Original Article

Low Current Intensity Plus an Ineffective Dose of Morphine Affect Conditioning Place Preference Through Different Pathways in the Lateral Habenula

Elahe Amohashemi, Parham Reisi, Hojjat Allah Alaei

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: The involvement of lateral habenula and the ineffective dose of morphine on reward-related learning and memory is less well-known. This study looked into the effects of electrical stimulation, $GABA_B$ receptor blockade, and a combination of both with morphine on conditioned place preference.

Materials and Methods: In this experiment, male rats were anesthetized with ketamine/xylazine (six rats in each group). A 5-day biased conditioned place preference paradigm was used for the behavioral test. The effects of electrical stimulation and phaclofen plus a low dose of morphine on the acquisition and expression phases were examined during conditioning sessions and before the test phase, respectively.

Results: The conditioning scores were reduced by antagonist injection during the acquisition phase. Interestingly, different intensities exhibited opposite effects on the acquisition phase. Conditioned place preference scores during the acquisition phase were significantly induced by 25 μA electrical stimulation, while conditioning scores were suppressed by electrical stimulation at 150 μA. Phaclofen (2 μg/rat) combined with high intensity induced aversion during the acquisition phase, while inhibiting expression. In contrast, high intensity with phaclofen (1 μg/rat) inhibited only the acquisition session. However, low intensity during the acquisition phase had an additive effect that was prevented by pretreatment with phaclofen (2 μg/rat), but this response was modified by the antagonist's low dose.

Conclusions: A behavioral technique called conditioned place preference is frequently used to evaluate learning that is related to rewards. Therefore, lateral habenula electrical stimulation and phaclofen plus morphine could affect place preference through the involvement of the reward system.

Keywords: Addiction, electrical stimulation, morphine

Address for correspondence: Prof. HojjatAllah Alaei, Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: alaei@med.mui.ac.ir
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Introduction

There is evidence that suggests that activation of the mu-opioid receptors in the lateral habenula may play the primary role in inducing the morphine reward's effects.[1,2] The mu-opioid receptors mediate some of the actions of morphine, including its rewarding and analgesic properties.[3,4]

The GABA neurotransmitter exerts inhibitory effects through receptors such as $GABA_A$, $GABA_B$, and $GABA_C$.[5] $GABA_B$ receptors have been proven to affect memory functions in several species.[6] The states of excitability and inhibition in the parts of the brain involved in processing information and processes associated with rewards may be regulated by the GABAergic system.[7] Addiction may be influenced by an imbalance of the neurotransmitters that limit learning and memory, GABA, and glutamate.[8]

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Lateral habenula can serve as a mediator for the rewarding effects of opiates since it is connected to the mesolimbic dopamine pathway, which links the ventral tegmental area to the nucleus accumbens. On the other hand, electrical stimulation of this area prevents the development of morphine-induced conditioned place preference.[9] Stimulating the pathway from the ventral tegmental area to the lateral habenula as well as the lateral habenula lesion also caused place preference behavior and increased ethanol consumption, respectively.[10,11]

Recently, the effect of lateral habenula's GABA receptors on the reward system has been established.[9] The lateral habenula has a high concentration of $GABA_B$ receptors and plays an essential role in regulating the dopamine system.[12] The involvement of lateral habenula's $GABA_B$ receptors in the reward circuits, particularly in response to a low dose of morphine, is not supported by any data. To this end, the present study was designed to evaluate the effects of lateral habenula electrical stimulation and intra-lateral habenula injections of phaclofen, alone and a combination of both, plus the ineffective dose of morphine on conditioned place preference.

Materials and Methods

Animals

Male adult Wistar rats (260–290 g) were purchased from the Isfahan University of Medical Sciences (Faculty of Pharmacy, Isfahan, Iran). The rats were housed under a 12-h light/dark cycle. The animals received standard free food and water. The study was allowed by the Isfahan University of Medical Sciences' Ethic Committee for Animal Use (IR.mui.MED.REC.1397.244), and all operations were carried out in compliance with those guidelines' institutional policies on animal care and the use of laboratory animals (National Institutes of Health Publication No. 85–23, revised 2010).

Animal groups

Morphine operation, electrical stimulation (25 μA) + morphine (acquisition phase), electrical stimulation (25 μA) + morphine (expression phase), electrical stimulation (25 μA) + saline (acquisition phase).

Electrical stimulation (150 μA) + morphine (acquisition phase), electrical stimulation (150 μA) + morphine (expression phase), electrical stimulation (150 μA) + saline (acquisition phase).

Phaclofen (1 μg/rat) + morphine (acquisition phase), phaclofen (1 μg/rat) + morphine (expression phase), phaclofen (1 μg/rat) + saline (acquisition phase).

Phaclofen (2 μg/rat) + morphine (acquisition phase), phaclofen (2 μg/rat) + morphine (expression phase), phaclofen (2 μg/rat) + saline (acquisition phase).

Electrical stimulation (25 and 150 μA) + phaclofen (1 μg/rat) + morphine, electrical stimulation (25 and 150 μA) + phaclofen (2 μg/rat) + morphine (n = 6 in all groups).

Drugs

Morphine (Temad, Iran, ip); Phaclofen is a selective $GABA_B$ receptors antagonist (Sigma-Aldrich, Germany, intra- lateral habenula); Urethane (Sigma-Aldrich, Germany, ip); Morphine, phaclofen, and urethane were dissolved in saline (0.9%); Ketamine and xylazine (Darou Pakhsh, Iran, ip).

Apparatus and the study protocol

To assess stimulus–reward associations, a place conditioning apparatus was used according to a biased procedure. The conditioned place preference apparatus involved two equal-sized (30 × 30 × 30 cm) and cue-different chambers (compartment A had black and white walls with a textured floor; compartment B was made from white walls and a smooth floor). The start box, as the third chamber (in cm) (C, 30 × 10 × 30), links to the A and B compartments. Chamber C is separated from the two main compartments by a guillotine door.

The procedure consists of 5 continuous days with three distinct phases: pre-conditioning, conditioning, and post-conditioning [Figure 1c]; Day 1 (pre-conditioning), Days 2, 3, and 4 (conditioning days), and Day 5 (post-conditioning session). Animals had access to the apparatus by opening the movable door of chamber C during the pre-conditioning and post-conditioning periods (duration 900 s). During the pre- and post-conditioning sessions, morphine was not administered. During the conditioning phase, the animals were given saline (s.c.) and morphine (s.c, 0.5 mg/kg) (in the alternate morning and afternoon designs, interval 6 h). There were six sessions in the conditioning session (45 min). Animals received drugs (intra-lateral habenula)

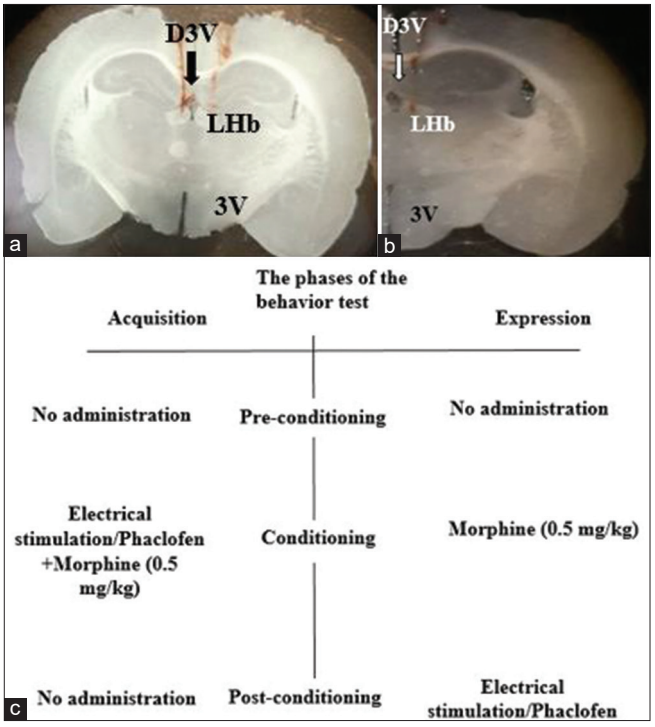

Figure 1: Coronal photomicrograph of bilateral microinjection (a) and unilateral electrical stimulation sites in lateral habenula (b). D3V, Dorsal 3rd ventricle; 3V, 3rd ventricle; LHb, Lateral habenula. Study protocol (c)

in three sessions while placed in a non-preferred compartment. In the remaining three sessions, saline (intra-lateral habenula) was administered while held in a preferred chamber.[9]

The method of electrical stimulation

The lateral habenula was exposed to electrical stimulation at two current densities (25 and 150 μA) at a specific frequency (25 Hz) (Stimulator Isolator A36O, WPI, USA). Different intensities were applied to the nucleus during the 3-day conditioning, 5 min before morphine administration (acquisition phase). Electrical stimulation of the lateral habenula was also performed to evaluate this change in the expression phase, 5 min before the test session.

Stereotaxic surgery and drug microinjections

The animals were anesthetized by injecting a mixture of ketamine and xylazine (100/10 mg/kg, ip)[9] and placed in a stereotaxic apparatus. According to the atlas of the rat brain, the lateral habenula coordinates in mm were: –3.9 (AP), 0.6-0.9 (ML), and 5.2 (DV). Finally, the two 22-gauge guide cannulas (bilaterally) and the stimulating electrode (unilaterally) were secured to anchor the jeweler's screws with dental cement. The drugs were microinjected into the lateral habenula using a 1-l Hamilton syringe that was attached to an injection needle (30 G) via a polyethylene tube.

Drug treatments

Determination of ineffective dose of morphine

Based on the morphine dose chart in the previous study, the low dose of morphine (0.5 mg/kg, s.c.) was selected. The involvement of the lateral habenula in the reward system has recently been discovered. The role of the lateral habenula in response to morphine is poorly understood.[9] Because there are no reports of its response to ineffective doses of morphine, this brain nucleus was chosen for our investigation

Effects of intra-lateral habenula microinjection of phaclofen and/or lateral habenula electrical stimulation on the expression and acquisition phases of conditioned place preference

To evaluate the effect of the agents on the acquisition, phaclofen (1 and 2 μg/rat)/saline (1 μl/rat) or electrical stimulation (25 and 150 μA), during the 3 days of the conditioning phase, 5 min before injection morphine or saline was used in lateral habenula. Also, to study the impact of agents on the expression phase, phaclofen or electrical stimulation was applied to the lateral habenula 5 min before the testing session.

Locomotor activity

The number of compartment crossings of rats in the conditioned place preference apparatus during 15 min (900 s) was employed to assess the rats' locomotion activity (in the post-conditioning session, the number of chamber crossings was recorded using the ANY-Maze software).

Histology

After the experimental sessions, the animals received deep urethane anesthesia, 0.9% saline and 10% formalin perfusion, brain removal and fixation in a formalin solution for 5 days, and coronal sectioning (50 m). The cannula and stimulating electrode were directed for the lateral habenula using the rat brain atlas as a guide, and the slices were analyzed to confirm this [Figure 1a and b].

Statistical analysis

The comparison between the experimental groups and motor activity was performed using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis. P value <0.05 was considered statistically significant. SPSS statistical software version 23 was used (Quantitative data).

Result

Effects of unilateral electrical stimulation of lateral habenula on the expression and acquisition phases

The effect of 25 and 150 μA electrical stimulation on the acquisition phase is shown in Figure 2a. In comparison to the morphine group, there was a significant difference between the groups receiving electrical stimulation at intensities of 25 or 150 μA [one-way ANOVA: F (2, 17) = 16.549, $P < 0.001$]. Interestingly, the acquisition phase was induced by morphine + 25 μA electrical stimulation ($P < 0.05$). However, an intensity of 150 μA + morphine reduced the conditioning scores compared to the morphine group ($P < 0.05$), indicating that high and low intensities of electrical stimulation could affect the reward system through a different pathway. However, electrical stimulation in groups receiving saline could not alter conditioned place preference scores [one-way ANOVA: F (2, 17) = 0.112, $P > 0.05$].

The expression was not significantly affected by electrical stimulation [one-way ANOVA (25 μA): F (2, 17) = 0.141, $P > 0.05$; (150 μA) F (2, 17) = 0.691, $P > 0.05$, Figure 2b].

Effects of microinjection of $GABA_B$ receptors antagonist within lateral habenula on the expression and acquisition phases

The effect of phaclofen (1 and 2 μg/rat) on the acquisition is shown in Figure 2c. An antagonist by itself did not affect place preference, according to a one-way ANOVA [F (2, 17) = 0.0793, $P > 0.05$]. However, inhibition of $GABA_B$ receptors in the morphine group induced a significant effect on morphine response during the acquisition phase [F (2, 17) = 7.725, $P < 0.01$]. The drug (2 μg/rat) prevented the place preference in comparison with the morphine group ($P < 0.01$), indicating that the high dose of phaclofen could suppress conditioning scores.

The expression phase were unaffected by phaclofen (1 and 2 μg/rat, intra-lateral habenula) during testing, as shown in Figure 2d [F (2, 17) = 1.289, $P > 0.05$].

Effects of blockade of $GABA_B$ receptors with a low dose of drug on electrical stimulation response during expression and acquisition phases

The effect of combining phaclofen (1 μg/rat) with two current intensities (25 and 150 μA) on the acquisition is

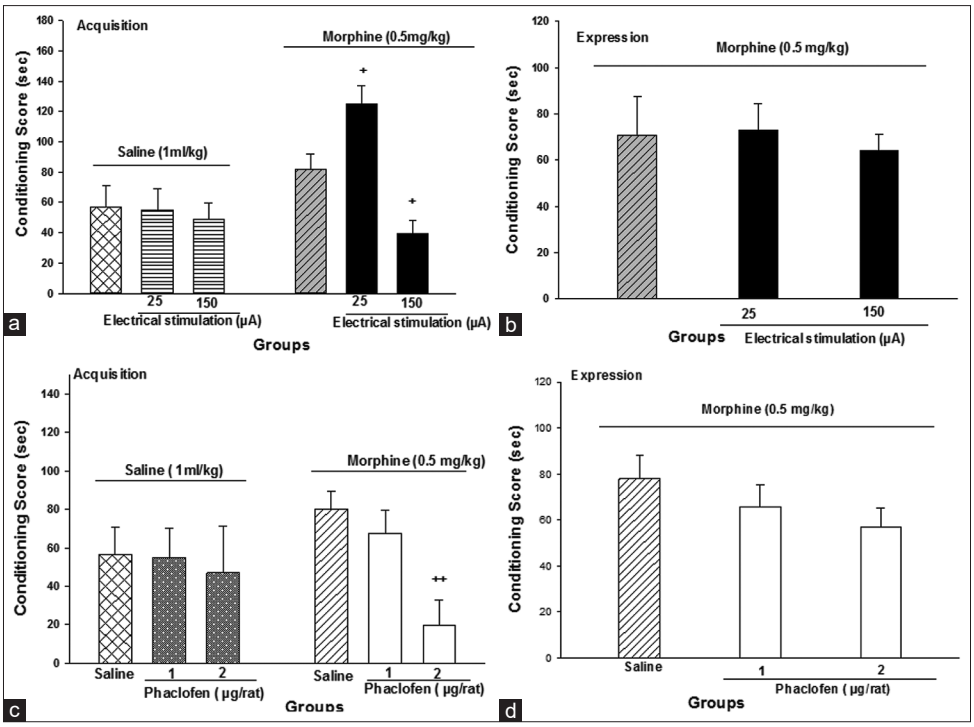

Figure 2: The effects of unilateral electrical stimulation of lateral habenula (a, b) and bilateral intra-lateral habenula infusions of phaclofen (c, d), either alone or in combination with morphine, on the acquisition (a, c). On the expression of place preference (b, d). The conditioning score was computed as the difference between the amount of time the animal spends in the morphine-associated box (testing) from the time it spends in the non-preference chamber (in the pre-conditioning stage). Data are expressed as averages ± S.E.M. of the six animals in each group $+P < 0.05$, $++P < 0.01$ demonstrates a significant difference compared to morphine

shown in Figure 3a. The results showed a significant impact of phaclofen + 25 μA electrical stimulation on conditioning scores [F (3, 23) = 8.556, $P < 0.001$]. Drug + electrical stimulation of 150 μA changed morphine response [F (3, 23) = 5.009, $P < 0.01$]. The intensity of 25 μA + phaclofen significantly elicited conditioning scores ($P < 0.05$). In contrast, antagonist + 150 μA electrical stimulation attenuated the conditioning scores compared to the morphine group ($P < 0.05$). Electrical stimulation with different intensities + a low dose of the drug could induce different responses in the acquisition phase.

Figure 3b portrays the effect of phaclofen (1 μg/rat) + electrical stimulation on the morphine response during the expression phase. Phaclofen did not alter the effect of electrical stimulation [One-way ANOVA: F (4, 29) = 0.0991, $P > 0.05$; F (4, 29) = 1.058, $P > 0.05$; respectively].

Effects of blockade of $GABA_B$ receptors with a high dose of the drug on electrical stimulation response during expression and acquisition phases

The interaction between 2 μg/rat dose of phaclofen and electrical stimulation during the acquisition is shown in Figure 3c. One-way ANOVA revealed that phaclofen reversed the response induced by electrical stimulation (25 μA) [F (3, 23) = 22.372, $P < 0.001$], while drug potentiated the effect of 150 μA electrical stimulation [F (3, 23) = 12.066, $P < 0.001$]. Phaclofen attenuated the conditioning scores induced by electrical stimulation (25 μA) ($P < 0.05$). Additionally, the antagonist + intensity of 150 μA decreased morphine response and induced aversion in conditioned place preference compared to the morphine group ($P < 0.001$, Figure 3c).

Figure 3d shows the effect of phaclofen (2 μg/rat) + electrical stimulation on the morphine response during the expression phase. Phaclofen reduced the effect of electrical stimulation (150 μA) on conditioning scores [one-way ANOVA: F (3, 23) = 3.636, $P < 0.05$]. The combination drug with high intensity significantly decreased morphine response ($P < 0.05$). However, high dose of antagonist + low intensity did not alter conditioning scores [one-way ANOVA: F (3, 23) = 0.790, $P > 0.05$]. The drug + high intensity could have a synergistic effect on the expression.

Effects of blockade of $GABA_B$ receptors into lateral habenula and/or electrical stimulation of this area on the locomotor activity

The electrical stimulation of lateral habenula and antagonist (phaclofen), both singly and combinatorically, and the injection of different doses of morphine did not change the locomotion (data are not shown).

Discussion

Data indicated that a lower dose of morphine, which did not cause conditioned place preference, combined with unilateral

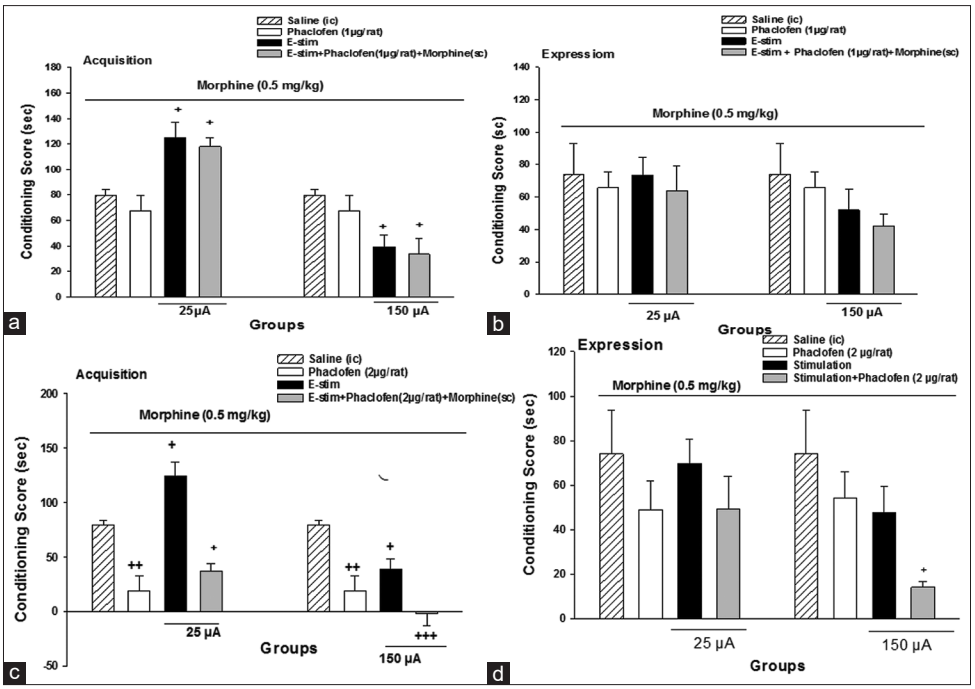

Figure 3: The effects of bilateral intra-lateral habenula pretreatment with phaclofen (1 µg/rat, a, b) and (2 µg/rat, c, d) on unilateral lateral habenula electrical stimulation response on the acquisition phase (a, c). On the expression of conditioned place preference (b, d). The change of preference is assessed as the difference between the amount of time the animal spends in the morphine-associated box (testing) and the time it spends in the non-preference chamber (in the pre-conditioning stage). Values are expressed as averages ± S.E.M. of the six animals in each group. $+P < 0.05$, $++P < 0.01$, $+++P < 0.001$ demonstrate a significant difference compared to morphine. E-stim = Electrical stimulation

electrical stimulation at a high intensity might significantly inhibit the acquisition; however, at a low intensity, the acquisition phase was increased [Figure 2a], while the retrieval processes did not significantly change [Figure 2b]. Recent publications pointed out that the lateral habenula unilateral electrical stimulation is effective in inducing the necessary effects; therefore, bilateral stimulation was unnecessary.[13] Consistent with the findings of others indicating that electrical stimulation of other areas of the brain with low and high electrical stimulation plus low doses of morphine elicited different responses in the acquisition phase.[14,15] However, to date, most research has examined the effect of high-intensity lateral habenula electrical stimulation on behavior.[13] The impact of low-intensity electrical stimulation of lateral habenula, in particular, on conditioned place preference has received very little scientific attention.

The presence of glutamatergic neurons, as well as glutamatergic inputs in lateral habenula, has been established.[1] Excitatory projections of lateral habenula to either the inhibitory neurons in the rostromedial tegmental nucleus (lateral habenula-rostromedial tegmental nucleus-ventral tegmental area pathway) or the dopamine neurons in the ventral tegmental area (lateral habenula-ventral tegmental area pathway). Appetitive reward learning via the lateral habenula-rostromedial tegmental nucleus-ventral tegmental area pathway may be influenced by high-intensity lateral habenula electrical stimulation.[16,17] On the other hand, other studies have determined that lateral habenula electrical stimulation activates excitatory outputs to the ventral tegmental area. There are different lateral habenula populations projected to the ventral tegmental area or rostromedial tegmental nucleus; activating them separately may induce opposite effects on mesolimbic dopamine release.[18] It is possible that lateral habenula electrical stimulation with low intensity plus an ineffective dose of morphine induces morphine response through the lateral habenula-ventral tegmental area pathway. High-intensity electrical stimulation could attenuate morphine response through the polysynaptic pathway [Figure 4].[18]

Infusions of $GABA_B$ receptors antagonist, phaclofen, into lateral habenula also in saline groups during conditioning days did not cause place preference [Figure 2c]. The drug (2 µg/rat) plus morphine (lower doses), which did not cause conditioned place preference by itself, reduced morphine response during the acquisition phase [Figure 2c]. Intra-lateral habenula microinjection of phaclofen before testing had no significant effects on place preference [Figure 2d]. Consistent with our observation, the activation of GABA receptors or blockade of $GABA_B$ receptors in lateral habenula results in reward and aversion, respectively.[9,19,20] Some brain regions send GABAergic outputs to the lateral habenula. Also, the high expression of $GABA_B$ receptors in this area of the brain has been proven.[12,21] Therefore, inhibition of $GABA_B$ receptors may change the balance of glutamate and GABA neurotransmitters and reduce the impact of GABA on lateral habenula. Finally, increases the activation of the rostromedial tegmental nucleus.[1]

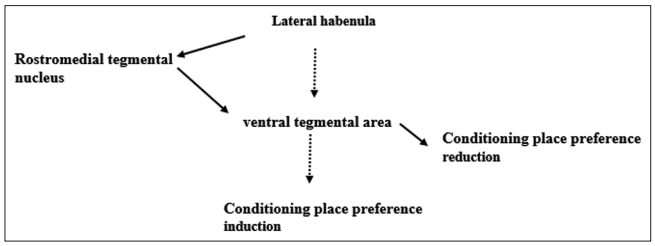

Figure 4: Activation of the lateral habenula-rostromedial tegmental nucleus-ventral tegmental area pathway leads to conditioned place preference inhibition (solid lines). Activation of the lateral habenula-ventral tegmental area pathway leads to conditioned place preference induction (dotted lines)

Moreover, phaclofen and electrical stimulation did not change locomotion. Therefore, it is possible that the motor impairment is not due to the decrease in conditioning scores.

Others have suggested that activating or inhibiting lateral habenula neurons can alter the level of dopamine released in areas like the medial prefrontal cortex and other regions that are important for learning and memory functions.[22,23] Microinjection of phaclofen into the lateral habenula may reduce learning, which in turn inhibits place preference, and may confirm that $GABA_B$ receptors in lateral habenula are involved in reward-related learning.

Considering the dose-dependent response of phaclofen in the morphine group, it was decided to investigate this effect by combining the ineffective dose of phaclofen with electrical stimulation. Evidence from a series of experiments showed that phaclofen (1 μg/rat) during conditioning moderated the enhancement of low-intensity morphine reward [Figure 3a]. The expression phase was not significantly affected by a low dose of the drug combined with electrical stimulation [Figure 3b]. However, a high dose of the drug plus an intensity of 25 μA significantly prevented the increase in the acquisition phase [Figure 3c], demonstrating that phaclofen reverses conditioning scores induced by the low intensity in a dose-dependent manner. Phaclofen (2 μg/rat) plus high intensity attenuated expression and produced an aversion in the acquisition phase [Figure 3c and d]. It seems that drug plus electrical stimulation (150 μA) could potentiate each other's effects on the increase of activation of neurons of the rostromedial tegmental nucleus, and regulate dopamine release in the midbrain. Lateral habenula may mediate dopamine neuron reward.

The neurons in the lateral habenula are frequently glutamatergic. They induce a significant impact on the neurons in the ventral tegmental area. As a result, additional research is needed to examine the influence of these neurons in the lateral habenula on the reward system. To summarize, research must be done to determine the cellular mechanisms in the lateral habenula in the development of mood disorders, drug addiction, and perhaps other illnesses.

There were limitations in this study, including the mortality of the rats used.

Conclusions

The lateral habenula projects on both ventral tegmental area dopamine neurons and rostromedial tegmental GABA neurons, which could potentially cause opposite behavioral responses. During the acquisition period, electrical stimulation of the lateral habenula at various levels led to opposite effects on conditioning scores. Electrical stimulation of 25 μA may activate lateral habenula glutamatergic projections to ventral tegmental area dopamine neurons and produce pleasurable effects similar to those of morphine by increasing dopamine release and involving the reward system, which in turn induces place preference. This indicated that the low intensity may increase memory and learning. Phaclofen and electrical stimulation at high intensity, acting via the lateral habenula-rostromedial tegmental nucleus-ventral tegmental area circuit, may decrease midbrain dopamine release and reduce the acquisition phase, which may help to prevent morphine dependency.

Ethics approval and consent to participate:

The study was allowed by the Isfahan University of Medical Sciences' Ethic Committee for Animal Use (IR.mui.MED.REC.1397.244), and all operations were carried out in compliance with those guidelines' institutional policies on animal care and the use of laboratory animals (National Institutes of Health Publication No. 85–23, revised 2010).

Availability of data and materials

Data will be made available upon request.

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Conflicts of interest

There are no conflicts of interest.

References

1. Margolis EB, Fields HL. Mu opioid receptor actions in the lateral habenula. PLoS One 2016;11:e0159097.
2. Almeida-Santos AF, Gobira PH, Souza DP, Ferreira RC, Romero TR, Duarte ID, *et al*. The antipsychotic aripiprazole selectively prevents the stimulant and rewarding effects of morphine in mice. Eur J Pharmacol 2014;742:139-44.
3. Fields HL, Margolis EB. Understanding opioid reward. Trends Neurosci 2015;38:217-25.
4. Henderson-Redmond AN, Yuill MB, Lowe TE, Kline AM, Zee ML, Guindon J, *et al*. Morphine-induced antinociception and reward in "humanized" mice expressing the mu opioid receptor A118G polymorphism. Brain Res Bull 2016;123:5-12.
5. Sallard E, Letourneur D, Legendre P. Electrophysiology of ionotropic GABA receptors. Cell Mol Life Sci 2021;78:5341-70.
6. Carletti R, Libri V, Bowery N. The GABA (B) antagonist cgp 36742 enhances spatial-learning performance and antagonizes baclofen-induced amnesia in mice. Br J Pharmacol 1993;109: 74-86.

7. Page CE, Coutellier L. Prefrontal excitatory/inhibitory balance in stress and emotional disorders: Evidence for over-inhibition. Neurosci Biobehav Rev 2019;105:39-51.
8. Wydra K, Golembiowska K, Zaniewska M, Kamińska K, Ferraro L, Fuxe K, *et al*. Accumbal and pallidal dopamine, glutamate and GABA overflow during cocaine self-administration and its extinction in rats. Addict Biol 2013;18:307-24.
9. 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.
10. Haack AK, Sheth C, Schwager AL, Sinclair MS, Tandon S, Taha SA. Lesions of the lateral habenula increase voluntary ethanol consumption and operant self-administration, block yohimbine-induced reinstatement of ethanol seeking, and attenuate ethanol-induced conditioned taste aversion. PLoS One 2014;9:e92701.
11. Batalla A, Homberg JR, Lipina TV, Sescousse G, Luijten M, Ivanova SA, *et al*. The role of the habenula in the transition from reward to misery in substance use and mood disorders. Neurosci Biobehav Rev 2017;80:276-85.
12. Meye FJ, Lecca S, Valentinova K, Mameli M. Synaptic and cellular profile of neurons in the lateral habenula. Front Hum Neurosci 2013;7:860.
13. Friedman A, Lax E, Dikshtein Y, Abraham L, Flaumenhaft Y, Sudai E, *et al*. Electrical stimulation of the lateral habenula produces enduring inhibitory effect on cocaine seeking behavior. Neuropharmacology 2010;59:452-9.
14. 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;67:832-6.
15. Kargari 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;231:187-92.
16. Ji H, Shepard PD. Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABAA receptor-mediated mechanism. J Neurosci 2007;27:6923-30.
17. Stamatakis AM, Jennings JH, Ung RL, Blair GA, Weinberg RJ, Neve RL, *et al*. A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. Neuron 2013;80:1039-53.
18. Wu J, Li X, Zhou P, Li X. M3 but not M4 muscarinic receptors in the rostromedial tegmental nucleus are involved in the acquisition of morphine-induced conditioned place preference. Eur J Pharmacol 2020;882:173274.
19. Barker DJ, Miranda-Barrientos J, Zhang S, Root DH, Wang H-L, Liu B, *et al*. Lateral preoptic control of the lateral habenula through convergent glutamate and GABA transmission. Cell Rep 2017;21:1757-69.
20. Zhang L, Hernández VS, Swinny JD, Verma AK, Giesecke T, Emery AC, *et al*. A GABAergic cell type in the lateral habenula links hypothalamic homeostatic and midbrain motivation circuits with sex steroid signaling. Translational psychiatry. 2018;8:1-14.
21. Golden SA, Heshmati M, Flanigan M, Christoffel DJ, Guise K, Pfau ML, *et al*. Basal forebrain projections to the lateral habenula modulate aggression reward. Nature 2016;534:688-92.
22. Goutagny R, Loureiro M, Jackson J, Chaumont J, Williams S, Isope P, *et al*. Interactions between the lateral habenula and the hippocampus: Implication for spatial memory processes. Neuropsychopharmacology 2013;38:2418-26.
23. Du CX, Liu J, Guo Y, Zhang L, Zhang QJ. Lesions of the lateral habenula improve working memory performance in hemiparkinsonian rats. Neurosci Lett 2018;662:162-6.