Pathophysiologic mechanisms of persistent orofacial pain

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Abstract: Nociceptive stimuli to the orofacial region are typically received by the peripheral terminal of trigeminal ganglion (TG) neurons, and nociceptive orofacial information is subsequently conveyed to the trigeminal spinal subnucleus caudalis and the upper cervical spinal cord (C1-C2). This information is further transmitted to the cortical somatosensory regions and limbic system via the thalamus, which then leads to the perception of pain. It is a well-established fact that the presence of abnormal pain in the orofacial region is etiologically associated with neuroplastic changes that may occur at any point in the pain transmission pathway from the peripheral to the central nervous system (CNS). Recently, several studies have reported that functional plastic changes in a large number of cells, including TG neurons, glial cells (satellite cells, microglia, and astrocytes), and immune cells (macrophages and neutrophils), contribute to the sensitization and disinhibition of neurons in the peripheral and CNS, which results in orofacial pain hypersensitivity.

Keywords: orofacial pain, spinal trigeminal nucleus, trigeminal ganglion, upper cervical spinal cord

Introduction

Physiological pain induced by noxious stimuli plays an important role in the body’s defense mechanism and is essential for supporting several life processes. Alternatively, chronic pathological pain is induced by noxious stimuli or persistent spontaneous pain hypersensitivity, leading to the impairment of processes involved in the body’s defense mechanism. Pathological orofacial pain conditions, such as trigeminal neuralgia, burning mouth syndrome, myofascial pain syndrome, and temporomandibular joint dysfunction, are considered variations of chronic pain, and they can be addressed by therapeutic intervention. Nevertheless, several aspects regarding the pathogenetic mechanisms of these variations remain unclear, and, consequently, a number of clinicians struggle to control abnormal pain hypersensitivity.

Orofacial inflammation, trigeminal nerve disturbances, and oral cancer are known to underlie nociceptive trigeminal neuronal hyperexcitability, leading to orofacial pain hypersensitivity, which is induced by various molecular signaling mechanisms that are closely associated with several mediators released from immune or glial cells [1,2]. Furthermore, the trigeminal sensory nucleus complex consists of primary nociceptive endings, sensitize voltage-gated sodium channel 1.8 (Nav1.8) by activating the protein kinase C, which results in the predisposition to generate action potentials [9,10]. The transient receptor potential (TRP) channel superfamily plays an important role in functions associated with sensing pain [11]. Furthermore, TRP vanilloid 1 (TRPV1) is activated in the presence of high-temperature conditions (>42°C), low pH, capsaicin, and vanilloid, while TRPA1 is activated by low temperatures (<15°C) and in the presence of numerous chemical irritants [12]. Facial inflammation subsequently reduces the acidity in the inflamed site, which increases TRPV1 expression in nociceptors. TRPV1 activation facilitates the intracellular influx of Ca²⁺, which sensitizes TRPA1, leading to orofacial cold hypersensitivity [13].

To the contrary, heat hypersensitivity following a facial skin incision is induced by the sensitization of TRPV1 through protein kinase A signaling and is facilitated by activating TRPA1 in the TG neurons that innervate the facial skin surrounding the incision [14]. Alternatively, there is a notable upregulation of artemin (ATN) mRNA expression, a member of the glial cell line-derived neurotrophic factor family, in the epithelial cells of tongue mucosa sampled from patients with burning mouth syndrome [5]. ATN signal upregulation, facilitated by the phosphorylation of p38 mitogen-activated protein kinase (MAPK) in the tongue mucosa, produces heat hypersensitivity in the tongue due to the hyperexpression of TRPV1 in nociceptors that innervate the tongue [5,15]. The p75 neurotrophin receptor (p75NTR) and tyrosine kinase receptor A (TrkA) act as NGF receptors in nociceptors in the primary nociceptive neurons [16]. NGF signaling via p75NTR and TrkA, which are expressed in nociceptive endings, has been shown to enhance the tetrodotoxin-resistant sodium current density and increase the threshold potential of Nav 1.8, which is etiologically associated with inflammatory mechanical allodynia [17,18].

Furthermore, the NGF/TrkA complex is formed after NGF successfully binds to TrkA at the nociceptor endings and is internalized in the endings to be retrogradely transported to the soma of the primary neuron [19]. Neuronal firing induces NGF secretion into the extracellular space in vitro, which increases the concentration of NGF in the culture medium [20,21]. Following local orofacial inflammation, NGF secreted from the TG neurons innervating the inflamed site binds to NGF receptors expressed in other TG neurons. This subsequently leads to an increased expression of TRPV1 within the intact TG neurons innervating the intact sites, which

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in turn contributes to ectopic orofacial pain [22]. Local orofacial inflammation also increases the activity of the P2X receptors, which are one of the adenosine triphosphate (ATP) receptors in TG neurons innervating the intact site. Increased P2X receptor activity is typically noted after the sensitization of primary afferent neurons, followed by ectopic orofacial mechanical allodynia [23].

**Neuronal communication among TG**

TG neurons innervating the injured inferior alveolar nerve release humoral factors, including cytokines and neuropeptides, along with nitric oxide (NO); this free-radical gas is an important neurotransmitter with many widespread effects. It is synthesized in the somata of primary neurons via NO synthase (NOS) signaling and is subsequently released to the extracellular space [24,25]. Synthesis using the NOS signaling pathway and the release of NO from these particular TG neurons are responsible for an increase in NO levels in the TG, which is associated with chronic orofacial ectopic mechanical allodynia [26]. Heat shock protein (Hsp70) that is produced in the pulpal tissues following tooth pulpitis is transported to the somata of the TG neurons, which innervate the inflamed pulp, and are released into the TG. The Hsp70 signaling via toll-like receptor (TLR) 4 in TG neurons innervates the tongue and thus enhances its neuronal excitability and leads to a condition known as tongue pain hypersensitivity [27]. However, these reports concurrently indicate that communication among TG neurons via various signaling pathways mediated by neurotransmitters contributes to orofacial pain hypersensitivity.

**Neuron and non-neuronal cell communication in TG**

Somata of the primary afferents, satellite glial cells, macrophages, and lymphocytes are typically present in the TG. The soma is surrounded by satellite glial cells; the gap between the soma and satellite glial cells is approximately 20 nm, and these cells communicate with each other using neurotransmitters [28]. Recent studies have revealed that morphological changes in satellite glial cells (soma and processes swelling) strongly correlates with their function [29]. Although the primary neuronal hyperactivity caused by peripheral inflammation or nerve injury plays an essential role in the morphology and function of satellite glial cells, the precise mechanism underlying this phenomenon currently remains unclear. It was observed that lingual nerve injury triggers a release of ATP molecules from the soma of TG neurons, which then bind to the P2Y1 receptors expressed in the satellite glial cells. The ATP-P2Y1 signaling activates the satellite glial cells, which induces hyperexcitability of the lingual nerve, followed by mechanical and heat hypersensitivity in the tongue [30]. Tooth pulp inflammation also leads to satellite cell activation in the TG, resulting in the hyperexpression of TRPV1 in the TG neurons that innervate the adjacent teeth, subsequently leading to pain hypersensitivity in the adjacent teeth [31].

The junction between satellite glial cells is constructed from a gap junction, which is known as a connexin and consists of two hemichannels [32]. Satellite glial cells are able to communicate with each other via their gap junctions; consequently, several ions and small molecules are able to pass between satellite glial cells [33]. Connexin 43 (Cx43) is a protein that is primarily observed in gap junctions that regulates the transport of a limited number of molecules between the satellite glial cells [34]. The presence of an infraorbital nerve injury increases Cx43 expression in TG neurons; furthermore, Cx43 levels decrease due to RNA interference in the TG’s reduced orofacial mechanical allodynia [35]. Inferior alveolar nerve injury also activates satellite glial cells by introducing morphological changes throughout the TG via gap junctions, and their activation results in extensive orofacial mechanical hypersensitivity [36]. These reports indicate that the large-scale activation of satellite glial cells in the TG under specific pathological orofacial conditions significantly enhances the excitability of the trigeminal neurons, which play a key role in ectopic orofacial pain.

Additionally, orofacial pathogenesis, such as orofacial inflammation and trigeminal nerve injury, leads to the infiltration and the subsequent accelerated activation of the inflammatory cells in the TG [37]. Incidentally, most of the macrophages that infiltrate the TG are supplied by the bone marrow [38]. Studies report that a peripheral nerve injury leads to the proliferation of resident macrophages in sensory ganglia [39-41]. Under specific pathological orofacial conditions, the infiltrated and resident macrophages alter their morphological appearance and demonstrate thicker ramifications and larger somata, which promotes other changes in the release of various neurotransmitters; therefore, changes in morphological appearance is presumed to indicate macrophage activation [42,43]. Furthermore, these macrophages are primarily categorized into two histological groups, each demonstrating specific functional properties [44]. The first group, known as M1 macrophage (the classically activated phenotype), is competent enough to release a large variety of proinflammatory cytokines and inflammatory mediators, which may be involved in the previous phases of local inflammatory responses. The second group of macrophages is known as M2 macrophages and is classified as the phenotype that is responsible for the anti-inflammatory response and tissue repair. A number of inflammatory mediators, including interferon-gamma, TNFα, and lipo-poly saccharide, activate M1 macrophages, and certain cytokines, such as interleukin (IL)-4 and IL-13, can possibly activate M2 macrophages [45-47]. Furthermore, signal transduction and activators of transcription 6 (STAT6) proteins are phosphorylated; STAT6-mediated gene transcription is facilitated in the M2 macrophage via IL-4 and IL-13 signaling and is involved in macrophage polarization [48]. The infiltration and activation of M1 and M2 macrophages may develop in not only the injured and inflamed region of the peripheral nerve but also the sensory ganglion [40]. Furthermore, TG neuronal excitability is regulated by certain chemical mediators, which are induced following orofacial pathogenesis by the functional transcellular communication between macrophages and TG neurons [49]. Long-lasting TG neuronal hyperexcitability further potentiates functional transcellular communication and initiates the enhancement of TG neuronal hyperexcitability, which leads to plastic changes in nociceptive neuronal excitability in the central nervous system (CNS). These plastic changes in TG neuronal transcellular communications are etiologically associated with orofacial pain hypersensitivity following orofacial peripheral nerve injury or inflammation [3]. Following peripheral nerve injury, the C-C chemokine receptor type 2 signaling in macrophages via chemokine C-C motif ligand 2 (CCL2) that is released into the TG from injured TG neurons may activate intra-ganglionic macrophages [50-52]. Moreover, a blockade of TLR2 signaling has yet to be associated with the hyperexpression of CCL2 in TG neurons. Additionally, the infiltration and activation of macrophages in the TG due to peripheral nerve injury, indicated by the enhancement of CCL2 signaling via TLR2, facilitate the infiltration of activated macrophages into the TG [53]. The intracellular signaling cascades, such as extracellular signal-regulated kinase (ERK) and p38 MAPK, regulate the release of TNFα from the macrophages [54-56]. Peripheral nerve injury of sensory neurons is known to accelerate the synthesis of substance P (SP), which is secreted into the sensory ganglion [57]. The secreted SP signaling promotes the release of TNFα via the ERK 1/2 and p38 MAPK signaling from the activated macrophages in the TG [58-60]. To summarize, TNFα or SP is released from activated macrophages after peripheral nerve injury in the orofacial region, subsequently resulting in the release of TNFα or SP signaling, which is responsible for TG neuronal hyperexcitability followed by orofacial pain hypersensitivity (Fig. 1) [61].

**Neuronal circuits in the trigeminal sensory nucleus complex**

Sensory information is integrated into the Vc, which consists of local circuit interneurons and projection neurons. A majority of the interneurons in the Vc are inhibitory gamma-aminobutyric acid (GABA)ergic and glycinergic neurons that suppress neuronal activity through the influx of Cl-, through GABAβ and glycine receptors. These neurons serve as the gate control of nociception. The balance between excitatory and inhibitory input into the neurons that transmit information to the CNS can be used to determine nociceptive information [62]. Local ablation of spinal glycinergic neurons using an intraspinal injection of AAV-FLEX diphtheria toxin A in glycine transporter 2-Cre mice shows a significant reduction in the mechanical threshold [63]. Additionally, prostaglandin E2, an inflammatory factor, downregulates the glycine receptor α3 subunit in the spinal dorsal horn, resulting in hyperalgesia [64]. Gate control in the Vc is regulated by GABAEergic neurons, and their reduction is observed seven days after inferior alveolar nerve transection [65]. Recently, an experiment that supports gate control theory was conducted using conditional ablation of somatosensory (SOM)-immunopositive neurons in lamina II of the spinal cord. SOM-immunopositive neurons receive both Aδ/C fiber-mediated noxious mechanical inputs and Aβ fiber-mediated non-noxious mechanical inputs, which are relayed via dynorphin-immunopositive neurons [66]. The loss
133 of Aβ fiber-mediated inputs onto SOM-immunopositive neurons caused mechanical allodynia by enhancing noxious mechanical inputs [66]. These data indicate that the transmission of noxious information from the spinal cord to higher brain regions is regulated by non-noxious information onto SOM-immunopositive neurons. Local circuit interneurons play an important role in pain sensation.

Sensory information that is integrated into a local, inter-neuronal circuit in the Vc is conveyed to the ventral posteromedial thalamic nucleus (VPM) and the parabrachial nucleus (PBN) by projection neurons [67]. The projection patterns are dependent on modality. In the case of a chronic constriction injury of the infraorbital nerve, C fiber-mediated responses are carried into both VPM and PBN. In contrast, mechanosensitive afferents preferentially terminate in the PBN, suggesting that Aδ fiber-mediated information is conveyed to the PBN [67]. Apart from the ascending pathway, direct monosynaptic input from TG neurons to the PBN is elucidated by genetic tools [68]. Distinct input patterns from the TG and Vc into the PBN might be instrumental in determining the severity of pain.

Abnormal pain
Glia cells play a crucial role in addressing chronic pain, which is typically resistant to therapeutics, including nonsteroidal anti-inflammatory drugs and opioids. After cellular activation, microglia retract their elaborated processes and enlarge their somata. The factors that induce microglial activation are primary afferent-derived chemokine (C-C motif) ligand 21, colony-stimulating factor 1 [69,70], de novo synthesis of lysophosphatidic acid, and ATP from spinal neurons [71,72]. Activated microglia release several proinflammatory cytokines, such as IL-1β, IL-6, and TNFα, and a brain-derived neurotrophic factor (BDNF) [73]. Among the proinflammatory cytokines, IL-1β potentiates glutamatergic neurotransmission and inhibits both GABAergic and glycinegic neurotransmission [74], resulting in hyperexcitation of the second-order neurons.
in spinal neurons with a lower net excitation. IL-1β derived from activated microglia releases BDNF, which acts on its receptor, CNTF/CNTF receptor-1 and -2 receptors in small DRG neurons of rats. Mediators Inflamm 2017, 2014925.


Conflict of interest

The authors have no conflict of interest to declare.

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