Abstract: Excitability of neurons in the trigeminal ganglion (TG), trigeminal spinal subnucleus caudalis (Vc), and upper cervical spinal cord (C1-C2) is greatly enhanced after orofacial inflammation and trigeminal nerve injury, and TG, Vc, and C1-C2 neurons remain sensitized long after such episodes. Sensitized neurons generate various molecules, which are released from nociceptive neurons in these areas and are involved in modulating the excitability of TG, Vc, and C1-C2 nociceptive neurons. Hyperexcitable nociceptive neurons also activate satellite glial cells in the TG and microglial cells and astrocytes in the Vc and C1-C2. Glial cell activation spreads throughout the TG, Vc, and C1-C2 and triggers the release of various molecules involved in modulating nociceptive neurons in TG, Vc, and C1-C2 neurons. These findings suggest that functional interaction between neurons and glial cells is critical in persistent orofacial pain associated with orofacial inflammation and trigeminal nerve injury.

Keywords: allodynia; hyperalgesia; trigeminal nerve; astrocyte; microglial cell; satellite glial cell.

Introduction
A barrage of action potentials is generated in trigeminal primary afferent neurons after orofacial or tooth pulp inflammation and trigeminal nerve injury (1). The excitability of trigeminal ganglion (TG) neurons persists long after inflammation and trigeminal nerve injury, and various molecules are generated in the TG during this time (2). These molecules, which include transient receptor potential channels, purinergic receptors, and neuropeptides, are transported to the peripheral and central terminals of TG neurons and act as ligand-gated receptors, transmitters, or neuromodulators. After these events, the excitability of TG neurons is further enhanced, which culminates in sensitization of TG neurons. High-frequency action potentials are generated and sent to the trigeminal spinal subnucleus caudalis (Vc) and upper cervical spinal cord (C1-C2). The activity of nociceptive neurons in Vc and C1-C2 is strongly enhanced, resulting in sensitization of Vc and C1-C2 nociceptive neurons.

A recent study reported that satellite glial cells (SGCs) in the TG are activated during orofacial inflammation and trigeminal nerve injury (3-5). The soma of TG neurons is tightly encircled by SGCs, which help modulate TG neuronal excitability and have roles in nutrition and structure formation (6). SGCs are activated and change their morphological features, which may become glial fibrillary acidic protein (GFAP)-immunoreactive after orofacial inflammation or trigeminal nerve injury. Activated SGCs also generate molecules involved in modulating TG neuronal excitability, and TG neurons also release various molecules (7). These findings suggest that neuron-satellite glial cell communication is a primary mechanism underlying the modulation of TG neuronal excitability associated with orofacial inflammation and trigeminal nerve injury (6). In addition, microglial cells and astrocytes in the Vc and C1-C2 are activated after orofacial inflammation and trigeminal nerve injury (2). These glial cells communicate with Vc and C1-C2 neurons, and this mechanism may be involved in modulating neuronal excitability (2).

To develop appropriate treatments for patients with persistent orofacial pain, it is important to identify the
mechanisms underlying neuron-glial cell communication in the TG after orofacial inflammation and trigeminal nerve injury.

Satellite glial cell activation associated with tooth pulp inflammation

Many previous studies used complete Freund’s adjuvant (CFA) to induce tooth pulp inflammation (4,8,9). Small dental paper points are soaked in a CFA emulsion (50% CFA and saline) and inserted into the tooth pulp to produce pulpal inflammation. One day after insertion of the CFA paper points, infiltration of inflammatory cells was significantly increased in tooth pulp, and this inflammatory state persisted longer than 1 week. We noted that masseter muscle electromyographic activity elicited by capsaicin application to tooth pulp was significantly greater in CFA-treated rats than in saline-treated rats (4).

A previous report found that extracellular signal-regulated kinase (ERK) is phosphorylated in dorsal root ganglion neurons within 5 minutes after intense noxious stimulation of the hind paw (10). The number of phosphorylated ERK-immunoreactive (pERK-IR) neurons is proportional to the intensity of a noxious stimulus (10). These results strongly suggest that ERK phosphorylation is a reliable marker of the excitability of TG neurons. Numerous pERK-IR cells were observed in the TG after CFA administration into tooth pulp, and many were encircled by activated SGCs (4). After blockade of SGC activation, the number of pERK-IR cells decreased, and masseter muscle EMG activity elicited by tooth pulp stimulation was significantly suppressed (4). It is highly likely that TG neurons are strongly activated after tooth pulp inflammation and that SGC activation is enhanced after that. This mechanism explains persistent orofacial pain resulting from tooth pulp inflammation.

Activation spreading of SGCs after trigeminal nerve injury

Trigeminal nerve injury and tooth pulp inflammation result in activation of SGCs in the TG (3). Numerous TG neurons were encircled by activated SGCs after inferior alveolar nerve transection (IANX), and many of these encircled neurons were present in the first and second branches of the trigeminal nerve (3).

Gap junction hemichannels facilitate the exchange of molecules between cells (11). Connexin 43 (Cx43) is the primary gap junction protein and is involved in the transport of small molecules between cells (12). Some previous studies reported that Cx43 is a necessary hemichannel in the exchange of small molecules within the TG (13,14). The Cx43 expression is strongly enhanced in the TG after IANX, and most of it is co-expressed with GFAP-IR cells (activated SGCs) (3). After intra-TG administration of the selective gap junction blocker Gap27 in IANX rats, the number of TG cells encircled by GFAP-IR cells was reduced, and the hypersensitivity spreading in the broad area of the face completely resolved. These findings suggest that Cx43 is a key molecule in the spreading of pain in the orofacial region after trigeminal nerve injury (3).

Involvement of the astrocyte glutamine-glutamate shuttle in persistent orofacial pain

Vc and C1-C2 nociceptive neurons become hyperexcitable after orofacial inflammation and trigeminal nerve injury (15-18). These neurons are sensitized a long time after this hyperexcitable state. The receptive field sizes were larger, spontaneous activities were greater and mechanical and heat-evoked responses were significantly stronger than those in sham-operated animals (15,19). Various molecules are generated in and released from nociceptive neurons in the Vc and C1-C2. Astrocytes take glutamate released from primary afferent terminals to produce glutamine, which is released from activated astrocytes. Glutamine is taken at the primary afferent terminals via the glutamine transporter, a process that enhances glutamate release from the terminals. A series of these processes involving glutamate and glutamine release is called the glutamine-glutamate shuttle (20). Existing evidence strongly suggests that this mechanism is essential for neuron-glial cell communication in the Vc and C1-C2 after orofacial inflammation and trigeminal nerve injury.

Microglial mechanisms underlying persistent orofacial pain

Microglial cells in the Vc and C1-C2 are activated after sensitization of nociceptive neurons associated with trigeminal nerve injury and orofacial inflammation (21,22). Activated microglial cells change their morphological features, e.g., shrinking of processes or soma swelling (23). Activated microglial cells express phosphorylated p38 and release various molecules (such as brain-derived neurotrophic factor [BDNF]) and cytokines after inflammation and tumor necrosis factor, cytokines, BDNF, ATP, prostaglandin, and nitric oxide after trigeminal nerve injury (24-26). These molecules bind their receptors, and the excitability of Vc and C1-C2 neurons is modulated. Microglial cell activation also spreads in the broad area of Vc and C1-C2, and nociceptive neurons become hyperactive because of microglial
cell activation. This process results in persistent extraterritorial pain in orofacial regions.

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References