda M. Mechanisms underlying morphine analgesic tolerance and dependence. Front Biosci 2009; 14:5260-5272.

3. Volkow ND, Fowler J, Wang G, Baler R, Telang F. Imaging dopamine's role in drug abuse and addiction. Neuropharmacol 2009; 56:3-8.

4. Wang TR, Moosa S, Dallapiazza RF, Elias WJ, Lynch WJ. Deep brain stimulation for the treatment of drug addiction. Neurosurg Focus 2018; 45:1-19.

5. Perlmutter JS, Mink JW. Deep brain stimulation. Annu Rev Neurosci 2006; 29:229-257.

6. Okun MS. Deep-brain stimulation for parkinson's disease. N Engl J Med 2012; 367:1529-1538.

7. Kisely S, Li A, Warren N, Siskind D. A systematic review and meta-analysis of deep brain stimulation for depression. Depress Anxiety 2018; 35:468-480.

8. Baldermann JC, Schueler T, Huys D, Becker I, Timmermann L, Jessen F, et al. Deep brain stimulation for tourette-syndrome: a systematic review and meta-analysis. Brain Stimul 2016; 9:296-304.

9. Alonso P, Cuadras D, Gabriël L, DENYS D, Goodman W, Greenberg BD, et al. Deep brain stimulation for obsessive-compulsive disorder: a meta-analysis of treatment outcome and predictors of response. PloS one 2015; 10:1-16.

10. Alaei H, Pour MG. Stimulation and transient inactivation of ventral tegmental area modify reinstatement of acquisition phase of morphine-induced conditioned place preference in male rats. Brain Res Bull 2021; 176:130-141.

11. Amohashemi E, Reisi P, Alaei H. Lateral habenula electrical stimulation with different intensities in combination with GABAB receptor antagonist reduces acquisition and expression phases of morphine-induced CPP. Neurosci Lett 2021; 759:135996-136001.

12. Kargar A, Ramshini E, Alaei H, Sedighi M, Oryan S. Different current intensities electrical stimulation of prelimbic cortex of mPFC produces different effects on morphine-induced conditioned place preference in rats. Behav Brain Res 2012; 231:187-192.

13. Basa V, Tran TLN, Caputo J, Guerin GF, Goeders NE, Wilden J. Intermittent bilateral deep brain stimulation of the nucleus accumbens shell reduces intravenous methamphetamine intake and seeking in Wistar rats. J Neurosurg 2017; 126:1339-1350.

14. Creed M, Pascoli VJ, Lüscher C. Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology. Science 2015; 347:659-664.

15. SABET KM, Masoudinia F, Khansefid N, BEHZADI Z. Opioid receptors of the central amygdala and morphine-induced antinociception. Iran Biomed J 2007; 11:75-80.

16. Freedman L, Cassell M. Distribution of dopaminergic fibers in the central division of the extended amygdala of the rat. Brain Research 1994; 633:243-252.

17. Bie B, Wang Y, Cai Y-Q, Zhang Z, Hou Y-Y, Pan ZZ. Upreregulation of nerve growth factor in central amygdala increases sensitivity to opioid reward. Neuropsychopharmacology 2012; 37:2780-2788.

18. Baxter MG, Murray EA. The amygdala and reward. Nat Rev Neurosci 2002; 3:563-573.

19. Bardo MT, Hertfield DT, Yates JR. Conditioned place preference as a preclinical model for screening psychopharmacotherapies for drug abuse. Nonclinical Assessment of Abuse Potential for New Psychopharmacicals: Elsevier; 2015; 151-196.

20. Prus AJ, James JR, Roscencrans JA. Conditioned Place Preference. 2nd ed. 2009.

21. Ghavipanjeh GR, Pourshanazari AA, Alaei H, Karimi S. The influence of electrical stimulation on dorsal raphe nucleus with different current intensities on morphine-induced conditioned place preference in male rats. Pharmacol Rep 2015; 67:832-836.

22. Ghavipanjeh GR, Pourshanazari AA, Alaei H, Karimi S, Nejad MA. Effects of temporary inactivation and electrical stimulation of the dorsal raphe nucleus on morphine-induced conditioned place preference. Malays J Med Sci 2015; 22:33-40.

23. Yan N, Chen N, Zhu H, Zhang J, Sim M, Ma Y, et al. High-frequency stimulation of nucleus accumbens changes in dopaminergic reward circuit. PLoS One 2013; 8:79318-79325.

24. Radadhami M, Ramshini E, Hosseini N, Karimi S, Alaei H. Effect of electrical stimulation of nucleus accumbens with low, median and high currents intensities on conditioned place preference induced by morphine in rats. Adv Biomed Res 2014; 3:14-19.

25. Paxinos G, Watson C. The Rat Brain in Stereotactic Coordinates: Hard Cover. 6th ed. 2006.

26. Hao Y, Yang J, Sun J, QI J, Dong Y, Wu CF. Lesions of the medial prefrontal cortex prevent the acquisition but not reinstatement of morphine-induced conditioned place preference in mice. Neurosci Lett 2008; 433:48-53.

27. Shi X-D, Wang G-B, Ma Y-Y, Ren W, Luo F, Cui C-L, et al. Repeated peripheral electrical stimulations suppress both morphine-induced CPP and reinstatement of extinguished CPP in rats. A rapidly accelerated expression of PPE and PPD mRNA in NAc implicated. Mol Brain Res 2004; 130:124-133.

28. Koob G, Volkow N. Neurocircuity of addiction. Neuropsychopharmacology 2010; 35:217-238.

29. Tzschentke TM. Review on CPP: measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 2007; 12:227-462.

30. Arjdmand A, Rezayof A, Zarrindast M-R. Involvement of central amygdala NMDA receptor mechanism in morphine state-dependent memory retrieval. Neurosci Res 2011; 69:25-31.

31. Madhavan A, Bonci A, Whistler JL. Opioid-induced GABA potentiation after chronic morphine attenuates the rewarding effects of opioids in the ventral tegmental area. J Neurosci 2010; 30:14029-14035.

32. Zhang Z, Tao W, Hou Y-Y, Wang W, Lu Y-G, Pan ZZ. Persistent pain facilitates response to morphine reward by downregulation of central amygdala GABAergic function. Neuropsychopharmacology 2014; 39:2263-2271.

33. Everitt BJ, Parkinson JA, Oimstede MC, Arroyo M, Robledo P, Robbins TW. Associative processes in addiction and reward the role of amygdala-ventral striatal subsystems. Ann N Y Acad Sci 1999; 877:412-438.

34. Gonzales C, Cheselet MF. Amygdalomaligral pathway: an arteriography study in the rat with Phaseolus vulgaris leucoagglutinin (PHA-L). J Comp Neurol 1990; 297:182-200.

35. Leshan RL, Opland DM, Louis GW, Leininger GM, Patterson CM, Rhodes CJ, et al. Ventral tegmental area leptin receptor neurons specifically project to and regulate cocaine-and amphetamine-regulated transcript neurons of the extended central amygdala. J Neurosci 2010; 30:5713-5723.

36. Nikolaus S, Wittsack H-J, Beu M, Antke C, De Souza Silva MA, Wickrath F, et al. Deep brain stimulation for tourette-syndrome: a meta-analysis of treatment outcome and meta-analysis of deep brain stimulation for depression. Depress Anxiety 2021; 6:9.

37. Rezayof A, Zarrindast M-R, Sahraei H, Haeri-Rohani A. Involvement of central amygdala and nucleus accumbens in morphine-induced conditioned place preference in male rats. Malays J Med Sci 2015; 22:33-40.
radial maze performance of rats. Pharmacol Biochem Behav 1997; 58:249-253.
41. Chesworth R, Corbit L. The contribution of the amygdala to reward-related learning and extinction. The Amygdala—Where Emotions Shape Perception, Learning and Memories. 2017;1:13.
42. Ono T, Nishijo H, Uwano T. Amygdala role in conditioned associative learning. Prog Neurobiol 1995; 46:401-422.
43. Khatami L, Khodagholi F, Motamedi F. Reversible inactivation of interpeduncular nucleus impairs memory consolidation and retrieval but not learning in rats: a behavioral and molecular study. Behav Brain Res 2018; 342:79-88.