Chemical stimulation of the lateral hypothalamus (LH) induces analgesia by forming neural circuitries with multiple brain regions. The involvement of hippocampal dopaminergic receptors in the LH stimulation-induced antinociception in specific pain models in animals has been documented. However, because the neural circuitries involved in the mediation of orofacial pain are not the same as those that mediate the other types of pain, the present study aims to detect the role of dopamine receptors within the dentate gyrus (DG) in the antinociceptive responses induced by LH stimulation in an animal model of orofacial pain. Male Wistar rats (220–250 g) were implanted with two separate cannulae into the LH and DG on the same side. D1- or D2-like dopamine receptor antagonist, SCH23390, or sulpiride (0.25, 1, and 4 μg) were microinjected into the DG, five minutes before intra-LH injection of carbachol (250 nM). The animals were then injected with formalin 1% (50 μL; sc) into the upper lip lateral to the nose and subjected to the orofacial formalin test. Intra-DG administration of SCH23390 or sulpiride attenuated the antinociceptive responses induced by intra-LH microinjection of carbachol during the orofacial formalin test. The findings of the current study suggest that chemical stimulation of the LH modulates orofacial pain, possibly through activation of the DG dopaminergic neurons. Due to the high incidence and prevalence of orofacial pain in the general population, understanding how such neuronal circuitry modulates nociceptive processing will advance the search for novel therapeutics. 

Behavioural Pharmacology 2023, 34:45–54

Keywords: D1-like dopamine receptor, D2-like dopamine receptor, dentate gyrus, lateral hypothalamus, orofacial pain, rat

Introduction

Inflammation in the orofacial region is known to frequently cause persistent pain in the orofacial region and is considered a serious public health issue. Central and peripheral mechanisms are involved in the persistent ectopic orofacial pain associated with orofacial inflammation (Imbe et al., 2001). The diagnosis, etiology, and management of orofacial pain are complex, multidisciplinary, and multifactorial processes (Crandall, 2018). Among all pain models, the formalin test is a widely used and the most reliable model for producing and quantifying nociception in the rat’s trigeminal region. Besides, the formalin test is considered more relevant to clinical pain than the other animal models of pain (Raboisson and Dallel, 2004). The applied chemical stimulus, that is, formalin injection into the orofacial receptive field, produces a typical biphasic nociceptive response: a brief early phase and a prolonged late phase (Shields et al., 2010). It has been believed that the early phase is caused by the direct effect of formalin chemical stimulation on the nociceptors, which causes the activation of the C-fibers, and the late phase is due to the inflammatory response in the peripheral tissue (Tjølsen et al., 1992).

Neurons containing orexin are localized exclusively in the lateral hypothalamus (LH), and their projections are widely distributed in multiple brain regions (Peyron et al., 1998). Chemical stimulation of LH attenuated the nociceptive responses in several animal pain models. For instance, it has been demonstrated that LH stimulation attenuated tonic pain through the dopaminergic receptors within the ventral tegmental area (VTA) following the rat’s paw formalin injection (Siahposht-Khachaki et al., 2021). Besides, chemical stimulation of LH could attenuate acute pain via orexin receptors within the dentate gyrus (DG) of the hippocampus (Brojenci et al., 2019). LH stimulation could also reduce hyperalgesia through orexin-A neurons in an animal model of neuropathic pain (Wardach et al., 2016). Moreover, the effect of LH stimulation on dopaminergic receptors within the VTA in attenuating orofacial pain has also been demonstrated.
(Matini et al., 2020). Orexin neurons project from the LH to dopaminergic neurons in the VTA, and VTA dopamine neurons project to the nucleus accumbens (NAc), amygdala, hippocampus, and prefrontal cortex, forming the mesocorticolimbic dopamine system (Korotkova et al., 2003).

The mesolimbic dopamine system modulates sensory and emotional aspects of chronic pain sensation (Yang et al., 2020). Dopamine belongs to the catecholamine family and binds to two types of dopamine receptor families: the D1-like receptor subtypes, including D1 and D5 receptors, and the D2-like receptor subtypes, including D2, D3, and D4 receptors (Missale et al., 1998). It has been reported that orofacial pain syndrome occurs more frequently in patients with dopamine dysregulation, such as Parkinson’s disease, than in the general population (Ford et al., 1996). Besides, a PET study on patients with burning mouth syndrome showed a decrease in the D1/D2 ratio and dopamine hypofunction in the nigrostriatal dopaminergic pathway (Hagelberg et al., 2003). It has been shown that dopaminergic receptors within the VTA (Matini et al., 2020) and NAc (Haghiparast et al., 2020) modulate the antinociceptive responses induced by chemical stimulation of the LH in an animal model of orofacial pain.

On the other hand, the hippocampal formation (HF) receives dopaminergic inputs from the VTA and substantia nigra and plays an essential role in pain modulation (Scatton et al., 1980). Dopamine receptors within the DG, as the main gateway of the HF participate in the antinociception induced by LH stimulation in animal models of acute and persistent inflammatory pain (Khaleghzadeh-Ahangar et al., 2021; Torkamand et al., 2021). The previous study demonstrated that dopamine receptors in the dorsal hippocampus suppress nociception induced by formalin injection into the orofacial region (Shamsizadeh et al., 2013). Because different types of pain based on their origins and different regions of the brain involved different mechanisms in modulating pain, this study aimed to investigate the potential role of dopamine receptors within the DG region in the antinociceptive responses induced by the chemical stimulation of LH using carbachol.

**Methods**

**Subjects and surgical procedures**

Seventy-eight adult male Wistar rats (220–250 g) were randomly chosen (Pasteur Institute, Tehran, Iran) and were kept in an animal house (3–4 rats per cage, temperature 22 ± 2°C, humidity 50 ± 10%) with a 12-h light/dark cycle and free access to food and water. All experiments followed the ethical guidelines of the National Academies of Science: ‘National Research Council, 2011, Guide for the Care and Use of Laboratory Animals: Eighth Edition, Washington, DC: The National Academies Press’. and were approved by the Research and Ethics Committee of the Faculty of Medicine, AJA University of Medical Sciences (IR.AJAUMS.REC.1400. 096), Tehran, Iran. Rats were randomized into control and treatment groups before any assessments were performed.

Animals were anesthetized with a mixture of ketamine 10% (100 mg/kg; intraperitoneally) and xylazine 2% (10 mg/kg; intraperitoneally) and were placed in a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). Two stainless steel guide cannulae (25-gauge) were unilaterally lowered and placed 1 mm above the LH and DG regions based on the Paxinos and Watson (2013) rat brain atlas (Paxinos and Watson, 2013). Accordingly, the coordinate for the LH was anteroposterior (AP) = 2.65 mm caudal to the bregma, (Lat) = ±1.3 mm lateral to the midline, and dorsoventral (DV) = 8.6 mm ventral from the skull surface, and for the DG was as follows: AP = −4.08 mm, Lat = ±2.2 mm, and DV = 3.6 mm from the skull surface. The guide cannulae were then fixed to the skull surface using dental acrylic cement. Seven days postoperative period was considered for the recovery.

**Drugs and drug administration**

In this study, carbachol (2-hydroxyethyl) trimethylammonium chloride carbamate (Tocris Bioscience, Bristol, UK) was diluted in saline (250 nM). The different solutions of D1-like dopamine receptor antagonist, SCH23390, (R)-(−)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine hydrochloride (Tocris Bioscience) were prepared in saline (0.25, 1, and 4 μg). Also, sulpiride, (S)-5-aminosulfonyl-N-[1-ethyl-2-pyrrolidinyl] methyl]-2-methoxybenzamide (Tocris Bioscience) as a selective D2-like dopamine receptor antagonist was dissolved in 12% dimethyl sulfoxide (DMSO) in different doses (0.25, 1, and 4 μg). All of the solutions were made freshly on the experiment day and microinjected into the LH or DG in 0.5 μL volume via a 1-μL Hamilton syringe using a polyethylene tube (PE-20) connected to a 30-gauge needle.

**Orofacial formalin test**

The animals were placed in the laboratory for 30 min before initiating the experiments during the acclimation period. Formalin 1% (50 μL; sc) was injected into the upper lip just lateral to the nose using a 29-gauge injection needle. To observe the nociceptive response after the formalin injections, rats were placed in a plexiglass box (30 × 30 × 30 cm) with a mirror angled at 45° under the surface of the box (Raboisson and Dalle, 2004). The time that animals spent rubbing the injected area was calculated in 15 blocks of 3 min for a total time of 45 min. A typical biphasic nociceptive response was observable after the formalin injection, the early phase between 0 and 3 min reflecting the direct chemical stimulation of the nociceptive terminals, and the late phase between 15
Experimental design
An effective dose of carbachol (250 nM/0.5 μL saline) was selected based on the previous study (Supplementary Fig. 2, Supplemental Digital Content 2, http://links.lww.com/BPHARM/A85) and microinjected into the LH to examine the effect of the chemical stimulation of LH on orofacial pain modulation (Haghparast et al., 2020). Different doses (0.25, 1, and 4 μg) of SCH23390 or sulpiride were microinjected into the DG area to investigate the role of intra-DG D1 and D2-like dopamine receptors in carbachol-induced analgesia, five minutes before the microinjection of carbachol (Fig. 1). Five minutes after the drug administration, formalin was injected into the orofacial region, and nociceptive behavior was evaluated. To investigate the effect of sole microinjection of D1- and D2-like dopamine receptors into the DG region, two experimental groups, received only the highest dose of SCH23390 or sulpiride (4 μg; intra-DG) before intra-LH administration of saline.

Histological verification
After the termination of experiments, animals were deeply anesthetized with a high dose of ketamine and xylazine and were transec tally perfused with 0.9% saline and 10% formaldehyde solution. Then, the rats' brain was harvested, and 50-μm coronal sections of the brain were prepared using a rotatory microtome. The cannulae placements in LH and DG regions were checked using the rat brain atlas (Paxinos and Watson., 2013). The final data analysis included only the animals with correct cannula placements (Fig. 2). Accordingly, 13 animals were excluded from the study, and 78 animals were included in the final data analysis. The number of animals in each group after removal due to misplaced cannula placement was as follows: the groups of animals received saline or DMSO into the DG region before intra-LH administration of carbachol, including saline–carbachol (n = 8), and DMSO–carbachol (n = 9) groups, the groups of animals received different doses of SCH23390 or sulpiride before LH administration of carbachol including; SCH23390 (0.25 μg)–carbachol (n = 8), SCH23390 (1 μg)–carbachol (n = 7), SCH23390 (4 μg)–carbachol (n = 8), sulpiride (0.25 μg)–carbachol (n = 8), sulpiride (1 μg)–carbachol (n = 8), and sulpiride (4 μg)–carbachol (n = 7). Two separate groups received intra-DG microinjection of the highest dose of SCH23390 or sulpiride before intra-LH administration of saline, including SCH23390 (4 μg)–saline (n = 7) and sulpiride (4 μg)–saline (n = 8).

Besides, since the volume of microinjection was very small (0.5 μL), the possibility of drug leakage was very low (Lohman et al., 2005).

Statistical analysis
Data analyses were done using GraphPad Prism 6.0 software. The results are expressed as mean ± SEM. The Kolmogorov–Smirnov goodness-of-fit test was then used to verify the normal distribution and homogeneity of variance. The one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests was used to evaluate the face rubbing time during the early or late phases of the orofacial formalin test. Besides, the best-fitted line to show the data on a scatter plot was drawn to estimate the effective dose of 50% (ED50) of SCH23390 or sulpiride. The ED50 values were estimated during the early and late phases of the orofacial formalin test. The effect size was also calculated by dividing the mean difference between the control and experimental groups during both phases of the orofacial formalin test by the SD of the population from which the different treatment groups were taken. P < 0.05 was considered to be statistically significant.

Results
Based on previous studies, formalin injection into the rat’s orofacial region induces a biphasic nociceptive response. Statistical analysis showed that the face rubbing time in formalin-treated group is significantly more than that of saline-treated group [formalin injection effect: F (1, 150) = 762.5, P < 0.0001; time effect: F (14, 150) = 21.13, P < 0.0001; formalin injection and time interaction effects: F (14, 150) = 19.93, P < 0.0001; Supplementary Fig. 1, Supplemental Digital Content 1, http://links.lww.com/BPHARM/A84; Adapted from Haghparast et al. (2020); Springer Nature license agreement number 5244730959475]. Furthermore, one-way ANOVA followed by Dunnett’s multiple comparison test demonstrated that different doses of the intra-LH microinjection of carbachol (62.5, 125, and 250 nM/0.5 μL saline) significantly attenuated the face rubbing time during both the early [F (3, 21) = 4.57, P = 0.0151; Supplementary Fig. 2, left panel, Supplemental Digital Content 2, http://links.lww.com/BPHARM/A85] and late [F (3, 21) = 18.83, P < 0.0001; Supplementary Fig. 2, right panel, Supplemental Digital Content 2, http://links.lww.com/BPHARM/A85] phases of orofacial formalin test (Adapted from Haghparast et al., 2020; Springer Nature license agreement number 5244730959475). Therefore, in the present study, the dose of 250 nM carbachol was chosen for the chemical stimulation of the LH in the next experiments on D1- and D2-like dopamine receptors in the DG region of the hippocampus.

The blockade of D1-like dopamine receptors within the dentate gyrus region attenuated the antinociceptive responses induced by lateral hypothalamus stimulation
In order to find out the role of D1-like dopamine receptors within the DG region in antinociception-induced by
chemical stimulation of LH, the groups which received different doses of SCH23390 into the DG region before intra-LH microinjection of carbachol (SCH23390–carbachol groups) were compared to the saline–carbachol group. One-way ANOVA followed by Tukey’s multiple comparison test indicated that intra-DG microinjection of D1-like dopamine receptor antagonist, SCH23390 before intra-LH carbachol administration (250 nM/0.5 µL saline), significantly increased face rubbing behavior during early [F (4, 37) = 4.38, P = 0.006; \( \eta^2 = 0.34 \); Fig. 3, left panel] and late [F (4, 37) = 30.51, P<0.0001; \( \eta^2 = 0.73 \); Fig. 3, right panel] phases of orofacial pain compared to saline–carbachol group. The results also showed that the highest dose of SCH23390 (4 µg/0.5 µL saline) could completely block the antinociceptive responses induced by carbachol; so, there was no significant difference between SCH23390 (4 µg)-carbachol and SCH23390-saline groups in both early (P = 0.99) and late phases (P = 0.94) of the orofacial formalin test.

Furthermore, as it has been depicted in Fig. 4, a comparison of the different doses of SCH23390 in the percentage of the changes in face rubbing time in the carbachol–control group (intra-LH microinjection; 250 nM/0.5 µL saline) between the early and late phases of orofacial formalin test has been made. Accordingly, the best-fitted line to represent the data on the scatter plot was drawn by the trendline equation option in the excel software (Version 2013) to estimate the effective dose of 50% (ED 50). As shown in Fig. 4, the antinociceptive response elicited by carbachol microinjection was reversed by a lower dose of SCH23390 in the late phase (ED50 = 0.53) than that of the early phase (ED50 = 1.44) during the orofacial formalin test.

The blockade of D2-like dopamine receptors within the dentate gyrus region attenuated the antinociceptive responses induced by lateral hypothalamus stimulation

To understand the role of D2-like dopamine receptors within the DG region in antinociception-induced by chemical stimulation of LH, the groups which received different doses of sulpiride into the DG region before intra-LH microinjection of carbachol (sulpiride–carbachol groups) were compared to the DMSO–carbachol group. As it has been shown in Fig. 5, ordinary one-way ANOVA followed by Tukey’s multiple comparison tests indicated that intra-DG administration of D2-like dopamine receptor antagonist, sulpiride before intra-LH carbachol microinjection (250 nM/0.5 µL saline), could significantly increase the face rubbing response during early [F (4, 39) = 5.64, P = 0.0013; \( \eta^2 = 0.32 \); Fig. 5, left panel] and late [F (4, 39) = 25.19, P<0.0001; \( \eta^2 = 0.69 \); Fig. 5, right panel] phases of orofacial pain compared to the saline-carbachol group. The results also showed that the highest dose of Sulpiride (4 µg/0.5 µL DMSO) completely blocked the antinociceptive responses induced by intra-LH microinjection of carbachol; so there was no
significant difference between sulpiride (4 µg)–carbachol and sulpiride–saline groups during early ($P = 0.99$) and late ($P = 0.99$) phases of the orofacial formalin test.

On the other hand, as shown in Fig. 6, the effect of different doses of sulpiride on the percentage of the changes in face rubbing time in comparison with the carbachol–control group (intra-LH microinjection; 250 nM/0.5 µL saline) is measured during the early and late phases of the orofacial formalin test. Accordingly, as shown in Fig. 6, the antinociception induced by carbachol was reversed by a lower dose of sulpiride in the late phase (ED50 = 0.93) compared to the early phase (ED50 = 1.47) of the orofacial formalin test.

**Discussion**

The present study illustrated that D1- and D2-like dopamine receptors located in the DG region of the hippocampus are essential for antinociceptive responses produced by chemical stimulation of LH during both early and late phases of orofacial pain. The significant findings of the present study were as follows: (1) Blockade of D1-like dopamine receptor via SCH23390 microinjection into this area attenuated the antinociception induced by intra-LH carbachol microinjection during early and late phases of the orofacial formalin test; (2) intra-DG microinjection of the D2-like dopamine receptor antagonist, sulpiride, significantly reversed the analgesia produced by the LH stimulation during both phases of orofacial pain; (3) the modulatory role of dopamine receptors within the DG region in the LH stimulation-induced antinociceptive responses during the late phase of orofacial pain was noticeably more prominent than that in the early phase.

According to the obtained results, the DG region of the hippocampus is involved in antinociception produced by the chemical stimulation of LH. Neuroanatomical tracing revealed that LH produces a vast network of connections by sending its neural projections to many brain areas and producing circuits orchestrating pain behaviors. For instance, optogenetic activation of axonal projections of LH neurons to the ventrolateral periaqueductal gray area attenuates noiception via preferentially targeting glutamatergic over gamma-aminobutyric acid (GABA) neurons in persistent inflammatory or neuropathic pain models. However, these neurons produce pathway-specific behavioral effects. So, LH projections to the lateral habenula regulate aversion but not nociception (Siemian et al., 2021). In this respect, it has been shown that the LH-DG neural circuitry participates in pain modulation...
via the LHS’s projections to the DG region in multiple animal models of acute and chronic pain (Brojeni et al., 2019; Khaleghzadeh-Ahangar et al., 2021; Torkamand et al., 2021). According to the results of immunohistochemistry studies, the c-fos expression as a sensitive and reliable marker of neuronal activity increased in the hippocampal DG and CA3 regions at two hours following subcutaneous injection of formalin in rats (Ceccarelli et al., 1999). Besides, a significant increase in the c-fos level in the hippocampus and a decrease in phosphorylated cyclic AMP-response element-binding protein were observed following condition place preference induced by intra-LH administration of carbachol (Haghparast et al., 2011). So, it seems that chemical stimulation of LH may activate LH-DG neural circuitry, which can be involved in orofacial pain modulation.

The present study results demonstrated that blockade of dopamine receptors within the DG region attenuates antinociceptive responses induced by chemical stimulation of LH following formalin injection into the orofacial region. Previously, the role of dopamine receptors within the DG region in LH-induced antinociception has been shown in acute and inflammatory pain models (Khaleghzadeh-Ahangar et al., 2021; Torkamand et al., 2021). Besides, dopamine receptors in the CA1 area were found to suppress orofacial pain (Shamsizadeh et al., 2013). There is a significant difference in the density and distribution of dopamine receptor subtypes along the various subregions of the hippocampus. For instance, D1-like dopamine receptors were detected in granule cells of the DG, while D2-like dopamine receptors were shown to be expressed in the hilus. Besides, both dopamine receptors were high density in CA1 and subicular areas (Edelmann and Lessmann, 2018). Although D1- and D2-like dopamine receptors share similar intracellular signaling mechanisms, D2-like dopamine receptors, unlike D1 and D5 receptors, deactivate the cyclic adenosine monophosphate (Beaulieu and Gainetdinov, 2011). It has been reported that adenosine compounds have pain-relieving properties with slow onset and long duration of action, leading to chronic pain alleviation more efficiently than acute pain (Hayashida et al., 2005). Therefore, given the different densities and patterns of distribution of dopamine receptor subtypes in various
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Hippocampal subregions and different mechanisms by which dopamine receptors contribute to pain modulation, it seems that different hippocampal subregions play different roles in pain perception. On the other hand, it has been documented that microinjection of N-methyl-D-aspartate (NMDA) receptor antagonists, cholinergic agonists, and anesthetics into the DG region attenuated nociceptive response. It seems that NMDA-sensitive mechanisms in the DG region of the hippocampus modulate both acute and tonic pain perception. In contrast, the CA1 region of the hippocampus modulates tonic pain behavior only, indicating possible differential involvement of hippocampal subregions in mediating pain processing [for review see Liu and Chen, 2009].

It seems that the functions of LH are directly attributed to the activation of orexin receptors or indirectly through neural projections to the other cells, such as dopaminergic neurons. We previously reported that the chemical stimulation of LH modulates orofacial pain by activating orexin receptors in the CA1 area of the hippocampus (Haghparast et al., 2018). It has been reported that a very low concentration of orexin attenuates mRNA expressions of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and NMDA receptor subunits in rat primary neuron cultures (Yamada et al., 2008). Orexin-containing neurons originated from the LH project to the brainstem noradrenergic, serotonergic, as well as dopaminergic cells (Peyron et al., 1998; Horvath et al., 1999; Brown et al., 2001). Previously it has been demonstrated that chemical stimulation of LH induces analgesia via activation of orexin receptors within the VTA (Ezzatpanah et al., 2016). The VTA comprises a heterogeneous population of dopaminergic and non-dopaminergic (glutamatergic and GABAergic) neurons (Yu et al., 2019). Dopaminergic projections of VTA to the hippocampus are only 6–18%. The most abundant non-dopaminergic neurons of the VTA are GABAergic and glutamatergic and release both

Fig. 4

A log dose-response curve of the effect of intra-DG administration of SCH23390 on the percentage of the changes in face rubbing time compared to the carbachol–control group during the early and late phases of formalin nociception. The effective dose (ED50) of SCH23390 in the late phase (ED50 = 0.53) was less than that in the early phase (ED50 = 1.44). DG, dentate gyrus.
GABA and glutamate to the granule cell layer of the DG (Ntamati and Lüscher, 2016). However, since the present study results demonstrated that administration of the highest dose of D1- or D2-like dopamine receptor antagonist within the DG region could dramatically block the antinociceptive effect of LH stimulation, it seems that at least in part, the role of dopaminergic neurons was more considerable.

The modulatory role of dopamine neurotransmitters in orofacial pain has been shown. Dopamine depletion of the nigrostriatal pathway caused a hyperalgesic response to orofacial pain (Maegawa et al., 2015). Besides, patients with burning mouth syndrome showed decreased endogenous dopamine levels in the putamen and dopamine hypofunction in the nigrostriatal dopaminergic pathway (Hagelberg et al., 2003). On the other hand, there is an interaction between the opioidergic and dopaminergic systems in the dorsal hippocampus leading to modulation of orofacial pain (Haghparast et al., 2014). Meyer et al. (2009) reported that injection of apomorphine as a dopamine receptor agonist into the ventrolateral periaqueductal gray induced robust antinociception during the hot-plate test in rats via inhibition of the GABAergic system, as a common mechanism between dopamine and opioids.

According to the present study results, the role of D1- and D2-like dopamine receptors of DG was noticeably more prominent in the late phase of orofacial formalin pain than in the early phase. Formalin injection into the orofacial receptive field provokes a biphasic orofacial pain and evoked activity in thinly myelinated Aδ and non-myelinated C fibers as well as trigeminal and spinal nociceptive neurons (Raboisson and Dallel, 2004). Increased firing impulses, following external noxious stimulation of the orofacial region, conducted through Aδ- or C-fibers in the central terminals and depolarize them, which subsequently causes the release of certain neurotransmitters such as glutamate, GABA, noradrenaline, serotonin, as well as dopamine [for review see Sessle (1987)]. The early phase of orofacial formalin nociception is attributed to activating non-myelinated afferent nerve fibers, C fibers. At the same time, the late phase results from inflammation of orofacial tissues and the functional changes in the dorsal horn leading to central and peripheral sensitization of trigeminal nociceptive neurons (Takeda et al.)
Therefore, differences in the modulatory role of the dopamine system in the early and late phases of orofacial pain may stem from different mechanisms by which dopamine receptors are involved in antinociception induced by LH stimulation.

Summing up, the present study suggests that chemical stimulation of LH using carbachol microinjection activates the DG dopaminergic neurons and so, participates as a part of neural circuitry that modulates tonic orofacial pain.

**Acknowledgements**

Funding for this study was provided by the grant (No. 97001607) from AJA University of Medical Sciences, Tehran, Iran. The AJA University of Medical Sciences had no further role in the design of the study, in the collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

A.H.: conceptualization, designing the study, data analysis and interpretation of data, and approval of the final article. A.H.: data acquisition, interpretation of data, and writing the article. M.Y.: designing the study, data interpretation, and final article approval. M.R.: writing the article and approval of the final article. L.G.: designing the study and approval of the final article. B.R.: data analysis and approval of the final article.

The datasets generated during and/or analyzed during the current study are not publicly available due to comparison with previous studies in this laboratory but are available from the corresponding author upon reasonable request.

This study was performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication, 8th edition, revised 2011). It was approved by the research and ethics committee of the Faculty...
of Medicine, AJA University of Medical Sciences (IR. AJAUMS.REC.1400.096), Tehran, Iran.

Conflicts of interest
There are no conflicts of interest.

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