ISSN: 2538-5887; Pathobiology Research. 2026; 29 (1): 7-12
DOI: 10.48311/mjms.2026.118821.82615

Type 2 dopamine receptors of the ventral tegmental area modulated the evoked anxiolytic effects of lateral hypothalamus in chronic pain in rat

Review History:
Received: 2026-01-07
Revised: 2026-03-12
Accepted: 2026-05-17

Article Type:
Original Research

Authors:
Maryam Kohandani[1]
Mohammad Taghi Mohammadi[2,3*]
Zahra Bahari[2,3]
Narges Marefati[2,3]
Alireza Mohammadi[3]
Mehdi Raei[4,5]

1. Student Research Committee, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Department of Physiology and Medical Physics, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3. Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
4. Department of Epidemiology and Biostatistics, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran.
5. Artificial Intelligence in Health Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

**** Corresponding author:***
Mohammad Taghi Mohammadi

ORCID: 0000-0003-0202-4236
Tell/ Fax: +98 2187555420
Email:
mohammadi.mohammadt@yahoo.com
mohammadi.mohammad.t@gmail.com

ABSTRACT

Introduction: Chronic pain is associated with the anxiety, mediated in part by dopaminergic circuits. The ventral tegmental area (VTA) is critical area in the mesocorticolimbic dopamine system and regulate emotional and motivational behaviors. Important input to the VTA is from lateral hypothalamus (LH). However, the role of D2-like dopamine receptors of VTA in modulating of LH-anxiolytic effects during chronic pain remains unclear.

Methods: Adult male rats were unilaterally implanted with two guide cannulae in the VTA and LH. Chronic constriction injury (CCI) was used to induce neuropathic pain. Animals divided into six groups as following: control, sham, CCI, CCI+Citicoline (cholinergic precursor for LH activation), CCI+ Sulpiride (D2-like receptor antagonist), and CCI+ citicoline+ Sulpiride groups. Animals received D2-like dopamine receptor antagonists (Sulpiride, 1 µg/1µl/rat) into the VTA before intra-LH injection of Citicoline (1 µg/1µl/rat) for 7 days after CCI induction. Anxiety-like behavior was assessed using the elevated plus maze (EPM) on days 1, 3, and 7 post-CCI.

Results: The CCI surgery significantly induced anxiety-like behaviors in all experimental days (p=0.001). We observed that intra-LH injection of citicoline significantly improved anxiety-like behaviors (p=0.001). It means activation of LH via citicoline has anxiolytic effects. Intra-VTA injection of sulpiride cannot significantly change anxiety. Additionally, intra-VTA administration of sulpiride after intra-LH injection of citicoline significantly reversed the anxiolytic effects of citicoline (p=0.001).

Conclusion: The present study support the hypothesis that targeting D2-like receptor signaling in the VTA, may represent a promising strategy to alleviate anxiety related to the neuropathic pain.

Keywords: Dopamine receptors, Ventral tegmental area, Lateral hypothalamus, Chronic pain, Anxiety-Like behaviors.

Introduction

Neuropathic pain may cause an unstable emotional state and induce negative emotions, most commonly, anxiety and depression disorders. However, the precise neurobiological mechanism (s) that linking neuropathic pain to anxiety disorders remain far from clear (1). Ample of the studies reported that neurons in the lateral hypothalamus (LH) could modulate various physiological and cognitive functions, including pain and emotional mood (2, 3, 4). For example, Siahposht-Khachaki et al., identified that intra-LH carbachol-induced antinociception during both phases of the formalin test (5). It has been reported that the LH neurons project to several areas of the brain such as VTA. Additionally, activation of LH neurons increases the firing rate of VTA dopamine neurons and it can modulate anxiety (6). The VTA dopamine system plays a central role in motivation, affective regulation, and stress responsiveness, and has emerged as a critical modulator of pain-related emotional states (7). Dopaminergic neurons originating in the VTA and project to multiple forebrain regions, including the prefrontal cortex (PFC), forming the mesocortical pathway that is essential for emotional control and executive function (7). Chronic pain has been shown to disrupt dopaminergic signaling within this circuitry, leading to altered reward processing, increased negative affect, and vulnerability to anxiety-like behaviors (8). Dopamine exerts its effects through multiple receptor subtypes, among which dopamine type 2 (D2)-like receptors are particularly important for regulating neuronal excitability, synaptic plasticity, and inhibitory control within dopaminergic circuits (9). D2-like receptors are expressed both presynaptically on dopaminergic neurons in the VTA and postsynaptically in target regions such as the PFC. Dysregulation of D2-like receptor signaling has been implicated in psychological disorders, including anxiety and depression, as well as in maladaptive responses to chronic stress. Notably, chronic pain states are associated with reduced dopamine release and altered D2-like receptor function, suggesting a potential mechanism that linking pain to anxiety (9). Despite growing recognition of the importance of dopamine in pain-affective interactions (10), the specific contribution of LH activation and its association with the D2-like receptors within the VTA in chronic pain remains insufficiently characterized. Understanding how D2-like receptor signaling in these region affects anxiety during chronic pain may provide critical insights into the neurocircuitry underlying pain-related affective disorders and identify novel therapeutic targets. Therefore, the present study aims to investigate the role of LH activation and dopamine D2-like receptors in the VTA in modulating anxiety-like behavior in a rat model of chronic pain.

Materials and Methods

Animals

Male adult Wistar rats weighing 200–250 g randomly choose and used in this study. All of the animals kept in an animal house with a 12 h light/ dark cycle, and had access to food and water ad libitum. The lights turned on at 7 A.M. and turned off at 7 P.M. All of the experiments performed according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication) (Approval Ethics Code: IR.BMSU.BLC.1404.044).

Surgery of guide cannula

Animals anesthetized by intraperitoneal injection of chloral hydrate (350 mg/kg, i.p) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). They implanted with two guide cannulae (23-gauge stainless steel needles) unilaterally into the LH and VTA hemispheres, 1 mm above the target areas. The following coordinates used for the LH and VTA respectively (11), the LH: AP = -2.65 mm, ML = 1.3 mm, and DV = 8.6 mm ventral from the skull surface; and for the VTA: AP = +4.8 mm, ML = 0.8 mm, and DV = 8.3 mm ventral from the skull surface. All animals were given 4 days' recovery period. Representative picture of rat brain coronal section reported in the Figure 1.

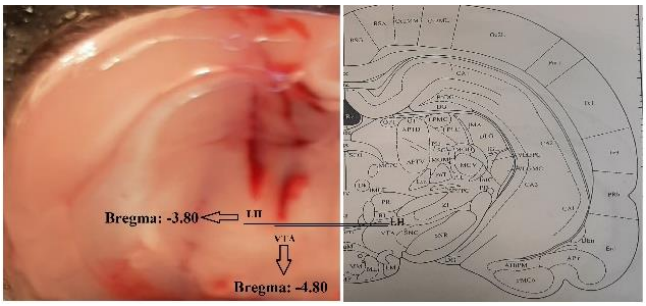

Figure 1. Representative picture of rat brain coronal section. Corresponding coronal brain picture illustrating the damage (red lines) caused by the guide cannula implantation.

Induction of neuropathic pain

Neuropathic pain (CCI model) induce, as it was previously introduced by Bennett and Xie (12). Briefly, after anesthetizing the animals with chloral hydrate (350 mg/kg, i.p), the left body of sciatic nerve (1 cm) exposed and then four loss ligatures (4/0 catgut) tied around the nerve, 1 mm apart, until a brief twitch in the hind limb observed. In sham animals, only the left sciatic nerve exposed, but not ligated.

Animal Groups

Animals divided into 6 groups (n=6 per each group): control, sham, CCI, CCI+ citicoline (citicoline injection in the LH), CCI+ sulpiride, CCI + citicoline +sulpiride (citicoline injection in the LH +sulpiride injection in the VTA) groups.

Experimental protocol

Four days after cannula insertion surgery, on day 0 (day 0 is equivalent to 5 days after cannula implantation surgery), CCI model of neuropathy performed. Drug administration began on day 1 (one day after CCI surgery) and continued for 7 days. In behavioral study, anxiety-like responses measured by elevated plus maze (EPM) on days 1, 3, and 7 after CCI surgery (15 minutes after drug injection). The effects of each drug examined in LH and VTA in experimental groups. The dosage of the drugs [(citicoline: 1 µg/1µl/rat) and (sulpiride: 1 µg/1µl/rat)] was based on the preliminary study.

Elevated Plus Maze (EPM)

The elevated plus maze consisted of 2 open and 2 closed arms, animals placed on the center platform of the maze, facing an open arm for 5 minutes. The percent of time spent in open arms and also the percent of entrance in open arms used as an index of anxiety-like behaviors. Less time spent in the open arms and less number of entrances to open arms were in favor of anxiety (13).

Statistical Analysis

The present data presented as mean ± standard error of the mean (SEM). Data analyzed using the SPSS software (version 19.0). Differences in measured parameters among different groups analyzed by using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. The differences considered to be significant when the probability was less than 0.05.

Results

Effects of intra-VTA administration of sulpiride as D2-like dopamine receptor antagonist on the LH-induced anxiolytic effects; spent time into open arms of EPM

As shown in the Figure 2A, One-way ANOVA followed by Tukey's HSD revealed that CCI surgery significantly decreased spent time into open arms in all experimental days (*$p<0.001$). Furthermore, we observed that intra-LH injection of citicoline significantly increased spent time into open arms in all experimental days (^$p<0.001$). It means activation of LH via citicoline has anxiolytic effects. Interestingly, intra-VTA injection of sulpiride cannot significantly change spent time into open arms in all experimental days as compared with the CCI group. Additionally, intra-VTA administration of sulpiride as D2-like dopamine receptor antagonist after intra-LH injection of citicoline significantly reversed the anxiolytic effects of citicoline on the all experimental days ($^{\$}p<0.001$).

Effects of intra-VTA administration of sulpiride as D2-like dopamine receptor antagonist on the LH-induced anxiolytic effects; the number of entrance into open arms of EPM

As shown in the Figure 2B, CCI surgery significantly decreased the number of entrance into open arms in all experimental days as compared with the sham group (*$p<0.001$). Furthermore, we observed that intra-LH injection of citicoline significantly increased the number of entrance into open arms in all experimental days (^$p<0.001$) as compared with the CCI group. It means activation of LH via citicoline has anxiolytic effects. Interestingly, intra-VTA injection of sulpiride cannot significantly change the number of entrance into open arms in all experimental days as compared with the CCI group. Additionally, intra-VTA administration of sulpiride after intra-LH injection of citicoline significantly reversed the anxiolytic effects of citicoline on the all experimental days ($^{\$}p<0.001$).

Effects of intra-VTA administration of sulpiride as D2-like dopamine receptor antagonist on the LH-induced anxiolytic effects; the spent time into close arms of EPM

As shown in the Figure 2C, One-way ANOVA followed by Tukey's HSD revealed that CCI surgery significantly increased spent time into close arms in all experimental days (*$p<0.001$).

Furthermore, we observed that intra-LH injection of citicoline significantly decreased spent time into close arms in all experimental days (^p<0.001). It means activation of LH via citicoline has anxiolytic effects. Interestingly, intra-VTA injection of sulpiride cannot significantly change spent time into close arms in all experimental days as compared with the CCI group. Additionally, intra-VTA administration of sulpiride as D2-like dopamine receptor antagonist after intra-LH injection of citicoline significantly reversed the anxiolytic effects of citicoline on the all experimental days ($^{\$}$p<0.001).

Effects of intra-VTA administration of sulpiride as D2-like dopamine receptor antagonist on the LH-induced anxiolytic effects; the number of entrance into close arms of EPM

As shown in the Figure 2D, CCI surgery significantly increased the number of entrance into close arms in all experimental days as compared with the sham group (*p<0.001). Furthermore, we observed that intra-LH injection of citicoline significantly decreased the number of entrance into close arms in all experimental days (^p<0.001) as compared with the CCI group. It means activation of LH via citicoline has anxiolytic effects. Interestingly, intra-VTA injection of sulpiride cannot significantly change the number of entrance into close arms in all experimental days as compared with the CCI group. Additionally, intra-VTA administration of sulpiride after intra-LH injection of citicoline significantly reversed the anxiolytic effects of citicoline on the all experimental days ($^{\$}$p<0.001).

Discussion

The major findings of the present study were as follows: (1) chemical activation of the LH by via injection of citicoline can induce anxiolytic effects in a model of neuropathic pain; (2) the blockade of D2-like dopamine receptors of the VTA neurons markedly decreased the anxiolytic effects of citicoline during neuropathic pain.

Pain is defined as a complex experience with a critical health concern for its various comorbidities and complications including psychological disorders (14). Psychological disorders including anxiety and depression, are common, with a comorbidity rate of up to 35% in patients with neuropathic pain (15). It has been reported that patients with neuropathic pain were4 times more highly likely to experience

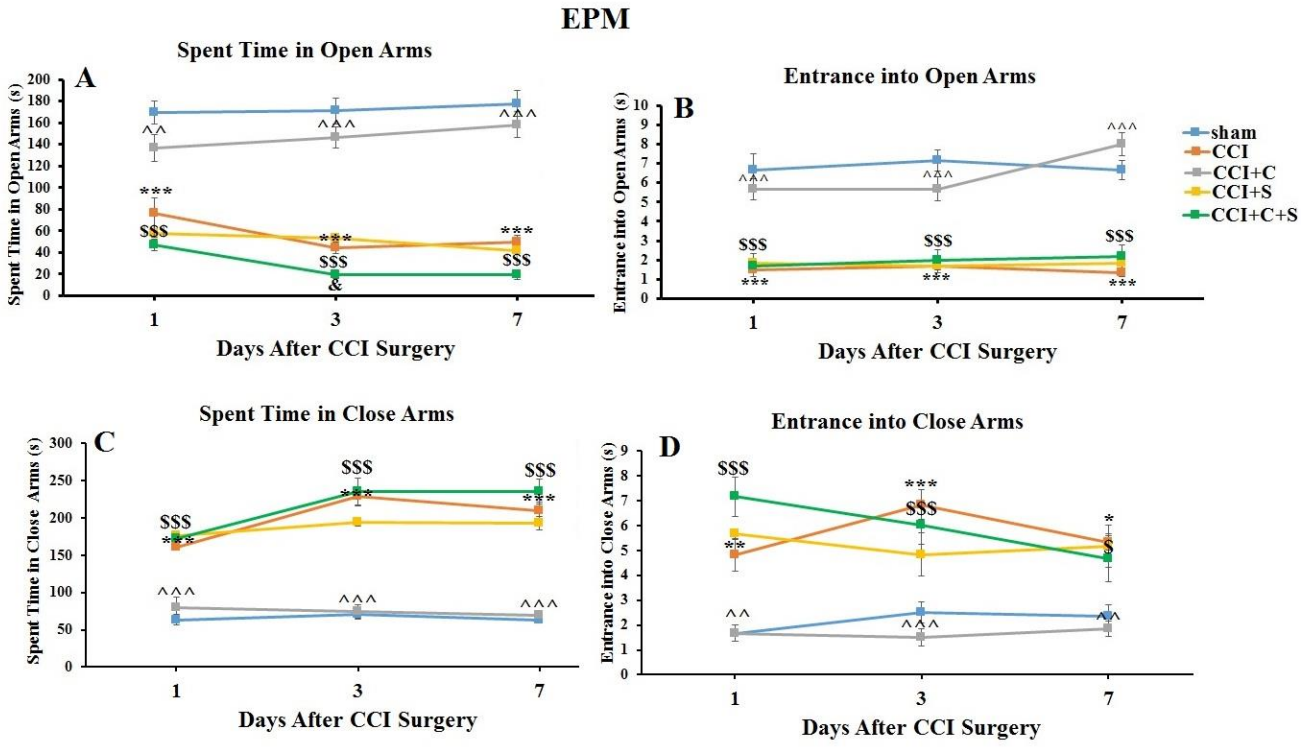

Figure 2: The effects of drug micro-injection on (A) spent time into open arms, (B) entrance into open arms, (C) spent time into close arms, and (D) entrance into close arms. The average of scores is shown during 1, 3, and 7 days after CCI surgery. Each point shows the mean ± SEM (n=6 in each group).
*** compared to the sham group; ^ compared to the CCI group; $ compared to the CCI+Citicholine group; & compared to the CCI+Sulpiride group.**

anxiety than those without neuropathic pain (16). This comorbidities of neuropathic pain have important effects on the quality of patient's life. However, the underlying mechanism (s) of neuropathic pain and anxiety is far from clear.

The LH is an important area that receives afferent input concerning internal homeostatic signals and relays projections to various brain areas and controlling different cognitive and pshycologic behaviors (17). It is well accepted that the afferents from LHA to the VTA is specifically related to the cognitive behaviors including pain perception and anxiety (17).

Dopamine release from VTA can mediate various cognitive behaviors (18). Interestingly, excitotoxic lesions of the LH induce several behavioral disturbances similar to those observed after dopamine-depletion in the VTA (18). Therefore, it is highly suggested that LH afferent fibers to the VTA is important in the modulating cognitive behaviors, probably by its effects on the VTA dopamine neurons.

Citicoline is consisting of cytosine, choline, ribose, and pyrophosphate. It is an important stimulator for the biosynthesis of acetylcholine (19). Additionally, citicoline perhaps have neuroprotective and cognitive improving activities via suppressing neuronal phospholipid membrane breakdown (19). It has been identified that citicoline has critical role in the improvement of various cognitive behaviors including memory, anxiety and depressive-like behavior (19). Therefore, in the present study, we hypothesized that micro-injection of citicoline within the LH indicates beneficial anxiolytic effects in neuropathic rats. Our data demonstrated that intra-LH injection of citicoline markedly improved all parameters related to the anxiety in neuropathic rats.

It has been demonstrated that the inhibition of the different receptors in the VTA decreased the intra-LH carbachol-induced analgesic effects in rats with inflammatory pain (5). Additionally, the electrophysiological study identified that activation of LH neurons can increase the increases activity of dopaminergic neurons in the VTA (5). It is well accepted that the VTA is consisting of various types of neurons. The most abundant neuron type is the dopamine neuron, which projects to the mesolimbic and mesocortical areas (20). Additionally, LH afferent projections to the VTA can modulate firing rate of dopaminergic neurons in the VTA (20). Hear, we observed that the suppression of D2-like receptors within VTA modulate the evoked anxiolytic effects of LH in neuropathic rats. The limitation of the present study was using the single dose of drugs. So, further research is necessary to determine the D2-like receptor signaling in the VTA during neuropathic pain.

Conclusion

The present study support the hypothesis that targeting D2-like receptor signaling in the VTA, may represent a promising strategy to alleviate anxiety related to the neuropathic pain. Future studies should investigate the precise cellular mechanisms and downstream pathways involved, as well as the long-term effects of such interventions.

Acknowledgements

This article is based on the Ph.D. thesis of Maryam Kohandani, in Medical Physiology.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Naranjo C, Del Reguero L, Moratalla G, Hercberg M, Valenzuela M, Failde I. Anxiety, depression and sleep disorders in patients with diabetic neuropathic pain: a systematic review. Expert Rev Neurother. 2019; 19(12):1201-9.
2. Sharpe MJ. The cognitive (lateral) hypothalamus. Trends Cogn Sci. 2024; 28(1):18-29.
3. Petrovich GD. Lateral hypothalamus as a motivation-cognition interface in the control of feeding behavior. Front Syst Neurosci. 2018; 12:14.
4. Goel M, Mittal A, Jain VR, Bharadwaj A, Modi S, Ahuja G, et al. Integrative functions of the hypothalamus: linking cognition, emotion and physiology for well-being and adaptability. Ann Neurosci. 2025; 32(2):128-42.
5. Siahposht-Khachaki A, Nazari-Serenjeh F, Rezaee L, Haghparast A, Rashvand M, Haghparast A. Dopaminergic receptors in the ventral tegmental area modulated the lateral hypothalamic stimulation-induced antinociception in an animal model of tonic pain. Neurosci Lett. 2021; 751:135827.

6. Nieh EH, Vander Weele CM, Matthews GA, Presbrey KN, Wichmann R, Leppla CA, et al. Inhibitory input from the lateral hypothalamus to the ventral tegmental area disinhibits dopamine neurons and promotes behavioral activation. Neuron. 2016; 90(6):1286-98.
7. Laviolette SR. Dopamine modulation of emotional processing in cortical and subcortical neural circuits: evidence for a final common pathway in schizophrenia? Schizophr Bull. 2007; 33(4):971-81.
8. Taylor AM, Becker S, Schweinhardt P, Cahill C. Mesolimbic dopamine signaling in acute and chronic pain: implications for motivation, analgesia, and addiction. Pain. 2016; 157(6):1194-8.
9. Ayano GJ. Dopamine: receptors, functions, synthesis, pathways, locations and mental disorders: review of literatures. J Ment Disord Treat. 2016; 2(120):2.
10. Geha P, Sulovari A, Freeman R, Gewandter JS. Dopaminergic modulation: revisiting a promising novel pathway for pharmacologic treatment of chronic pain. Pain. 2025; 166(10):2213-9.
11. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition. Elsevier; 2006.
12. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988; 33:87-107.
13. Tahmasebi L, Bahrami F, Sahraei H, Shankayi Z, Shahyad S, Bahari Z. Interaction effects of intracerebroventricular injection of crocin with the α2-adrenoceptors on memory deficit and hippocampal synaptic plasticity following chronic pain in rat. Brain Res Bull. 2022; 190:168-78.
14. Dang TN, Tien SN, Ochi R, Le Trung D, Nishio K, Kuwamura H, et al. Enhanced anxiety-like behavior induced by chronic neuropathic pain and related parvalbumin-positive neurons in male rats. Behav Brain Res. 2024; 459:114786.
15. Velly AM, Mohit S. Epidemiology of pain and relation to psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2018; 87:159-67.
16. Mills SE, Nicolson KP, Smith BH. Chronic pain: a review of its epidemiology and associated factors in population-based studies. Br J Anaesth. 2019; 123(2):e273-83.
17. Perez-Bonilla P, Santiago-Colon K, Leinninger GM. Lateral hypothalamic area neuropeptides modulate ventral tegmental area dopamine neurons and feeding. Physiol Behav. 2020; 223:112986.
18. Stratford TR, Wirtshafter D. Lateral hypothalamic involvement in feeding elicited from the ventral pallidum. Eur J Neurosci. 2013; 37(4):648-53.
19. Payamshad S, Khakpai F, Nasehi M, Zarrindast MR. Effect of citicoline and transcranial direct current stimulation on depressive-like behaviors in mice & quot. Behav Brain Res. 2023; 450:114495.
20. Harada M, Capdevila LS, Wilhelm M, Burdakov D, Patriarchi T. Stimulation of VTA dopamine inputs to LH upregulates orexin neuronal activity in a DRD2-dependent manner. Elife. 2024; 12:RP90158.