Tumor Necrosis Factor Alpha Signaling in Trigeminal Ganglion Contributes to Mechanical Hypersensitivity in Masseter Muscle During Temporomandibular Joint Inflammation

Reio Ito, DDS
Graduate Student
Department of Complete Denture Prosthodontics

Masamichi Shinoda, DDS, PhD
Associate Professor
Department of Physiology;
Division of Functional Morphology
Dental Research Center

Kuniya Honda, DDS, PhD
Postdoctoral Fellow
Department of Physiology

Kentaro Urata, DDS, PhD
Clinical Fellow
Department of Complete Denture Prosthodontics

Jun Lee, DDS, PhD
Assistant Professor
Department of Complete Denture Prosthodontics

Mitsuru Maruno, DDS
Graduate Student
Department of Complete Denture Prosthodontics

Kumi Soma, DDS
Graduate Student
Department of Pediatric Dentistry

Shinji Okada, DDS
Graduate Student
Department of Complete Denture Prosthodontics

Nobuhito Gionhaku, DDS, PhD
Professor
Department of Complete Denture Prosthodontics

Koichi Iwata, DDS, PhD
Professor
Department of Physiology;
Division of Functional Morphology
Dental Research Center

Nihon University School of Dentistry
Kandasurugadai, Chiyoda-ku
Tokyo, Japan

Correspondence to:
Dr Masamichi Shinoda
Associate Professor
Department of Physiology
Nihon University School of Dentistry
1-8-13 Kandasurugadai, Chiyoda-ku,
Tokyo 101-8310, Japan
Fax: +81-3-3219-8341
Email: shinoda.masamichi@nihon-u.ac.jp

Aims: To determine the involvement of tumor necrosis factor alpha (TNFα) signaling in the trigeminal ganglion (TG) in the mechanical hypersensitivity of the masseter muscle during temporomandibular joint (TMJ) inflammation. **Methods:** A total of 55 male Sprague-Dawley rats were used. Following injection of Complete Freund's Adjuvant into the TMJ, the mechanical sensitivities of the masseter muscle and the overlying facial skin were measured. Satellite glial cell (SGC) activation and TNFα expression in the TG were investigated immunohistochemically, and the effects of their inhibition on the mechanical hypersensitivity of the masseter muscle were also examined. Student *t* test or two-way repeated-measures analysis of variance followed by Bonferroni multiple comparisons test were used for statistical analyses. $P < .05$ was considered to reflect statistical significance. **Results:** Mechanical allodynia in the masseter muscle was induced without any inflammatory cell infiltration in the muscle after TMJ inflammation. SGC activation and an increased number of TNFα-immunoreactive cells were induced in the TG following TMJ inflammation. Intra-TG administration of an inhibitor of SGC activity or of TNFα-neutralizing antibody depressed both the increased number of TG cells encircled by activated SGCs and the mechanical hypersensitivity of the masseter following TMJ inflammation. **Conclusion:** These findings suggest that persistent masseter hypersensitivity associated with TMJ inflammation was mediated by SGC–TG neuron interactions via TNFα signaling in the TG. *J Oral Facial Pain Headache 2018;32:75–83. doi: 10.11607/ofph.1854*

Keywords: *extraterritorial pain, satellite glial cell, temporomandibular joint, trigeminal ganglion, tumor necrosis factor alpha*

Temporomandibular joint (TMJ) inflammation frequently causes chronic pain in the TMJ and orofacial regions, including in the masticatory muscles.[1,2] Many TMJ inflammation patients suffer from masticatory dysfunction because jaw movements cause severe pain during mastication, and many also complain of myofascial pain in the masseter and temporalis muscles.[3,4] Eventually, the impaired food intake and disruption in orofacial motor functions associated with TMJ inflammation reduce the quality of life (QoL) in these patients. To develop appropriate treatments for widespread orofacial pain related to TMJ inflammatory pain, it is important to understand the mechanisms underlying masseter muscle pain related to TMJ inflammation.

Sensitivity to a variety of non-noxious and noxious stimuli to the TMJ region can be enhanced under orofacial inflammatory conditions.[5–7] Various immune cells appear in the TMJ region during TMJ inflammation that release several cytokines and inflammatory mediators, further sensitizing primary afferent neurons.[2,8] Satellite glial cells (SGCs) are also activated in the trigeminal ganglion (TG) in association with orofacial inflammation or trigeminal nerve injury.[9,10] SGCs and neurons in the TG are located close to each other (within less than 10 μm), suggesting that SGCs and TG neurons may have strong functional interactions. SGCs are known to undergo morphologic changes, developing large cell bodies and thick processes following activation.[9] Activated SGCs are also known to show glial fibrillary acidic protein (GFAP) immunore-

activity,[10] and activation of SGCs has been reported to spread widely throughout the TG via Connexin 43 (Cx43) signaling.[11] Cx43 is the primary gap junction protein involved in the transport of small molecules between cells.[12] These findings suggest that activated SGCs release molecules that may affect neuronal activity in a wide area of the TG.

Tumor necrosis factor (TNF) is one of the major cytokines produced in inflamed peripheral tissue.[13,14] TNF consists of three subtypes—TNFα, TNFβ, and lymphotoxin-β—that are released from macrophages in the region of the inflammation.[15] Because TNFα binds to the TNF receptor (TNFR) and its signaling is involved in the translocation of nuclear factor kappa B (NFκB) into the cell nucleus, it is believed to be a key molecule regulating the cellular immune system.[14] It has also been reported that plasma and synovial fluid levels of TNFα are significantly higher in patients who suffer from TMJ pain[16–18]; thus, TNFα is also thought to play a pivotal role in the modulation of orofacial pain due to TMJ inflammation.[19]

The aim of the present study was to determine the involvement of TNFα signaling in the TG in the mechanical hypersensitivity of the masseter muscle during TMJ inflammation. For this purpose, administration of Complete Freund's Adjuvant (CFA) into the TMJ capsule in rats was performed to cause persistent inflammation in the TMJ, an approach that has been widely used in TMJ pain studies.[5,6]

Materials and Methods

Animals

Male Sprague-Dawley rats (n = 55; 200 to 300 g, Japan SLC) were used in this study. The animals were housed in a temperature-controlled room (23 ± 1.0°C) under a 12/12–hour light/dark cycle with ad libitum access to food and water. The study was approved by the Nihon University Animal Experimentation Committee and was conducted in accordance with the guidelines of the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals and the International Association for the Study of Pain.[20] The number of rats used in the study was reduced to the minimum required for statistical analysis.

TMJ Inflammation Model

Rats were deeply anesthetized with an intraperitoneal (ip) injection of sodium pentobarbital (50 mg/kg; Schering Plough). A 50% solution of CFA (Sigma-Aldrich) dissolved in saline or vehicle solution (isotonic saline) alone was slowly administered with a 27-gauge needle (25 μL) into the left TMJ through the skin immediately below the posteroinferior border of the zygomatic arch. The CFA-administered rats (ie, TMJ-inflamed group; n = 35) were, while under deep anesthesia, subjected daily for 3 days to passive vertical jaw movements (interincisal vertical dimension: 20 mm, 1 Hz, 30 minutes/day) by using a passive vertical jaw-moving device. This was also performed daily in the vehicle-administered rats (ie, control group; n = 20). At 3 days after CFA or vehicle administration, rats (n = 45) were deeply anesthetized with sodium pentobarbital (80 mg/kg, ip) and perfused with saline. Horizontal sections of the masseter muscles from rats in both groups were stained with hematoxylin-eosin and analyzed under the light microscope.

Head Withdrawal Threshold to Mechanical Stimulation

Five days prior to CFA or vehicle treatment, rats were trained daily to calmly protrude their snout from a hole in the front wall of a small chamber for 5 minutes.[11] Following daily training and 1 day before CFA or vehicle administration, mechanical stimuli were applied to the left masseter muscle with a digital force gauge equipped with a flat rounded tip (5 mm in diameter; Attonic) to measure the mechanical head withdrawal threshold (HWT). The rats could escape freely from the mechanical stimulation at any time during the measurements. The HWT was also measured with von Frey filaments (Touch-Test Sensory Evaluator, North Coast Medical). Application of mechanical stimuli with von Frey filaments to the masseter muscle and to the facial skin overlying the masseter muscle was performed three times a day at 5-minute intervals, and the HWT for that day was defined as the mean value of these three experiments in each rat. Behavioral testing was performed from day 1 to 7 after CFA/vehicle administration in 10 rats (n = 5 from each group) under blinded conditions. All rats that were subjected to behavioral testing showed no motor deficits.

Intra-TG Administration of Fluorocitrate and TNFα-Neutralizing Antibody

Rats were placed in a stereotactic apparatus under deep anesthesia with sodium pentobarbital (50 mg/kg, ip). Following exposure, the skull was perforated (diameter 1 mm), and a guide cannula was implanted into the left TG in CFA-administered rats (n = 15). The guide cannula was fixed to the skull by using stainless steel screws and dental cement. The rats were allowed to recover for several days before administration of vehicle, satellite cell inhibitor Fluorocitrate (FC) (8 μmol/day, Cell Signaling) dissolved in 10% dimethyl sulfoxide, or TNFα-neutralizing antibody (TNFαNA) (1:20, R&D systems), carried out by using a 30-gauge injection needle inserted through the guide cannula. FC, TNFαNA, or vehicle (n = 5 in each group) was administered

once daily into the TG at 0, 1, 2, and 3 days after CFA injection, and HWTs were measured on days 1, 2, and 3, as described above. These rats were then sacrificed with the other 30 rats sacrificed for immunohistochemical analyses, as described above.

Immunohistochemistry

To identify the TG cells innervating the masseter muscle, 30 mL of FluoroGold (FG) (3% hydroxystilbamidine) (Fluorochrome) dissolved in saline was injected into the left masseter muscle with a 27-gauge needle 4 days before CFA or vehicle injection. At 3 days after injection, rats were transcardially perfused with isotonic saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under deep anesthesia produced by sodium pentobarbital (80 mg/kg, ip), and the left TGs were removed. TGs were kept in 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose for 24 hours and then embedded in TissueTek (Sakura Finetek) at –20°C. Then, 15-μm horizontal sections of TGs were prepared, and every 10th section (5 sections per TG) was collected and mounted onto MAS-coated Superfrost Plus microscope slides (Matsunami).

The sections were dried overnight, washed with 0.01 M PBS, and incubated with mouse anti–glial fibrillary acidic protein (GFAP) monoclonal antibody (1:1000; Merck Millipore), rabbit anti-TNFα (1:200; Abcam), or rabbit anti-Cx43 polyclonal antibody (1:200; Sigma-Aldrich) in 0.01 M PBS containing 4% normal goat serum for blocking unknown antigens and 0.3% Triton X-100 (Sigma-Aldrich) for 3 days at 4°C. Sections were washed in 0.01 M PBS and then incubated in Alexa Fluor 568 anti-rabbit IgG (1:200; Thermo Fisher Scientific) and/or Alexa Fluor 488 anti-mouse IgG (1:200; Thermo Fisher Scientific) in 0.01 M PBS for 2 hours at room temperature. After rinsing, the sections were coverslipped in mounting medium (Thermo Fisher Scientific), and immunopositive cells were analyzed with a BZ-9000 system (Keyence).

The mean numbers of FG-labeled TG neurons and of FG-labeled TG cells encircled by GFAP-immunoreactive (IR) cells were calculated first and presented as size-frequency histograms (every 200 mm^2) in both the TMJ-inflamed and control groups. The mean numbers of FG-labeled TG neurons in the regions of the first-second (V1-V2) and third (V3) branches of the trigeminal nerve were also calculated. FG-labeled TG cells that had over half of their soma perimeters encircled by GFAP-IR cells or by FG-labeled TNFα-IR cells were identified, and the relative numbers of those TG cells were calculated from the following formula using SensivMeasure (Mitani): 100 × the number of FG-labeled TG cells surrounded by GFAP-IR or FG-labeled TNFα-IR cells/total number of FG-labeled TG cells. No labeling was observed in the absence of primary antibodies under the same conditions.

Statistical Analyses

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed with Student *t* test or two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test. A *P* value of less than .05 was considered to reflect statistical significance.

Results

Nocifensive Behavior and Masseter Muscle Inflammation

The HWT in response to mechanical stimulation of the masseter muscle in the TMJ-inflamed group was significantly lower compared to that in the control group (Fig 1a). In contrast, no change in the HWT to mechanical stimulation of the lateral facial skin was observed in the TMJ-inflamed group (Fig 1b). Pathologic changes, such as infiltration of inflammatory cells or degeneration, could not be detected in the masseter muscle in either the TMJ-inflamed or control groups (Figs 1c and 1d).

SGC Activation and Cx43 Expression

A number of FG-labeled TG cells encircled by GFAP-IR cells were observed, and the distribution of these cells displayed network-like morphologic features (Fig 2a). The number of these cells in the TMJ-inflamed group (31.2 ± 5.5) was significantly greater than that in the control group (7.7 ± 1.0; $P < .05$) (Fig 2b). Many GFAP-IR cells expressing Cx43 were also observed in the V1-V2 and V3 regions in the TG (Fig 2c). There were no significant differences in the total number of FG-labeled TG cells between the TMJ-inflamed and control groups (Fig 2d). Although there were no significant differences between groups, TMJ inflammation significantly increased the number of FG-labeled TG cells encircled by GFAP-IR cells in all cell-size groups in the TMJ-inflamed group (Fig 2e). FG-labeled TG cells were significantly more numerous in the V3 region than in the V1-V2 region ($P < .01$) in both the TMJ-inflamed group and the control group (Fig 2f).

TNFα Expression in the TG

FG-labeled TG cells showed TNFα immunoreactivity in both the TMJ-inflamed group and the control group (Fig 3a). The mean proportion of FG-labeled TG cells encircled by TNFα-IR cells in the TMJ-inflamed group (33.5% ± 2.9%) was significantly greater than that in the control group (5.0% ± 0.2%; $P < .01$) (Fig 3b).

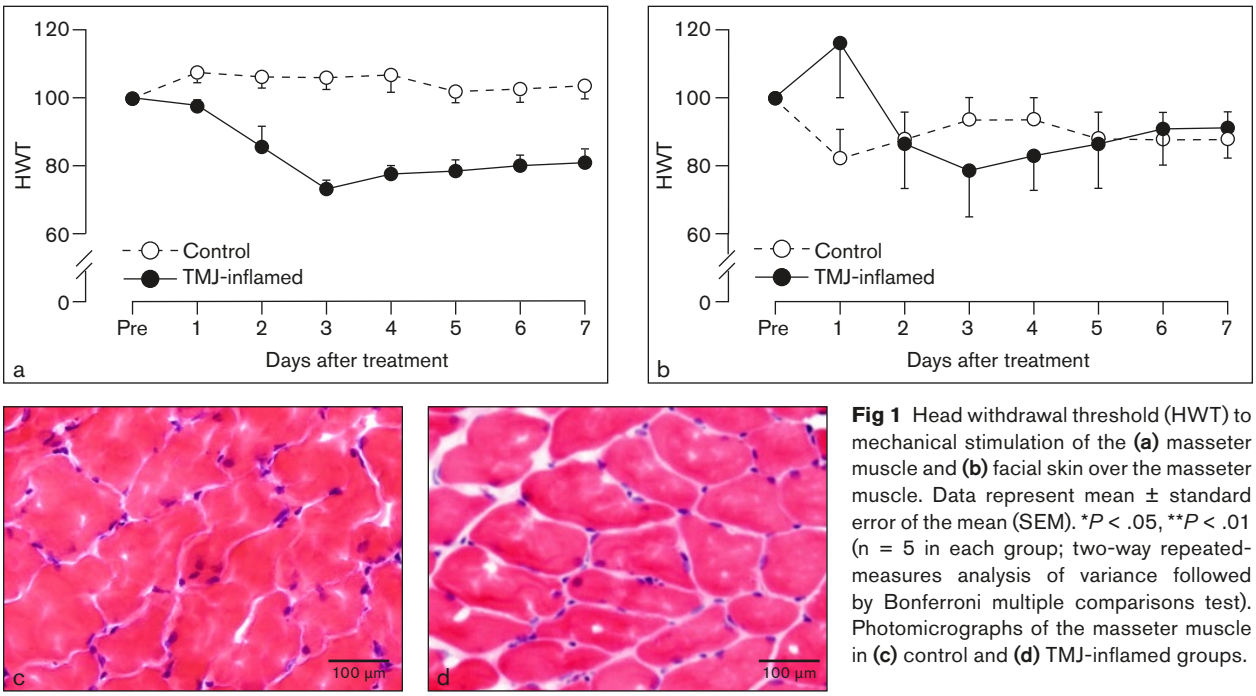


Fig 1 Head withdrawal threshold (HWT) to mechanical stimulation of the **(a)** masseter muscle and **(b)** facial skin over the masseter muscle. Data represent mean ± standard error of the mean (SEM). **P* < .05, ***P* < .01 (n = 5 in each group; two-way repeated-measures analysis of variance followed by Bonferroni multiple comparisons test). Photomicrographs of the masseter muscle in **(c)** control and **(d)** TMJ-inflamed groups.

Effect of Intra-TG Administration of FC or TNFαNA on SGC Activation and Mechanical Hypersensitivity of Masseter Muscle

FC is a potent inhibitor of SGC activation.[21] In the TMJ-inflamed group, the number of FG-labeled TG cells encircled by GFAP-IR cells was significantly reduced following 3 successive days of FC administration into the TG compared to the vehicle-administered group (vehicle: 23.8 ± 2.3; FC: 3.0 ± 0.2; $P < .01$) (Figs 4a and 4b). Successive intra-TG administration of TNFαNA also suppressed the increase in FG-labeled TG cells encircled by GFAP-IR cells in the TMJ-inflamed group (vehicle: 21.9 ± 0.9; TNFαNA: 13.3 ± 0.7; $P < .05$) (Figs 4c and 4d). The decreased HWT of the masseter muscle in the TMJ-inflamed group was significantly increased following successive intra-TG administration of FC or TNFαNA (Day 3; vehicle: 63.5% ± 1.4%, FC: 81.1% ± 2.1% [$P < .05$], TNFαNA: 97.7% ± 0.5% [$P < .01$]) (Fig 4e).

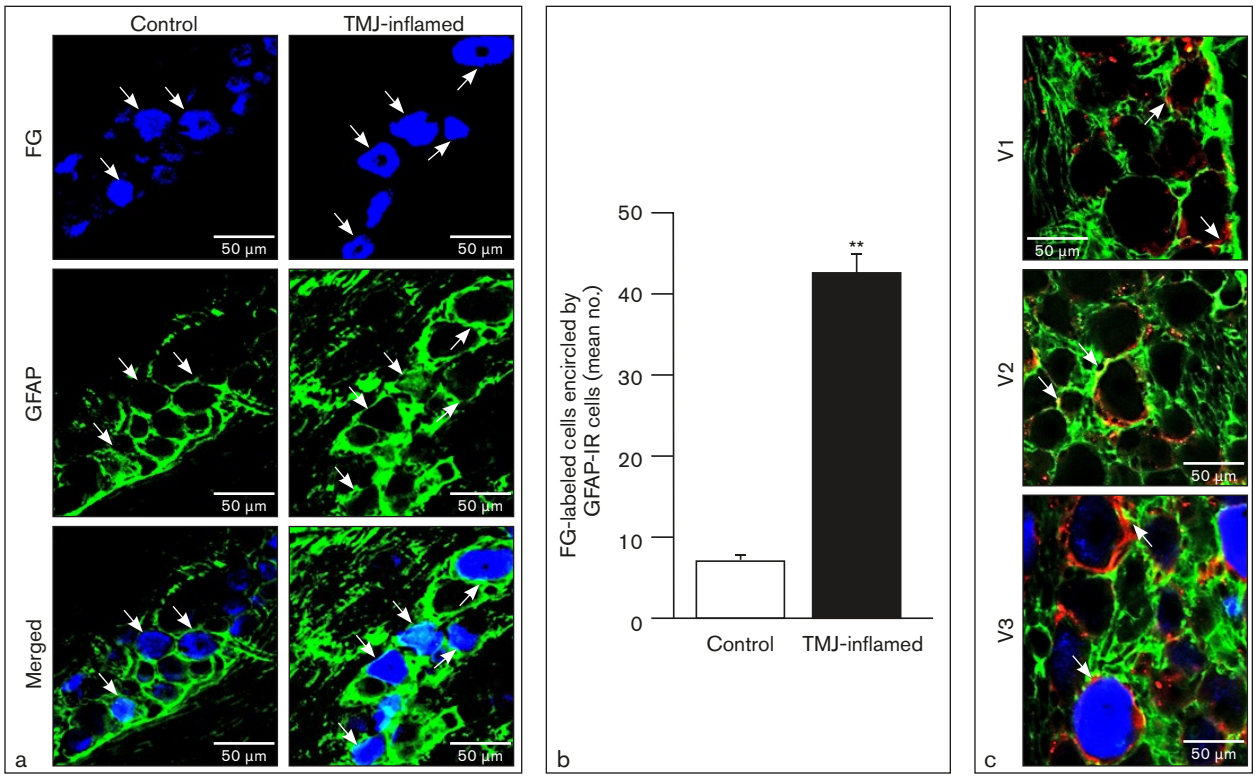
Effect of Intra-TG Administration of FC on TNFα Expression in TG Cells

FG-labeled TG cells expressing TNFα were confirmed in the TMJ-inflamed group (Fig 5a), but their mean proportion was significantly smaller in rats administered FC than in control rats (vehicle: 35.6% ± 2.4%, FC: 25.8% ± 1.3%; $P < .05$) (Fig 5b). This finding indicates that SGC activation after TMJ inflammation was involved in the TNFα expression in TG cells.

Discussion

The present study evaluated the mechanisms underlying masseter muscle pain originating from TMJ inflammation. Specifically, the role of TNFα signaling in the activation of SGCs in the TG and SGC–TG neuron interactions following TMJ inflammation were examined by using a rat model of TMJ inflammation.

Large-diameter pressure probes have been reported to be the most suitable for assessing the mechanical pain threshold of deep tissue, and small-diameter pressure probes, such as von Frey filaments, as appropriate for measuring the threshold of superficial tissues.[22] In the present study, the HWT to mechanical stimulation of the masseter muscle in the TMJ-inflamed group was significantly lower compared to that of the control group, although no significant change in response to stimulation of the lateral facial skin was observed in either group. These results indicate that TMJ inflammation induced mechanical allodynia in the masseter muscle but not in the facial skin overlying the masseter, implying that the excitability of TG neurons innervating the masseter was enhanced by TMJ inflammation. In addition, there were no significant changes in the total number of TG cells innervating the masseter muscle, and these cells were observed in all cell-size groups in both the TMJ-inflamed and control groups, suggesting that neuronal excitabilities of putative nociceptive TG neurons—as well as low-threshold and



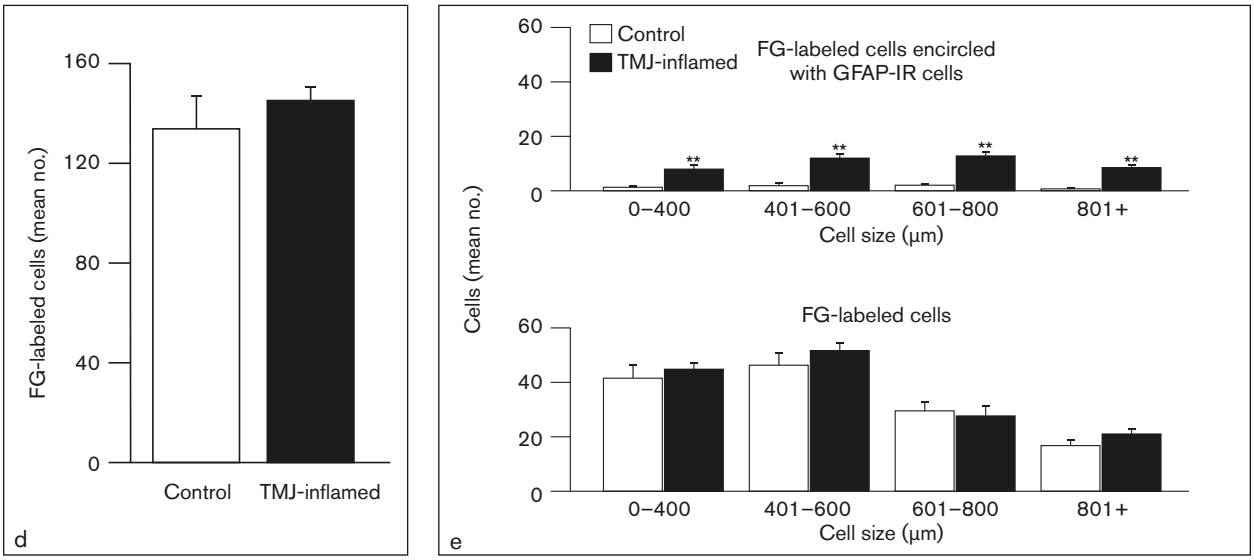
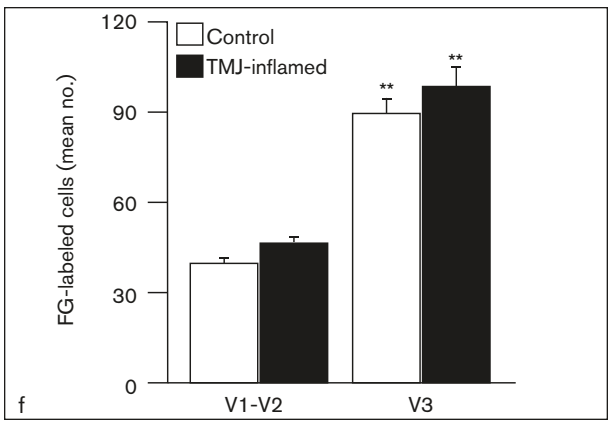
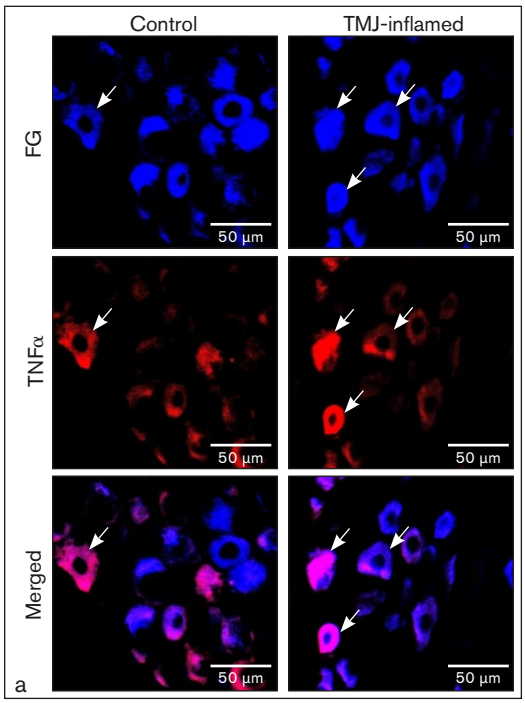


Fig 2 Glial fibrillary acidic protein (GFAP) and Connexin 43 (Cx43) expression in the TG. All data represent mean ± standard error of the mean (SEM). **(a)** Photomicrographs of FG-labeled TG cells and GFAP-immunoreactive (IR) cells *(arrows)* and **(b)** the mean number of TG cells encircled by GFAP-IR cells in both groups. **P* < .05 (n = 5 in each group; Student *t* test). **(c)** Cx43 expression in GFAP-IR cells *(arrows)* in the areas of the V1, V2, and V3 regions of the trigeminal nerve in the TMJ-inflamed group. **(d)** Mean number of FG-labeled TG cells in both groups. **(e)** Size-frequency histograms illustrating the distribution of FG-labeled TG cells and FG-labeled TG cells encircled by GFAP-IR cells in both groups by cell size. **(f)** Mean number of FG-labeled cells in the V1-V2 and V3 regions of the trigeminal nerve in both groups. ***P* < .01 vs V1-V2 region (n = 5 in each group; Student *t* test).





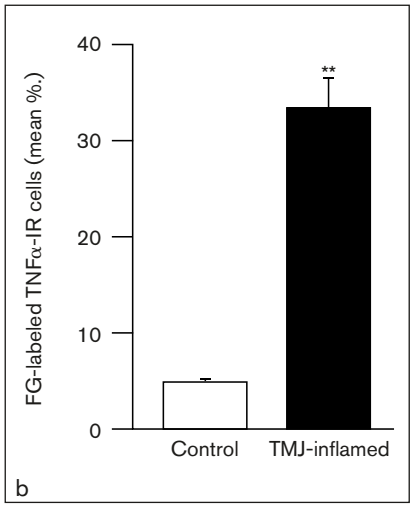


Fig 3 Tumor necrosis factor alpha (TNFα) expression in the TG. All data represent mean ± standard error of the mean (SEM). **(a)** Photomicrographs of FG-labeled TG cells and TNFα-IR cells *(arrows)* in both groups. **(b)** Mean proportion of FG-labeled cells encircled by TNFα-IR cells in both groups. $^{**}P < .01$ (n = 5 in each group; Student *t* test).

high-threshold mechanosensitive TG neurons innervating the masseter—were enhanced following TMJ inflammation. Furthermore, TG neurons innervating the masseter were extensively distributed over the TG, although they were mainly distributed in the V3 region. This result suggests that excitatory changes in TG neurons innervating the masseter muscle following TMJ inflammation may exert a major influence on the excitability of other TG neurons innervating other parts of the orofacial region.

Moreover, in addition to the peripheral sensitization uncovered by the present study, recent evidence indicates that central sensitization can also occur following injection of algesic chemicals into the TMJ capsule.[5,23,24] Central sensitization of medullary nociceptive neurons receiving inputs from the masseter muscle is thus likely to be induced by TMJ inflammation, resulting in the enhancement of nocifensive reflexes evoked by mechanical stimulation of the masseter muscle.

It has been reported that TG neurons are strongly activated following trigeminal nerve injury or orofacial inflammation.[9,10,25] A barrage of action potentials associated with trigeminal nerve injury or orofacial inflammation is generated in the trigeminal nerve.[26] High-frequency action potentials appear at the somata of TG neurons, which subsequently release various molecules such as substance P, calcitonin gene-related peptide (CGRP), nerve growth factor (NGF), or adenosine triphosphate (ATP), resulting in activation of the SGCs.[27,28] Activated SGCs become GFAP immunopositive, are observed around the somata of TG neurons,[10] and release several neuropeptides and ATP, molecules known to be involved in the modulation of TG neuronal activity.[10] Activated SGCs have been observed in regions of the TG beyond the V3 branch of the trigeminal nerve following lingual nerve injury or transection of the V3 branch.[10] In the present study, many FG-labeled TG cells and GFAP-IR cells were observed over a wide area of the TG covering the V2 region, in which TG neurons innervating the masseter muscle were present following TMJ inflammation. Furthermore, the number of TG cells encircled by GFAP-IR cells was significantly higher in the TMJ-inflamed group than in the control group. These findings suggest that TMJ inflammation induces activation of SGCs in a wide area of the TG covering the V2 and V3 regions, and this activation may be involved in the hyperexcitability of TG neurons innervating the masseter muscle following TMJ inflammation. Moreover, TMJ inflammation significantly increased the number of TG cells encircled by activated SGCs in all cell-size groups, indicating that neuronal excitability was likely enhanced not only in nociceptive TG neurons innervating the masseter muscle, but in non-nociceptive TG neurons as well.

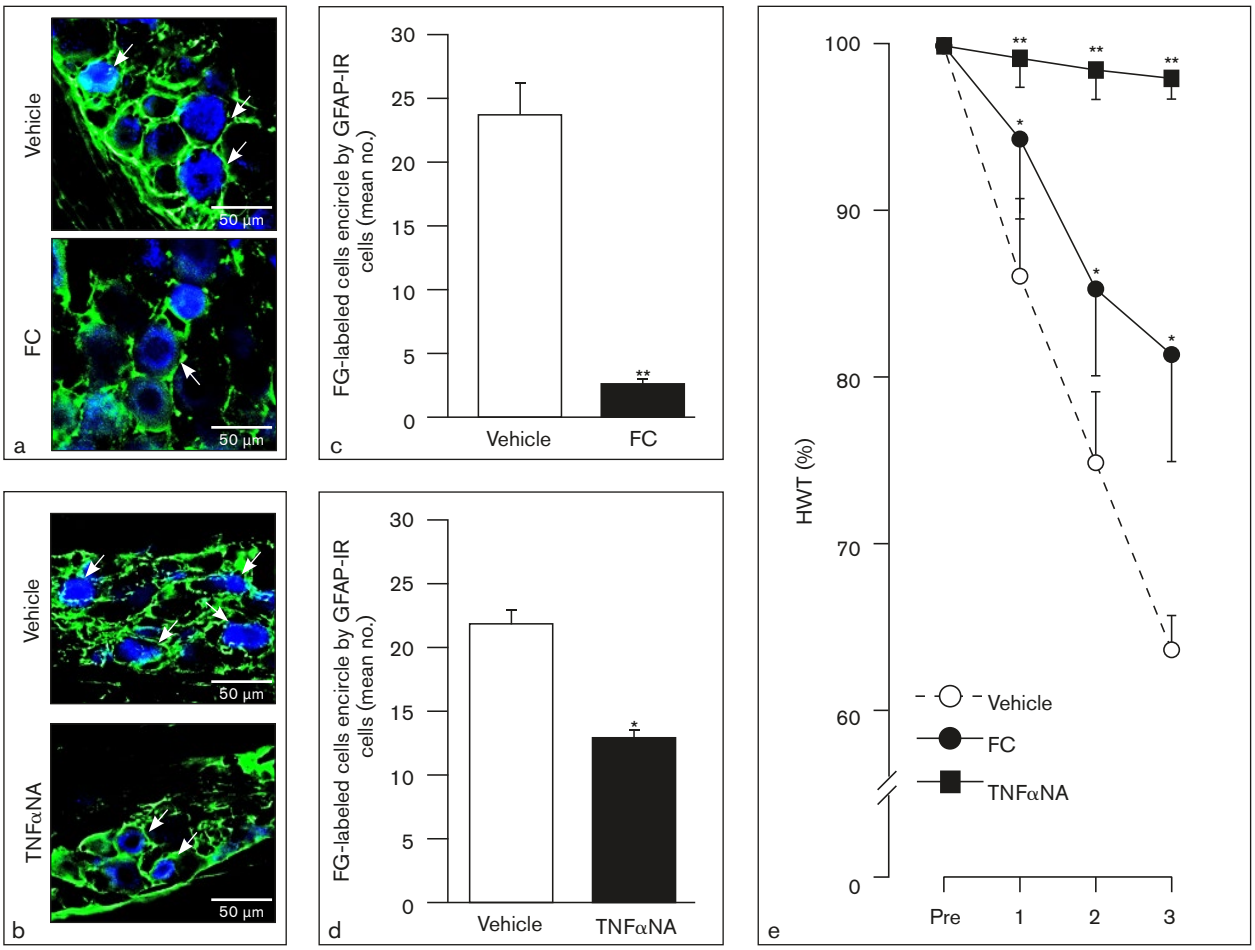


Fig 4 Effects of intra-TG administration of Fluorocitrate (FC) and tumor necrosis factor alpha neutralizing antibody (TNFαNA) on GFAP expression and mechanical sensitivity of the masseter muscle. Photomicrographs of FG-labeled TG cells encircled by GFAP-IR cells *(arrows)* after administration of **(a)** FC or **(b)** TNFαNA in the TMJ-inflamed group. The mean number of FG-labeled TG cells encircled by GFAP-IR cells following **(c)** FC or **(d)** TNFαNA administration in the TMJ-inflamed group. **P* < .05, ***P* < .01 (Student *t* test). **(e)** Relative HWT to mechanical stimulation of the masseter muscle following successive vehicle, FC, or TNFαNA administration into the TG in the TMJ-inflamed group. **P* < .05, ***P* < .01 vs vehicle-administered group (n = 5 in each group; two-way repeated-measures analysis of variance followed by Bonferroni multiple comparisons test).

Cx43 is a gap junction component thought to be involved in mechanisms underlying the widespread SGC activation within the TG.[11] Blockade of Cx43 activation in the TG reduced GFAP expression and suppressed orofacial mechanical nocifensive reflex behavior.[11] Many GFAP-IR cells were merged with Cx43-IR cells in the TMJ-inflamed group in the present study, and so it is highly likely that SGC activation occurred in the TMJ-inflamed group via Cx43 signaling and that this mechanism is involved in SGC activation in an extensive area within the TG following TMJ inflammation.

TNFα is widely accepted as a proinflammatory cytokine involved in the enhancement of primary neuronal excitability, which contributes to pain hypersensitivity.[14] TNFα is upregulated in sensory ganglion neurons and in microglial cells and macrophages associated with peripheral nerve injury or tissue inflammation and is released from these cells during inflammation.[13,15,29] An increase in the plasma concentration of TNFα has also been reported in blood samples of patients who suffer from TMJ pain.[16] TNFα released from glial cells has been shown to bind to TNFα receptors in sensory ganglion neurons and initiate signaling involved in the activation of these neurons. Furthermore, cultured sensory ganglion cells show an increase in transient receptor potential vanilloid 1 expression following TNFα treatment.[19] Moreover, several lines of evidence indicate that local injection of TNFα causes thermal and mechanical hypersensitivity at the injection site, and blockade of TNFα by TNFα-neutralizing agents alleviates this hypersensitivity.[19,30,31]

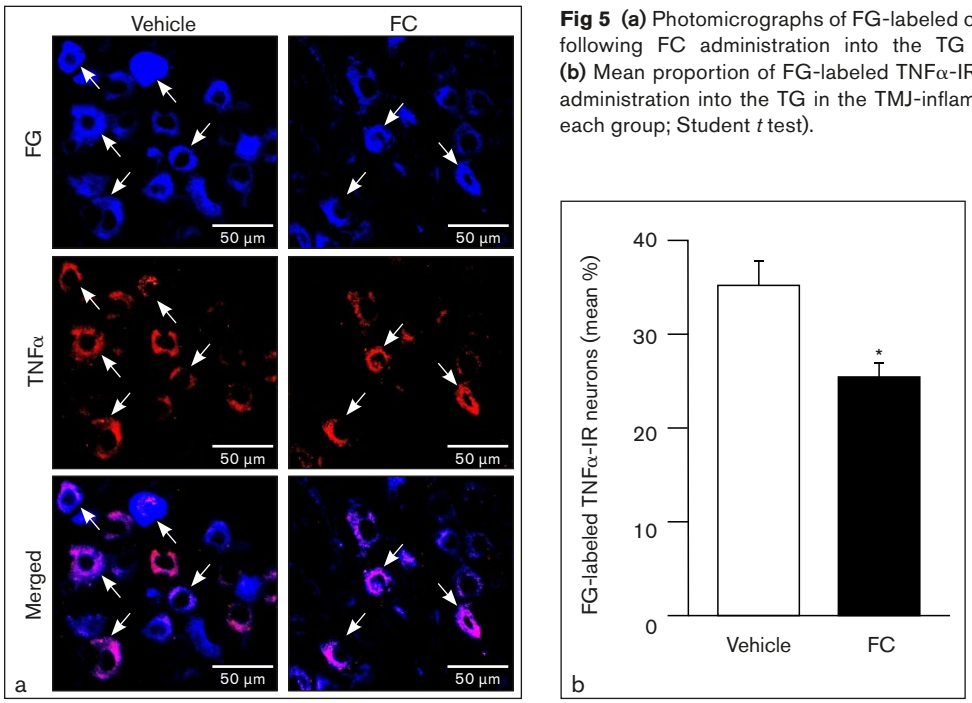


Fig 5 **(a)** Photomicrographs of FG-labeled cells expressing TNFα *(arrows)* following FC administration into the TG in the TMJ-inflamed group. **(b)** Mean proportion of FG-labeled TNFα-IR cells following FC or vehicle administration into the TG in the TMJ-inflamed group. *$P < .05$ (n = 5 in each group; Student *t* test).

In the present study, a significant increase in expression of TNFα was observed in TG cells and in GFAP-IR cells. Continuous administration of TNFαNA in the TG restored mechanical sensitivity of the masseter muscle in the TMJ-inflamed group. Furthermore, the number of FG-labeled cells encircled by GFAP-IR cells in the TMJ-inflamed group was significantly reduced by TNFαNA administration compared to that in the vehicle-administered group, and the number of TNFα-IR cells was significantly reduced following FC injection into the TG. Moreover, it was reported that GFAP-IR cells expressed Cx43. Together, these data indicate that the SGC activation via Cx43 signaling following TMJ inflammation enhanced TNFα expression in TG neurons in an extensive area within the TG, and TNFα signaling in TG neurons may be involved in further activation of SGC via Cx43 signaling within the TG.

Conclusions

A significant enhancement of mechanical sensitivity of the masseter muscle, an increase in TNFα-IR cells, and hyperactivation of SGCs expressing Cx43 in the TG in the TMJ-inflamed group were observed in the present study. The inhibition of SGC hyperactivation and the neutralization of TNFα in the TG caused a significant suppression of masseter muscle hypersensitivity. These findings suggest that SGC–TG neuron interactions via TNFα signaling in the TG play a pivotal role in masseter muscle pain hypersensitivity associated with TMJ inflammation, and TNFα signaling may be a therapeutic target for treatment of widespread deep orofacial pain in patients who have TMJ pain. TMJ pain frequently occurs in patients with temporomandibular disorders (TMD), which occur predominantly in young or elderly women,[32,33] but a limitation of the present study is that the mechanisms underlying masseter muscle pain associated with TMJ pain in females were not addressed because only male rats were used. Moreover, it is unclear whether TNFα signaling in the TG also plays a predominant role in masseter muscle pain associated with TMD. Further studies are needed to address these unresolved matters.

Acknowledgments

This study was supported by the Uemura Fund, Nihon University School of Dentistry, a grant from Dental Research Center, Nihon University School of Dentistry, KAKENHI (Grant-in-Aid for Challenging Exploratory Research 15K15748), and MEXT-Supported Program for the Strategic Research Foundation at Private Universities 2013–2017. The authors declare no potential conflicts of interest regarding this article.

References

1. Stoustrup P, Kristensen KD, Verna C, Küseler A, Herlin T, Pedersen TK. Orofacial symptoms related to temporomandibular joint arthritis in juvenile idiopathic arthritis: Smallest detectable difference in self-reported pain intensity. J Rheumatol 2012;39:2352–2358.
2. Hawkins JL, Durham PL. Prolonged jaw opening promotes nociception and enhanced cytokine expression. J Oral Facial Pain Headache 2016;30:34–41.
3. Fernández-de-las-Penas C, Svensson P. Myofascial temporomandibular disorder. Curr Rheumatol Rev 2016;12:40–54.
4. Bonato LL, Quinelato V, De Felipe Cordeiro PC, De Sousa EB, Tesch R, Casado PL. Association between temporomandibular disorders and pain in other regions of the body. J Oral Rehabil 2017;44:9–15.
5. Iwata K, Tashiro A, Tsuboi Y, et al. Medullary dorsal horn neuronal activity in rats with persistent temporomandibular joint and perioral inflammation. J Neurophysiol 1999;82:1244–1253.
6. Shinoda M, Ozaki N, Asai H, Nagamine K, Sugiura Y. Changes in P2X3 receptor expression in the trigeminal ganglion following monoarthritis of the temporomandibular joint in rats. Pain 2005;116:42–51.
7. Harriott AM, Dessem D, Gold MS. Inflammation increases the excitability of masseter muscle afferents. Neuroscience 2006;141:433–442.
8. Villa G, Ceruti S, Zanardelli M, et al. Temporomandibular joint inflammation activates glial and immune cells in both the trigeminal ganglia and in the spinal trigeminal nucleus. Mol Pain 2010;6:89.
9. Matsuura S, Shimizu K, Shinoda M, et al. Mechanisms underlying ectopic persistent tooth-pulp pain following pulpal inflammation. PLoS One 2013;8:e52840.
10. Katagiri A, Shinoda M, Honda K, Toyofuku A, Sessle BJ, Iwata K. Satellite glial cell P2Y12 receptor in the trigeminal ganglion is involved in lingual neuropathic pain mechanisms in rats. Mol Pain 2012;8:23.
11. Kaji K, Shinoda M, Honda K, Unno S, Shimizu N, Iwata K. Connexin 43 contributes to ectopic orofacial pain following inferior alveolar nerve injury. Mol Pain 2016;12.
12. Chen MJ, Kress B, Han X, et al. Astrocytic CX43 hemichannels and gap junctions play a crucial role in development of chronic neuropathic pain following spinal cord injury. Glia 2012;60:1660–1670.
13. Wei XH, Zang Y, Wu CY, Xu JT, Xin WJ, Liu XG. Peri-sciatic administration of recombinant rat TNF-alpha induces mechanical allodynia via upregulation of TNF-alpha in dorsal root ganglia and in spinal dorsal horn: The role of NF-kappa B pathway. Exp Neurol 2007;205:471–484.
14. Rothman SM, Huang Z, Lee KE, Weisshaar CL, Winkelstein BA. Cytokine mRNA expression in painful radiculopathy. J Pain 2009;10:90–99.
15. Niu YL, Guo Z, Zhou RH. Up-regulation of TNF-alpha in neurons of dorsal root ganglia and spinal cord during coronary artery occlusion in rats. Cytokine 2009;47:23–29.
16. Park JW, Chung JW. Inflammatory cytokines and sleep disturbance in patients with temporomandibular disorders. J Oral Facial Pain Headache 2016;30:27–33.
17. Nordahl S, Alstergren P, Kopp S. Tumor necrosis factor-alpha in synovial fluid and plasma from patients with chronic connective tissue disease and its relation to temporomandibular joint pain. J Oral Maxillofac Surg 2000;58:525–530.
18. Fredriksson L, Alstergren P, Kopp S. Tumor necrosis factor-alpha in temporomandibular joint synovial fluid predicts treatment effects on pain by intra-articular glucocorticoid treatment. Mediators Inflamm 2006;2006:59425.
19. Xu JT, Xin WJ, Zang Y, Wu CY, Liu XG. The role of tumor necrosis factor-alpha in the neuropathic pain induced by Lumbar 5 ventral root transection in rat. Pain 2006;123:306–321.
20. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110.
21. Shimizu K, Guo W, Wang H, et al. Differential involvement of trigeminal transition zone and laminated subnucleus caudalis in orofacial deep and cutaneous hyperalgesia: The effects of interleukin-10 and glial inhibitors. Mol Pain 2009;5:75.
22. Takahashi K, Taguchi T, Itoh K, Okada K, Kawakita K, Mizumura K. Influence of surface anesthesia on the pressure pain threshold measured with different-sized probes. Somatosens Mot Res 2005;22:299–305.
23. Tashiro A, Okamoto K, Bereiter DA. Chronic inflammation and estradiol interact through MAPK activation to affect TMJ nociceptive processing by trigeminal caudalis neurons. Neuroscience 2009;164:1813–1820.
24. Lam DK, Sessle BJ, Hu JW. Glutamate and capsaicin effects on trigeminal nociception II: Activation and central sensitization in brainstem neurons with deep craniofacial afferent input. Brain Res 2009;1253:48–59.
25. Nakagawa K, Takeda M, Tsuboi Y, et al. Alteration of primary afferent activity following inferior alveolar nerve transection in rats. Mol Pain 2010;6:9.
26. Kitagawa J, Takeda M, Suzuki I, et al. Mechanisms involved in modulation of trigeminal primary afferent activity in rats with peripheral mononeuropathy. Eur J Neurosci 2006;24:1976–1986.
27. Iwata K, Imamura Y, Honda K, Shinoda M. Physiological mechanisms of neuropathic pain: The orofacial region. Int Rev Neurobiol 2011;97:227–250.
28. Shinoda M, Asano M, Omagari D, et al. Nerve growth factor contribution via transient receptor potential vanilloid 1 to ectopic orofacial pain. J Neurosci 2011;31:7145–7155.
29. Takahashi N, Kikuchi S, Shubayev VI, Campana WM, Myers RR. TNF-alpha and phosphorylation of ERK in DRG and spinal cord: Insights into mechanisms of sciatica. Spine (Phila Pa 1976) 2006;31:523–529.
30. Chatterjea D, Paredes L, Martinov T, et al. TNF-alpha neutralizing antibody blocks thermal sensitivity induced by compound 48/80-provoked mast cell degranulation. F1000Res 2013;2:178.
31. Mizukoshi K, Sasaki M, Izumi Y, Miura M, Watanabe M, Amaya F. Activation of p38 mitogen-activated protein kinase in the dorsal root ganglion contributes to pain hypersensitivity after plantar incision. Neuroscience 2013;234:77–87.
32. Cairns BE. Pathophysiology of TMD pain—Basic mechanisms and their implications for pharmacotherapy. J Oral Rehabil 2010;37:391–410.
33. Shaefer JR, Holland N, Whelan JS, Velly AM. Pain and temporomandibular disorders: A pharmaco-gender dilemma. Dent Clin North Am 2013;57:233–262.