Published in final edited form as:
Exp Neurol. 2008 December ; 214(2): 209–218. doi:10.1016/j.expneurol.2008.08.005.

Modulation of paratrigeminal nociceptive neurons following temporomandibular joint inflammation in rats

Yoko Yamazaki[a], **Ke Ren**[b], **Masahiko Shimada**[a], and **Koichi Iwata**[c,d,e,*]

a *Department of Orofacial Pain Management, Tokyo Medical and Dental University, Bunkyo-ku, Yushima, Tokyo 113-8549, Japan*

b *Department of Neural and Pain Sciences, University of Maryland Dental School, Baltimore, Maryland 21201, USA*

c *Department of Physiology, Nihon University School of Dentistry, Chiyoda-ku, Tokyo 101-8310, Japan*

d *Division of Functional Morphology, Dental Research Center, Nihon University School of Dentistry, Chiyoda-ku, Tokyo 101-8310, Japan*

e *Division of Applied System Neuroscience Advanced Medical Research Center, Nihon University Graduate School of Medical Science, 30-1 Ohyaguchi-Kamimachi Itabashi, Tokyo 173-8610, Japan*

Abstract

To evaluate the involvement of paratrigeminal nucleus (Pa5) nociceptive neurons in temporomandibular joint (TMJ) inflammation-induced pain and its autonomic correlates, we conducted behavioral, single unit recording and Fos immunohistochemical studies in anesthetized rats. Nocifensive behaviors to mechanical, heat or cold stimulation of the lateral face over the TMJ region were significantly enhanced in the TMJ-inflamed rats for 10–14 days after injection of complete Freund's adjuvant (CFA) into the TMJ and gradually decreased at the end of the 14-day observation period. Lowering of the nocifensive threshold in TMJ-inflamed rats lasted longer in vagus nerve-transected rats than vagus nerve-intact rats. A large number of Fos-like immunoreactive (LI) cells were observed in the Pa5, and half of them were retrogradely labeled with Fluorogold (FG) injected into the parabrachial nucleus. Background activity of Pa5 wide dynamic range and nociceptive specific neurons was significantly higher in the TMJ-inflamed rats when compared with controls. Responses to mechanical stimuli were significantly higher in NS neurons in the TMJ-inflamed rats. All thermal responsive Pa5 neurons were exclusively sensitive to cold and the response to cold was significantly higher in the TMJ-inflamed rats compared with control rats. Vagus nerve stimulation significantly decreased responses to mechanical and cold stimuli as well as the background activity in TMJ-treated rats but not in TMJ-untreated rats. The present findings suggest that populations of Pa5 neurons are nociceptive and involved in TMJ inflammation-induced pain as well as in autonomic processes related to TMJ pain.

Keywords

Trigeminal nerve; TMJ; Pa5; Vagus nerve; Hyperalgesia; Fos; Nociceptive neuron; Complete Freund's adjuvant

*Corresponding author. Department of Physiology, Nihon University School of Dentistry, Chiyoda-ku, Tokyo 101-8310, Japan. Fax: +81 3 3219 8341. E-mail address: E-mail: iwata-k@dent.nihon-u.ac.jp (K. Iwata).

Introduction

Temporomandibular joint disorders (TMJD) are recurrent diseases frequently observed in the dental clinic. TMJD patients have a variety of symptoms such as jaw movement disorder and TMJ pain, and TMJD is involved in stress-related disorders characterized by somatic and psychologic complaints such as fatigue, sleep disturbances, anxiety and depression (Korszun et al., 1996; Bush et al., 1989; Hansdottir and Bakke, 2004; Schellhas et al., 1989). It is also suggested that alternations of stress response hormones occur in response to somatic symptoms associated with chronic TMJ pain (Korszun et al., 2002). It is possible that the stress-related autonomic alteration occurs in TMJD patients (Ushiyama et al., 2008). However, the neural mechanisms underlying orofacial pain and related autonomic responses associated with TMJ inflammation remain unclear.

The paratrigeminal (Pa5) nucleus is a small nucleus located within the dorsal lateral medullary spinal trigeminal tract (Paxinos and Watson, 1986). Pa5 neurons are thought to be involved in autonomic functions such as controlling the cardiovascular system (Yu et al., 2002; Yu and Lindsey, 2003). The experimental TMJ inflammation results in expression of Fos proteins in Pa5 neurons as well as in the trigeminal spinal subnucleus caudalis (Vc) (Hathaway et al., 1995; Zhou et al., 1999; Bereiter et al., 2002). Based on these data, we hypothesized that Pa5 neurons are involved in processing trigeminal nociceptive information as well as autonomic information related to nociception. However, the type of nociceptive neurons in Pa5 that are involved in the processing of this information is not known.

Inflammation has been thought to be a source of the TMJ pain in TMJD patients. A number of rat models with TMJ inflammation has been developed (Hathaway et al., 1995; Imbe et al., 2001). Complete Freund's adjuvant (CFA), mustard oil, formalin, adenosine triphosphate (ATP) and glutamate are frequently used to produce TMJ inflammation in rats (Bereiter et al., 2002; Imbe and Ren, 1999; Lam et al., 2005; Oliveira et al., 2005; Roveroni et al., 2001). Among them CFA is known as a reliable irritant to produce long lasting inflammation after local injection and to elicit acute and long lasting pain. In the present study, therefore, we used CFA to produce persistent inflammation of the TMJ capsule in rats.

Inflammation of the TMJ is associated with deep tissue pain that is often referred to the cutaneous site (Imbe et al., 2001, Iwata et al., 1999). This allows assessing hyperalgesia induced by injection of CFA into the TMJ capsule through the facial skin site without invasive manipulations. It is also well known that many trigeminal sensory neurons receive convergent inputs from the facial skin and TMJ (Tashiro et al., 2007). We hypothesize that the excitability of Pa5 neurons that also receive cutaneous inputs is modulated by TMJ inflammation. Thus, to investigate the role of Pa5 in TMJ inflammatory hyperalgesia, we examined nocifensive behavior and neuronal activity of Pa5 neurons in rats after TMJ inflammation in response to noxious stimulation of the facial skin.

Previous studies have shown that vagus nerve stimulation modulates Pa5 neuronal activities (Rutherfurd et al., 1992). It is also known that modulation of Pa5 neuronal activity is associated with changes in arterial blood pressure (Balan et al., 2004; Yu and Lindsey 2003). Furthermore, esophageal acid stimulation or nociceptive stimuli to the tongue or laryngopharyngeal mucosa has been found to increase Fos protein expression in Pa5 neurons (Carstens et al., 1995; Suwanprathes et al., 2003; Boucher et al., 2003). In addition, electrical stimulation of the tongue or hypoglossal nerve has also been found to induce Fos expression in Pa5 neurons (Bereiter et al., 2006). Indeed, the Pa5 appears to have a complex role in nocifensive behavior (Koepp et al., 2006). A Pa5 lesion did not alter the latency for radiant heat-induced withdrawal of the paw, although it reduces the paw withdrawal threshold for mechanical stimulation. Furthermore, a Pa5 ablation reduced formalin-induced nocifensive behavior. Sensory afferents

in the vagus nerve are known to modulate the function of pain pathways (Randich and Gebhart 1992). Indeed, electrical stimulation of the vagus nerve has been found to change the nociceptive neuronal activity in the medullary and spinal dorsal horn (Bossut and Maixner 1996; Chandler et al., 1991; Ren et al., 1993).

Anatomical tracing studies have revealed that Pa5 neurons not only receive primary afferent inputs from the inferior alveolar nerve, but also have strong connections with the parabrachial nucleus (PBN), the solitary tract nucleus (STN) and medullary reticular formation (Caous et al., 2001). Based on these studies, the authors have suggested that the Pa5 nucleus relays trigeminal nociceptive information to other brain areas such as the PBN and the STN. These previous studies suggest that Pa5 neurons are involved in trigeminal nociception as well as pain-related autonomic function.

In the present study, we evaluated the importance of Pa5 nociceptive neurons in mechanisms underlying the trigeminal pathological pain in rats. We focused on Pa5 nociceptive neurons and studied their involvement in TMJ inflammation-induced nocifensive behavior and analyzed to what extent the responses of these neurons to nociceptive stimuli are modulated by the vagus nerve.

Materials and methods

The present experiments were approved by the Animal Experimentation Committee of Nihon University School of Dentistry, and animals were treated according to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Adult male Sprague–Dawley (SD) rats (220–440 g) were used in all experiments.

TMJ CFA injection

Male SD rats weighing 220–440 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and complete Freund's adjuvant (CFA, 0.05 ml) was injected through the facial skin into the left side of the TMJ capsule. The CFA was suspended in an oil/saline (1:1) emulsion; an injection of saline instead of CFA served as a control in behavior and Fos experiments. The rats were tested for mechanical, heat and cold allodynia and used for Fos or single neuron recording study at three days after CFA injection. Nocifensive behavior in response to mechanical, heat and cold stimuli was clearly present at this time.

Behavioral test

Seventy five male SD rats that had limited access to water (100 ml/kg/day for 1 week) were used for the behavioral study. The animals were able to drink water through a hole in the front panel of the plastic cage. They were trained to continue drinking water during noxious mechanical stimulation of the lateral face. The mechanical stimulation (1, 6, 15 and 26 g) was applied with von-Frey monofilaments through a small whole (1 cm in diameter) in the lateral wall of the plastic cage daily for 14 days after CFA injection. The mechanical stimulation or heat stimulation was applied to the lateral face at 2 mm below the posterior edge of the zygomatic arc. The nocifensive withdrawal behavior was defined as withdrawing the head from the hole of the front panel. The head withdrawal threshold to increasing mechanical stimulus intensity was measured. Hargreaves type thermal stimulator was used to measure the heat escape latency (Hargreaves et al., 1988). The withdrawal latency was defined as the time lag between the onset of the head withdrawal and the onset of the heater. For cold stimulation, 50 μl of acetone solution was applied to the lateral face with microsyringe and the number of face groomings was counted. CFA injection was performed (TMJ CFA group for withdrawal threshold measurement, withdrawal latency measurement and face grooming frequency, n=5 each measurement). Before CFA injection and one day after injection, all animals were tested

with von-Frey hairs, heat and cold stimuli. Each hair was applied twice at an interval of 5 s. The rat was probed by the same filament twice and the two values were averaged. The escape threshold intensity was defined as the bending force of the first hair to evoke an escape response. For measurement of the cold nocifensive behavior, the number of face grooming was counted for 1 min. For vagus nerve transection study, a 15-mm incision was made along the mid-line of the ventral portion of the neck skin reaching the collar bone in the rats anesthetized with sodium pentobarbital (50 mg/kg/i.p.). The left vagus nerve and surrounding connective tissues were locally anesthetized with 2% lidocaine and neck muscles were carefully reflected. Then the vagus nerve, sympathetic nerve and carotid artery were exposed and the vagus nerve freed from the connective tissues. The vagus nerve was tightly ligated at two points (≈1 mm apart) and transected at the middle of these two ligations (about 7.0 mm rostral from the collar bone). Then, CFA or saline was injected into the left TMJ capsule.

Fos and Fluorogold (FG) immunohistochemistries

Fifteen male SD rats were used for Fos and Fluorogold (FG) tracing experiments. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p), and FG was injected into the left PBN, 10.0 mm caudal from the bregma and 2.0 mm lateral from the mid-line. Three days later CFA or saline was injected into the left TMJ (n=5 per treatment). Three days after CFA injection, animals were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and perfused through the aorta with 100 ml of 0.02 M phosphate-buffered saline (PBS, pH 7.4) followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered solution (PBS, pH 7.4). The medulla and the upper cervical spinal cord were removed, post fixed in the same fixative for 3 days at 4 °C with agitation, and transferred to 30% sucrose solution (w/v) in PBS for several days for cryoprotection. Fifty-micron-thick sections were cut on a freezing microtome, and every fourth section was collected in PBS. The FG injection sites were examined under a fluorescent microscope before an immunohistochemical treatment.

Free-floating tissue sections were rinsed in PBS, 3% normal goat serum (NGS) in PBS for 1 h, then incubated for 72 h with rabbit anti-Fos (1:10,000: c-*fos* ab-5, Oncogene, MA, USA) in 3% NGS at 4 °C. After three washes in PBS with 0.75% Triton X-100 and in PBS, the sections were incubated with biotinylated secondary IgG (1:200; Vector Labs, Burlingame, CA, USA) for 24 h at 4 °C. Following rinses in PBS with Triton three times, the sections were incubated in peroxidase-conjugated avidin–biotin complex (1:100; ABC, Vecter Labs) for 2 h at room temperature. To develop the ABC reaction product, the sections were incubated in 0.035% 33′-diaminobenzidine-tetra HCI (DAB; Sigma), 0.2% nickel ammonium sulfate, and 0.05% peroxide in 0.05 M Tris-buffer (TB, pH 7.4). After the Fos immunohistochemistry, sections were rinsed in PBS, 3% normal goat serum (NGS) in PBS for 1 h, then incubated for 24 h with rabbit anti-FG (1:5000: Chemicon, USA) in 3% NGS at room temperature. Sections were treated as described before to develop the ABC reaction product. Finally, the sections were rinsed in PBS, mounted on gelatin-coated slides, dehydrated in alcohols, cleared in xylene, and covered with Eukitt (O. Kindler, Germany).

Cells with black deposits in the nuclei were considered as Fos protein-LI cells. Camera lucida drawings of these sections were made. The number of Fos protein-L1 cells was counted per section per rat. The mean number of Fos protein-LI cells of all sections (number of Fos protein-LI cells per section) was calculated across five rats.

Single neuron recording

Thirty four SD male rats were used for single neuron recording experiments (control rats: n=17 and CFA-injected rats: n=17). Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the trachea and left external jugular veins were cannulated to allow artificial respiration and intravenous administration of drugs. Anesthesia was maintained with halothane

(2–3%) mixed with oxygen during surgery. The rats were mounted on a stereotaxic frame, and the medulla was exposed. A mineral oil pool was made with the skin flap. During recording session, rats were immobilized with pancuronium bromide (1 mg/kg/h, i.v.) and ventilated artificially. The expired CO_2 concentration was monitored and maintained between 3.0–4.0%. Rectal temperature was maintained at 37–38 °C by a thermostatically controlled heating pad, and the electrocardiogram was monitored. If the heart rate was increased after mechanical or thermal stimulation of the receptive fields, the percentage of halothane was increased. Enamel-coated tungsten microelectrodes (impedance =10–12 MΩ, 1000 Hz, FHC) were protruded perpendicularly to the brain surface into the Pa5 about 1.3 mm rostral to the obex and 2.5 mm lateral from the mid-line (Yu et al., 2003) in 2 μm steps ipsilateral to CFA injection. The Pa5 neurons were searched by applying mechanical stimulation (pressure or brush) to the facial skin. When a single neuron was isolated, the responses to mechanical stimulation of the facial skin were carefully examined and the RFs were mapped.

Mechanical stimuli were applied to the most sensitive areas of the RFs. Mechanical stimuli consisted of brushing with a camel hair brush, graded pressure produced by von-Frey filaments and pinch produced by a small arterial clip. In order to avoid sensitization due to repeated stimulation, noxious mechanical stimuli were applied to only small areas of the RFs in each neuron. If the non-noxious RFs of first and second encountered nociceptive neurons overlapped with each other, the second neuron was not included in the analysis. Each neuron was classified either as 1) a LTM neuron being a neuron that showed only transient firing at the onset and termination of the mechanical stimulus or had tonic responses during mechanical stimulation of the RFs and decreased firing frequency after noxious mechanical stimulation; 2) a WDR neuron being a neuron that responded to both non-noxious and noxious mechanical stimuli and increased its firing frequency as stimulus intensity increased; or 3) an NS being a neuron that responded exclusively to noxious mechanical stimulation of the RFs. To avoid sensitization of the RFs by noxious stimulation, we did not use repeated noxious stimuli to search for NS neurons. If a neuron showed weak responses to a pressure stimulus and not to brushing, noxious pinch was applied to verify if it was an NS neuron.

After characterization of Pa5 neurons with mechanical stimuli, thermal stimuli (heating and cooling) were applied to the most sensitive area of the cutaneous mechanical RF. The tip of the thermal probe was 5 mm in diameter, and the rate of temperature change was set at 10 °C/s. The structure of the thermal probe used in this study has previously been described in detail (Iwata et al., 1995). Before an application of the thermal stimulus to the RF, the surface temperature was adapted to 38 °C for 180 s. Skin heating ranged from 44–50 °C and lasted for 10 s. Cold stimuli were consisted of cooling of the skin to 5–30 °C. The thermal stimuli were applied to the RFs at 190-second intervals (adaptation time: 180 s, stimulus time: 10 s) to avoid sensitization of peripheral nociceptors. Neuronal responses were recorded in the hard disk of the microcomputer, and firing frequency was analyzed off-line. After recording, lesions were made at the recording site by passing direct current of 20 μA for 10 s.

The waveform of single or multiple neuronal activities was analyzed off-line. The waveform of each neuron was identified using Spike 2 software (CED). PSTHs (peristimulus time histograms; bin width=1 s) were generated in response to each stimulus. Background activities were first recorded for 10 s before application of mechanical or thermal stimulus, and they were subtracted from the neuronal responses during analysis. The mean firing frequency (spikes/s) during mechanical or thermal stimulation was calculated. Stimulus–response (S–R) functions of each nociceptive neuron were obtained in response to the mechanical (brush, graded pressure or pinch) or thermal (heat: 44–50 °C, cold: 5–30 °C) stimuli. The mechanical or thermal stimulation of the RFs was considered to have induced an effect when the peak firing frequency at 5 s after mechanical and 10 s (one trial for each neuron with 180 s intervals)

after thermal stimulation differed from the mean background discharge rate by ±2 S.D. The RFs of all neurons were drawn to scale on standard diagrams of a rat head.

We also tested the effect of vagus nerve electrical stimulation on Pa5 neuronal responses. For electrical stimulation of the left vagus nerve (ipsilateral to the recording side), the vagus nerve was exposed as described previously and tightly ligated at about 3.0 mm rostral from the collar bone. Then the bipolar silver wire electrodes (inter electrode distance: 0.5 mm) were placed 1.0 mm rostral from the point of ligation. After identification of nociceptive neurons in Pa5 the template of the wave form was made for each neuron. The electrical pulses (duration: 0.2 ms, intensity: 0.2 mA, frequency: 10 Hz, stimulus time: 5 s for mechanical and 10 s for thermal stimuli) were applied to the ipsilateral vagus nerve during mechanical or thermal stimulation of the receptive field. We were able to isolate the electrical artifact and generate PSTHs from nociceptive neurons during electrical stimulation of the vagus nerve. We did not observe any differences in behavior and Fos expression between TMJ-untreated and TMJ-vehicle (saline) injected rats. Thus, we used TMJ-untreated rats for single neuronal recording as a control.

After the recording session the animals were killed by an over dose of sodium pentobarbital (80 mg/kg, i.p.) and then the brain was removed. Then the rat brain was immersed 4 days in 4% formalin and 50 um thick serial sections along the path of the penetration electrode were cut from the medulla including Pa5 and stained with thionin. The location of Pa5 was defined according to the rat brain map by Paxinos and Watson (1986). We only analyzed neuronal activity when histological examination showed that the neurons were located inside the Pa5.

Statistical analysis

Results from Fos, neuronal recording, head withdrawal latency and head withdrawal threshold were presented as mean±SEM. Statistical analysis was performed with repeated measures ANOVA followed by Dunnett's test for the behavioral data and one way ANOVA followed by Dunnett's test for the Fos analysis. Student *t*-test or Welch's *t*-test was also used for a comparison between CFA-injected rats and vagus nerve-transected rats with CFA injection in behavioral study, Fos/FG analysis and neuronal recording data where appropriate. Differences were considered significant at $p<0.05$.

Results

Nocifensive behavior

The lateral face region was shaved every other day to measure the mechanical escape threshold, heat escape latency or grooming frequency (number/min) before and after CFA or saline injection into the TMJ capsule. Mechanical, heat or cold stimulus was applied to the lateral facial skin over the TMJ capsule. We did not observe significant changes in weight after TMJ CFA injection (before CAF injection: 376.0±8.4 g, 3 days after CFA injection: 381.2±6.7 g, $n=15$). After CFA injection into the TMJ capsule the head withdrawal threshold to mechanical stimulation of the lateral face was significantly decreased at the side ipsilateral to CFA injection (Fig. 1). The head withdrawal latency to heat stimulation of the lateral face was also significantly decreased in the rats with TMJ CFA injection compared with those before CFA injection. The number of groomings was significantly increased at the ipsilateral side of TMJ CFA injection after acetone application to the lateral face (Figs. 1E and F). A decrease of the mechanical escape threshold was observed at the contralateral side of CFA injection. On the other hand, the shortening of the escape latency to heating of the face and the increase of grooming frequency was not observed at the contralateral side of CFA injection (Figs. 1B and D). These behavioral data suggest that the mechanical and heat allodynia are developed in the lateral facial skin following TMJ CFA injection. In this model, the decrement of head withdrawal threshold to mechanical stimulation of the face, shortening of the head withdrawal

latency to heating of the lateral face and the increase in the face grooming frequency lasted for at least 10 days. The mechanical head withdrawal threshold was gradually increased and head withdrawal latency to heating of the face was elongated and face grooming frequency was reduced after 10 days (Figs. 1A, C and E). However, we did not observe complete recovery of mechanical escape threshold, heat escape latency and face grooming frequency in the CFA-injected rats during the 14-day observation period.

The mechanical escape threshold was significantly lower in vagus nerve-transected rats at 7–14 days after CFA injection compared with vagus nerve-intact rats (Fig. 1A). We also observed that the mechanical escape threshold in the contralateral side to CFA injection was significantly lower in vagus nerve-transected rats compared with vagus nerve-intact rats (Fig. 1B). The heat and cold nocifensive behavior were also significantly lower in vagus nerve-transected rats at 10–14 days after CFA injection compared with vagus nerve-intact rats as illustrated in Figs. 1C and D.

Fos and/or FG-LI cells in Pa5 after TMJ CFA injection

FG was injected into the PBN ipsilateral to TMJ CFA injection site as illustrated in Fig. 2A. A large number of Pa5 neurons were retrogradely labeled with FG 6 days after FG injection into the PBN ipsilateral to the CFA injection site. FG-labeled neurons exhibited brown staining (Figs. 2B–F) and Fos-LI cells were detected as neurons with black nuclei indicated by the arrows (Fig. 2C). Double labeled neurons appeared with brown cytoplasm and black nuclei and are indicated by arrows (Fig. 2D). We observed a significantly larger number of Fos-LI cells bilaterally in the Pa5 at 3 days after CFA injection into the TMJ capsule as compared with that after saline injection into the capsule (Fig. 2G: ipsilaterally to injection, Fig. 2I: contralateral side to injection). The number of FG/Fos-LI cells in Pa5 was also significantly larger at the ipsilateral side of CFA injection compared with saline-treated rats as illustrated in Fig. 2H. A half of Fos-LI cells expressed at the ipsilateral side of CFA injection was also labeled with FG retrogradely transported from the PBN (CFA in Figs. 2G and H). We observed many FG-labeled cells and Fos-LI cells in the ipsilateral side to FG injection into the Pa5, but no FG/Fos-LI cells in Pa5 (Fig. 2F). We could not observe any behavioral differences between naive and vehicle (saline) injected rat. Thus, we used the naive rats as a control.

Pa5 neuronal activity

Fifty six nociceptive neurons were identified in the Pa5, and 43 nociceptive neurons responded to mechanical and cold stimuli. Fig. 3 shows the location of WDR and NS neurons identified by the histological examination. WDR and NS neurons were intermingled with each other within the Pa5. All Pa5 nociceptive neurons responded to mechanical and cold stimuli of the receptive fields, but did not respond to heating of the receptive fields. In general, the background activity of WDR and NS neurons showed regular firing pattern. WDR and NS neurons recorded from the Pa5 showed significantly higher background activity in the rats with TMJ CFA injection compared with those of control rats (CFA, WDR: 4.93±2.34, NS: 3.09 ±0.98; control, WDR: 0.70±0.40; NS: 1.04±0.47, $p<0.05$). Mechanical stimulus–response functions of WDR and NS neurons are illustrated in Fig. 4. WDR neurons responded to non-noxious mechanical stimulation of the receptive fields and increased their firing frequency following graded mechanical stimulation of the receptive fields in both control and TMJ-inflamed rats (Figs. 4A and B). NS neurons did not respond to non-noxious stimuli and their firings were graded following increase in the noxious mechanical stimulus intensity (Figs. 4C and D). The mechanically evoked responses of NS neurons were significantly higher in inflamed rats than control rats (Figs. 4C and D). In the present study, we did not encounter any heat responsive units from the Pa5 in the control and CFA-treated rats. All units responsive to thermal stimulation were classified as cold-sensitive ($n=34$). WDR and NS neurons showed slight increases in their firing frequency following decreases in stimulus temperature from 30

to 5 °C. Cold-evoked responses of these neurons were much larger in WDR neurons from TMJ-inflamed rats as compared with those of control rats (Fig. 5). We did not observe any differences in receptive filed properties of WDR and NS neurons between the TMJ-inflamed and control rats.

Effect of vagus nerve electrical stimulation on Pa5 neurons

Electrical stimulation of the vagus nerve induced significant modulation on responses of Pa5 nociceptive neurons. As shown in Figs. 6A and C, the responses of WDR neurons to mechanical stimuli were significantly reduced following electrical stimulation of the vagus nerve in control and CFA-treated rats. For NS neurons a significant reduction of mechanically evoked responses were observed in the TMJ-inflamed rats, but not in control rats (Figs. 6B and D). We also observed a significant reduction of cold responses in WDR and NS neurons after vagus nerve stimulation in TMJ-inflamed rats, whereas no significant changes in cold responses in WDR and NS neurons could be found in control rats (Figs. 7A and B). Background activity of WDR and NS neurons was significantly decreased after vagus nerve stimulation in the TMJ-inflamed rats as illustrated in Fig. 8A. However, we did not observe any effect of vagus nerve stimulation on the background activity of WDR and NS neurons in control rats (Fig. 8A). The receptive field size was not affected by the electrical stimulation of the vagus nerve in the control and CFA-treated rats (Fig. 8B).

Discussion

An inflammation in deep structures such as the knee joint is known to produce a strong activation of small diameter primary afferent fibers compared with a superficial inflammation, resulting in a strong activation of peripheral and central nervous system (CNS) nociceptive neurons (Schaible and Schmidt, 1988; Imbe et al., 2001; Iwata et al., 1999; Zhou et al., 1999). Mustard oil injection into the masseter muscle induces significant increases in the activity of Vc neurons (Hu et al.,1992). It has been reported that the CFA injection into the TMJ capsule produces significant increases in background activity, heat evoked responses and expansion of the receptive fields of Vc nociceptive neurons, whereas subcutaneous injection of CFA produces only a very weak effecton Vc neuronal activity compared with that of the TMJ-inflamed rats (Iwata et al., 1999). These results indicate that TMJ inflammation triggers sensitization of both Vc neurons and primary afferent fibers. In addition to spinal trigeminal nucleus, many neurons in the Pa5 are activated following inflammation of the orofacial regions, as shown by Fos protein expression (Bereiter et al., 2002, 2005; Ogawa et al., 2003; Shimizu et al., 2006). These findings suggest that the inflammation in structures/tissues innervated by the trigeminal nerves such as TMJ and masseter muscle produces strong activation of both Pa5 neurons and Vc neurons. However, the effect of TMJ inflammation on Pa5 neuronal activity has not been studied. Here we demonstrated that a population of Pa5 neurons is nociceptive, and that WDR and NS neurons in Pa5 show significant increases of background activity and evoked responses after TMJ inflammation. These findings are consistent with the observation that there was a significant increase in Fos-LI cells in the Pa5 following CFA injection into the TMJ capsule. We further showed that responses of Pa5 WDR and NS neurons were enhanced after transection of the vagus nerve and depressed after vagus nerve stimulation, suggesting that Pa5 neuronal activity was modulated by vagus afferents.

Enhancement of Pa5 neuronal activity following TMJ inflammation

It is well known that TMJ inflammation induces a variety of changes in the TMJ region such as jaw movement disorder and TMJ pain (Bush et al., 1989; Schellhas et al., 1989; Quinn and Bazan 1990). The long lasting knee joint inflammation following carrageenan injection sensitizes small diameter nerve fibers in the capsule, resulting in an increase in primary afferent activity (Cairns et al., 1998). It has been reported that the 3rd branch of the trigeminal nerve

projects directly from the TMJ to Pa5 neurons (Lapa and Watanabe 2005). This result strongly suggests that the afferent fibers innervating the TMJ capsule send sensory information from the TMJ capsule to Pa5 neurons. We observed a significant increase in Fos-LI cells in the Pa5 ipsilateral to CFA injection side at 3 days after its injection. This may be the result of a direct activation of primary afferent neurons innervating the TMJ capsule by CFA. We also observed Fos-LI cells in the contralateral Pa5 after CFA injection. The mechanism of an effect of TMJ inflammation at the contralateral side of CFA injection is unknown. Many studies have revealed that TMJ CFA injection induces a strong bilateral expression of Fos-LI cells in the Vi/Vc transition zone (Sato et al., 2005; Zhou et al., 1999). It has been reported that some primary afferent fibers cross the mid-line and reach the contralateral side (Jacquin et al., 1990). These findings suggest that the contralateral Fos expression is likely to be result of direct and indirect inputs from the TMJ capsule. Some researchers have also speculated that the neurotrophic factors released from the injured nerves are involved in the contralateral Fos expression after the inflammation (Imbe et al., 2001; Ogawa et al., 2003).

It has been reported that glial cells in the spinal dorsal horn are activated after peripheral inflammation or peripheral nerve injury (Hains and Waxman 2006). Glial activation in the Vc has also been reported to extend into more than half of the Vc (Xie et al., 2007). A similar glial mechanism may be involved in the bilateral Fos expression in the Pa5 after a unilateral TMJ inflammation. It has also been suggested that activation of the autonomic system contributes to an enhancement of bilateral neuronal activity in the Vi/Vc transition zone of the TMJ-inflamed rats (Bereiter et al., 2002, 2005). However, the exact neural mechanism underlying the contralateral effect of peripheral inflammation is not known.

In the present study, FG was injected into the PBN and the population of FG/Fos double labeled cells was analyzed in TMJ-CFA-injected, TMJ-saline and TMJ-untreated control rats. The number of FG/Fos-LI cells was significantly increased after CFA injection into the TMJ. The present findings suggest that the PBN-projection neurons in Pa5 are also involved in relaying neuronal hyperexcitability to higher CNS areas.

We also analyzed the electrophysiological properties of Pa5 nociceptive neurons in TMJ-inflamed and control rats. Pa5 nociceptive neurons could be classified as WDR and NS neurons, similar to that in the spinal or medullary dorsal horn. The general response properties of these two types of nociceptive neurons were similar to those of Vc neurons as reported previously (Iwata et al., 1999). A particularly interesting difference between Pa5 and Vc neurons concerns their thermal responses. We did not encounter any Pa5 nociceptive neurons that responded to heat stimulation of the receptive fields and all thermal responsive nociceptive neurons we recorded from Pa5 were classified as cold-sensitive. As reported previously, many nociceptive neurons in Vc increased their excitability following TMJ inflammation, as shown by an increase of background activity and heat response as well as by an enlargement of the receptive field size (Iwata et al., 1999). In comparison, Pa5 nociceptive neurons increased their background activity and cold response without an increase in the receptive field size, and they did not respond to heat. These findings suggest that Pa5 plays a role in processing mechanical and cold nociceptive information in the rats with TMJ inflammation and also may be involved in other function such as autonomic regulation.

Vagus afferent modulation of Pa5 neurons

To clarify the possible involvement of vagus afferents on modulation of Pa5 nociceptive neurons we studied the effect of vagus nerve transection on nocifensive behavior and the effect of electrical stimulation of the vagus nerve on Pa5 neuronal activity. It has been reported that vagus afferent fibers are involved in modulation of trigeminal nociceptive neurons and opioid-induced anti-nociception (Bereiter et al., 2002, 2005). In human and animal studies, low intensity vagus nerve stimulation produced reduction of the thermal pain threshold (Ren et al.,

1993; Ness et al., 2000). Furthermore, previous single neuron recording studies have noted that low intensity stimuli or rapid conducting pathways produce nociception, whereas high intensity stimuli or slow conducting pathways produce anti-nociception (Thurston and Randich 1995; Bossut and Maixner, 1996). We also observed that mechanical head withdrawal threshold was significantly decreased and head withdrawal latency to heat stimulation of the face was significantly longer and face grooming frequency was significantly higher at 10–14 days in the vagus nerve-transected rats with CFA injection compared with CFA-injected rats. These data suggest that sensory afferents in the vagus nerve are involved in modulation of the ascending pain pathways. In the present study, the stimulus intensity used was at a level above the small diameter Aδ fiber threshold (Hu et al., 1992). We observed strong inhibition of Pa5 nociceptive responses during electrical stimulation of the vagus nerve. These data suggest that slow conducting sensory fibers in the vagus nerve are activated by electrical stimulation used in the present study, and that these fibers contribute to depression of Pa5 nociceptive neuronal activities. The effect of vagus nerve transection on nocifensive behavior was also studied in TMJ-inflamed rats as illustrated in Fig. 1. We did not observe an acute change in nocifensive behavior in TMJ-inflamed rats as that seen in the rats with vagus nerve electrical stimulation. The discrepancy of vagus nerve inputs between single neuronal activity in Pa5 and nocifensive behavior data may be explained by the existence of multiple ascending pathways from the vagus nerve. Electrical stimulation of the vagus nerve may activate short ascending pathway to Pa5 from the vagus nerve, whereas the vagus nerve transection affects a variety of ascending pathways such as those through the trigeminal spinal subnucleus caudalis and nucleus tractus solitarii. These may cause different modulation of Pa5 neuronal activity and nocifensive behavior following vagus nerve stimulation and transection in TMJ-inflamed rats.

Concluding remarks

The present study demonstrated that TMJ-inflamed rats show a decrease of the head withdrawal threshold to mechanical stimulation of the lateral face, a shortening of the head withdrawal latency to heat stimulation, and an increase of face grooming after cold stimulation of the lateral face in the TMJ-inflamed rats, suggesting that mechanical and thermal allodynia occurs in the rats with TMJ inflammation. The change in nocifensive behavior in TMJ-inflamed rats was gradually decreased at 10–14 days after CFA injection, whereas noxious escape threshold lasted longer in CFA-injected rats with vagus nerve transaction than vagus nerve-intact rats. Pa5 WDR and NS neurons dominantly received inputs from mechanical and cold-sensitive afferents. Furthermore, the number of Fos-LI cells in Pa5 and Pa5 nociceptive neurons that received noxious information from the trigeminal region was significantly increased after TMJ inflammation. In addition, vagus nerve stimulation decreased the activity of Pa5 nociceptive neurons.

These data strongly suggest that Pa5 nociceptive neurons play an important role in relaying trigeminal nociceptive information to higher CNS regions, and that their activity is modulated by vagus afferents.

Acknowledgements

We thank Prof. Cools for correcting English grammar and language. This study was supported in part by Research grants from Sato and Uemura Funds from Nihon University School of Dentistry, and Grant from Dental Research Center, Nihon University School of Dentistry; Nihon University multidisciplinary research grant; a grant from the Ministry of Education, Culture, Sports, Science, and Technology to promote multidisciplinary research projects “Brain Mechanisms for Cognition, Memory and Behavior” and “Translational Research Network on Orofacial Neurological Disorders” at Nihon University, and Japan–Canada (CIHR) Joint Health Research Program. KR is supported by NIH grant DE 11964.

Abbreviations

Pa5 paratrigeminal

TMJ temporomandibular joint

TMD temporomandibular disorder

Vc trigeminal spinal subnucleus caudalis

Vi trigeminal spinal subnucleus interpolaris

Fos-LI cells Fos-like immunoreactive cells

WDR wide dynamic range

NS noxious specific

LTM low threshold mechano-receptive

CFA complete Freund's adjuvant

CNS central nervous system

PBN parabrachial nucleus

STN solitary tract nucleus

FG Fluorogold

PSTH peristimulus time histogram

References

Balan JA, Caous CA, Yu YG, Lindsey CJ. Barosensitive neurons in the rat tractus solitarius and paratrigeminal nucleus: a new model for medullary, cardiovascular reflex regulation. Can J Physiol Pharmacol 2004;82:474–484. [PubMed: 15389294]

Bereiter DA, Bereiter DF, Ramos M. Vagotomy prevents morphine-induced reduction in Fos-like immunoreactivity in trigeminal spinal nucleus produced after TMJ injury in a sex-dependent manner. Pain 2002;96:205–213. [PubMed: 11932076]

Bereiter DA, Okamoto K, Bereiter DF. Effect of persistent monoarthritis of the temporomandibular joint region on acute mustard oil-induced excitation of trigeminal subnucleus caudalis neurons in male and female rats. Pain 2005;117:58–67. [PubMed: 16043292]

Bereiter DA, Cioffi JL, Bereiter DF, Zardeneta G, Milam SB. Local blockade of integrins in the temporomandibular joint region reduces Fos-positive neurons in trigeminal subnucleus caudalis of female rats produced by jaw movement. Pain 2006;125:65–73. [PubMed: 16762506]

Bush FM, Whitehill JM, Martelli MF. Pain assessment in temporomandibular disorders. Cranio 1989;7:137–143. [PubMed: 2611904]

Bossut DF, Maixner W. Effect of cardiac vagus afferent electrostimulation on the responses of trigeminal and trigeminothalamic neurons to noxious orofacial stimulation. Pain 1996;65:101–109. [PubMed: 8826496]

Boucher Y, Simons CT, Cuellar JM, Jung SW, Carstens MI, Carstens E. Activation of brain stem neurons by irritant chemical stimulation of the throat assessed by c-*fos* immunohistochemistry. Exp Brain Res 2003;148:211–218. [PubMed: 12520409]

Cairns BE, Sessle BJ, Hu JW. Evidence that excitatory amino acid receptors within the temporomandibular joint region are involved in the reflex activation of the jaw muscles. J Neurosci 1998;18:8056–8064. [PubMed: 9742172]

Caous CA, Buck HS, Lindsey CJ. Neuronal connections of the paratrigeminal nucleus: a topographic analysis of neurons projecting to bulber, pontine and thalamic nuclei related to cardiovascular, respiratory and sensory functions. Auton Neurosci Basic Clin 2001;94:14–24.

Carstens E, Saxe I, Ralph R. Brainstem neurons expressing c-Fos immunoreactivity following irritant chemical stimulation of the rat's tongue. Neuroscience 1995;69:939–953. [PubMed: 8596661]

Chandler MJ, Hobbs SF, Bolser DC, Foreman RD. Effect of vagus afferent stimulation on cervical spinothalamic tract neurons in monkeys. Pain 1991;44:81–87. [PubMed: 2038494]

Hains BC, Waxman SG. Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J Neurosci 2006;26:4308–4317. [PubMed: 16624951]

Hansdottir R, Bakke M. Joint tenderness, jaw opening, chewing velocity, and bite force in patients with temporomandibular joint pain and matched healthy control subjects. J Orofacial Pain 2004;18:108–113. [PubMed: 15250430]

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988;32:77–78. [PubMed: 3340425]

Hathaway CB, Hu JW, Bereiter DA. Distribution of Fos-like immunoreactivity in the caudal brainstem of the rat following noxious chemical stimulation of the temporomandibular joint. J Comp Neurol 1995;356:444–456. [PubMed: 7642805]

Hu JW, Sessle BJ, Raboisson P, Dallel R, Woda A. Stimulation of craniofacial muscle afferents induces prolonged facilitatory effects in trigeminal nociceptive brain-stem neurones. Pain 1992;48:53–60. [PubMed: 1738575]

Imbe H, Ren K. Orofacial deep and cutaneous tissue inflammation differentially upregulates preprodynorphin mRNA in the trigeminal and paratrigeminal nuclei of the rat. Mol Brain Res 1999;67:87–97. [PubMed: 10101236]

Imbe H, Iwata K, Zhou QQ, Zou S, Dubner R, Ren K. Orofacial deep and cutaneous tissue inflammation and trigeminal neuronal activation. Implications for persistent temporomandibular pain. Cells Tissues Organs 2001;169:238–247. [PubMed: 11455119]

Iwata K, Kanda K, Tsuboi Y, Kitajima K, Sumino R. Fos induction in the medullary dorsal horn and C1 segment of the spinal cord by acute inflammation in aged rats. Brain Res 1995;678:127–139. [PubMed: 7620882]

Iwata K, Tashiro A, Tsuboi Y, Imai T, Sumino R, Morimoto T, Dubner R, Ren K. Medullary dorsal horn neuronal activity in rats with persistent temporomandibular joint and perioral inflammation. J Neurophysiol 1999;82:1244–1253. [PubMed: 10482744]

Jacquin MF, Chiaia NL, Rhoades RW. Trigeminal projections to contralateral dorsal horn: central extent, peripheral origins, and plasticity. Somatosens Mot Res 1990;7:153–183. [PubMed: 2378191]

Koepp J, Lindsey CJ, Motta EM, Rae GA. Role of the paratrigeminal nucleus in nocifensive responses of rats to chemical, thermal and mechanical stimuli applied to the hind paw. Pain 2006;122:235–244. [PubMed: 16616416]

Korszun A, Hinderstein B, Wong M. Comorbidity of depression with chronic facial pain and temporomandibular disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:496–500. [PubMed: 8936511]

Korszun A, Young EA, Singer K, Carlson NE, Brown MB, Crofford L. Basal circadian cortisol secretion in women with temporomandibular disorders. J Dent Res 2002;81 (4):279–283. [PubMed: 12097314]

Lam DK, Sessle BJ, Cairns BE, Hu JW. Neural mechanisms of temporomandibular joint and masticatory muscle pain: a possible role for peripheral glutamate receptor mechanisms. Pain Res Manag 2005;10:145–152. [PubMed: 16175250]

Lapa RCRS, Watanabe I. Synaptic contacts established by inferior alveolar nerve fibres in the paratrigeminal nucleus: an electron microscopic study in the rat. Arch Oral Biol 2005;50:73–79. [PubMed: 15598419]

Ness TJ, Fillingim RB, Randich A, Backensto EM, Faught E. Low intensity vagal nerve stimulation lowers human thermal pain thresholds. Pain 2000;86:81–85. [PubMed: 10779664]

Ogawa A, Ren K, Tsuboi Y, Morimoto T, Sato T, Iwata K. A new model of experimental parotitis in rats and its implication for trigeminal nociception. Exp Brain Res 2003;152:307–316. [PubMed: 12898097]

Oliveira MC, Parada CA, Veiga MC, Rodrigues LR, Barros SP, Tambeli CH. Evidence for the involvement of endogenous ATP and P2X receptors in TMJ pain. Eur J Pain 2005;9:87–93. [PubMed: 15629879]

Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates. Vol. 2. Academic Press; New York: 1986.

Quinn JH, Bazan NG. Identification of prostaglandin E2 and leukotriene B4 in the synovial fluid of painful, dysfunctional temporomandibular joints. J Oral Maxillofac Surg 1990;48:968–971. [PubMed: 2168477]

Randich A, Gebhart GF. Vagal afferent modulation of nociception. Brain Res Rev 1992;17:77–99. [PubMed: 1327371]

Ren K, Zhuo M, Randich A, Gebhart GF. Vagal afferent stimulation-produced effects on nociception in capsaicin-treated rats. J Neurophysiol 1993;69:1530–1540. [PubMed: 8389827]

Roveroni RC, Parada CA, Cecilia M, Veiga FA, Tambeli CH. Development of a behavioral model of TMJ pain in rats: the TMJ formalin test. Pain 2001;94:185–191. [PubMed: 11690732]

Rutherfurd SD, Widdop RE, Sannajust F, Louis WJ, Gundlach AL. Expression of c-*fos* and NGFI-A messenger RNA in the medulla oblongata of the anaesthetized rat following stimulation of vagal and cardiovascular afferents. Brain Res Mol Brain Res 1992;13:301–312. [PubMed: 1320720]

Sato T, Kitagawa J, Ren K, Tanaka H, Tanabe A, Watanabe T, Mitsuhashi Y, Iwata K. Activation of trigeminal intranuclear pathway in rats with temporomandibular joint inflammation. J Oral Sci 2005;47:65–69. [PubMed: 16050485]

Schaible HG, Schmidt RF. Time course of mechanosensitivity changes in articular afferents during a developing experimental arthritis. J Neurophysiol 1988;60:2180–2195. [PubMed: 3236065]

Schellhas KP, Wilkes CH, Baker CC. Facial pain, headache, and temporomandibular joint inflammation. Headache 1989;29:229–232. [PubMed: 2714973]

Shimizu K, Asano M, Kitagawa J, Ogiso B, Ren K, Oki H, Matsumoto M, Iwata K. Phosphorylation of extracellular signal-regulated kinase in medullary and upper cervical cord neurons following noxious tooth pulp stimulation. Brain Res 2006;1072:99–109. [PubMed: 16442086]

Suwanprathes P, Ngu M, Ing A, Hunt G. c-Fos immunoreactivity in the brain after esophageal acid stimulation. Am J Med 2003;115:31S–38S. [PubMed: 12928072]

Tashiro A, Okamoto K, Milam SB, Bereiter DA. Differential effects of estradiol on encoding properties of TMJ units in laminae I and V at the spinomedullary junction in female rats. J Neurophysiol 2007;98:3242–3253. [PubMed: 17928557]

Thurston CL, Randich A. Responses of on and off cells in the rostral ventral medulla to stimulation of vagal afferents and changes in mean arterial blood pressure in intact and cardiopulmonary deafferented rats. Pain 1995;62:19–38. [PubMed: 7478705]

Ushiyama T, Nakatsu T, Yamane S, Tokutake H, Wakabayashi H, Ishimura K, Maeta H. Heart rate variability for evaluating surgical stress and development of postoperative complications. Clin Exp Hypertens 2008;30:45–55. [PubMed: 18214733]

Yu YG, Lindsey CJ. Baroreceptor-sensitive neurons in the rat paratrigeminal nucleus. Auton Neurosci Basic Clin 2003;105:25–34.

Yu YG, Caous CA, Balan AC, Rae GA, Lindsey CJ. Cardiovascular responses to sciatic nerve stimulation are blocked by paratrigeminal nucleus lesion. Auton Neurosci Basic Clin 2002;98:70–74.

Xie YF, Zhang S, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ. Involvement of glia in central sensitization in trigeminal subnucleus caudalis (medullary dorsal horn). Brain Behav Immun 2007;21:634–641. [PubMed: 17055698]

Zhou QQ, Imbe H, Dubner R, Ren K. Persistent Fos protein expression after orofacial deep or cutaneous tissue inflammation in rats: implications for persistent orofacial pain. J Comp Neurol 1999;412:276–291. [PubMed: 10441756]

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110. [PubMed: 6877845]

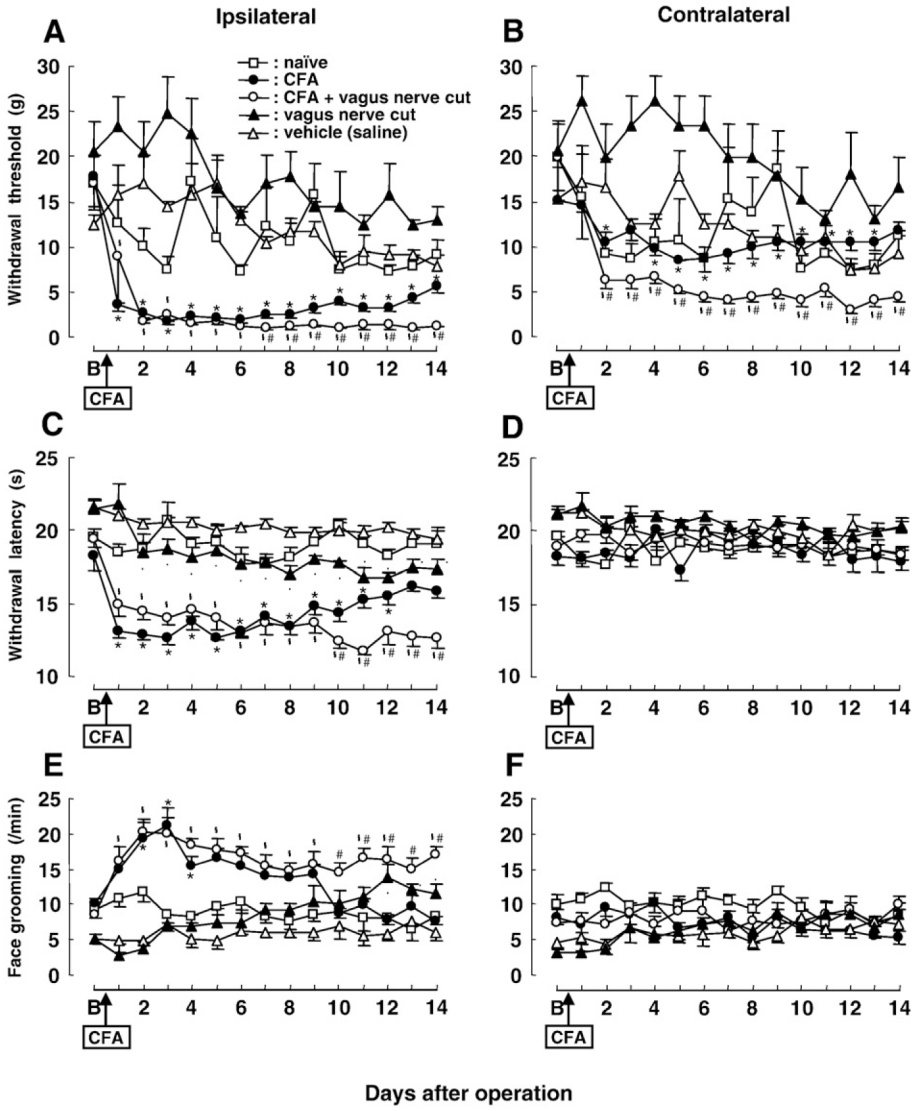

Fig. 1.

The mechanical head withdrawal threshold, heat head withdrawal latency and the number of face grooming events after cold stimulation in TMJ-untreated naive rats, TMJ-vehicle (saline) injected rats, TMJ-CFA-injected rats and TMJ-CFA injected rats with vagus nerve transection (CFA+vagus nerve cut). Mechanical (A) ipsilateral side to CFA injection and (B) contralateral side to CFA injection), heat (C) ipsilateral side to CFA injection and (D) contralateral side to CFA injection) or cold stimulus (E) ipsilateral side to CFA injection and (F) contralateral side to CFA injection) was applied to the lateral face and head withdrawal threshold to mechanical stimulation, head withdrawal latency to heat stimulation and face grooming frequency to acetone application (/min) were measured. *, §, †: $p<0.05$ (vs. before injection). #: $p<0.05$ (CFA vs. CFA+vagus nerve cut).

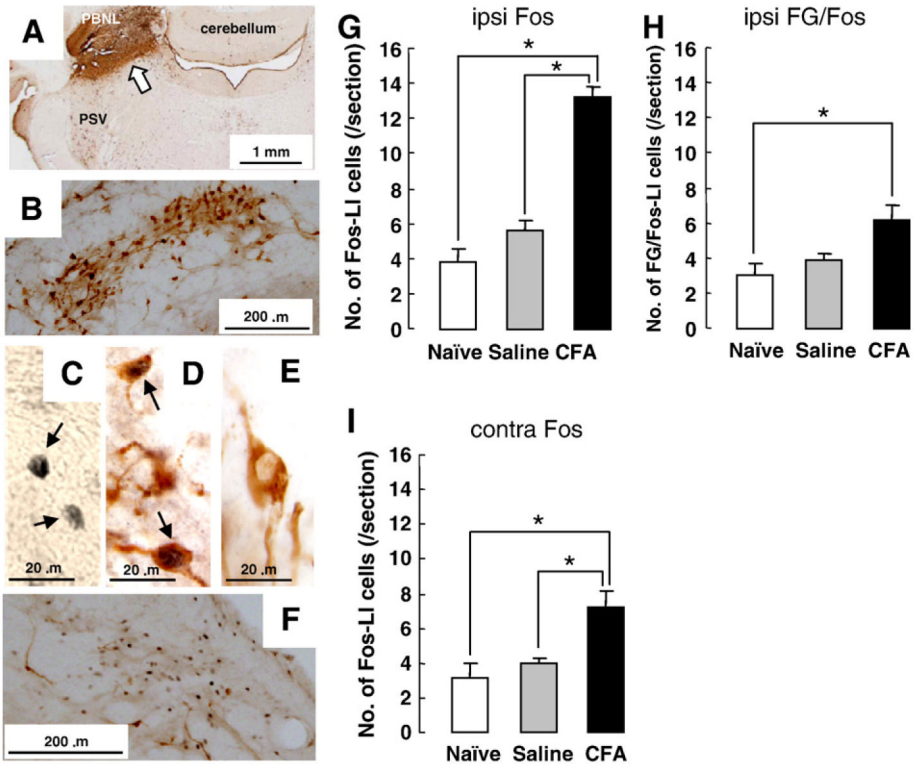

Fig. 2.

Fos-LI cells in the Pa5 after CFA or saline injection into the TMJ. Fluorogold injection site (A), retrogradely labeled FG and Fos/FG positive cells (B, C. D, E and F) and the mean number of Fos-LI cells (G) ipsilateral side to CFA injection and (I) contralateral side to CFA injection), Fos/FG-LI cells in the ipsilateral side to CFA injection (H). A: FG injection site indicated by the arrow. B: Pa5 with Fos-LI cells and Fos/FG-LI cells in the ipsilateral side to TMJ CFA injection. C: Fos-LI cells in the ipsilateral side to TMJ CFA injection as indicated by the arrows. D: Fos/FG-LI cells in the ipsilateral side to TMJ CFA injection as indicated by the arrows. E: A FG-LI cell in the ipsilateral side to TMJ CFA injection. F: Fos-LI cells in the contralateral side to TMJ CFA injection. G: Mean number of Fos-LI cells in the ipsilateral side to TMJ CFA injection. H: Mean number of FG/Fos-LI cells in the ipsilateral side to TMJ CFA injection. I: Mean number of Fos-LI cells in the contralateral side to TMJ CFA injection. PBNL: parabrachial nucleus lateralis, PSV: trigeminal spinal nucleus principalis. *: $p<0.05$(vs. TMJ-untreated or TMJ-vehicle, as indicated).

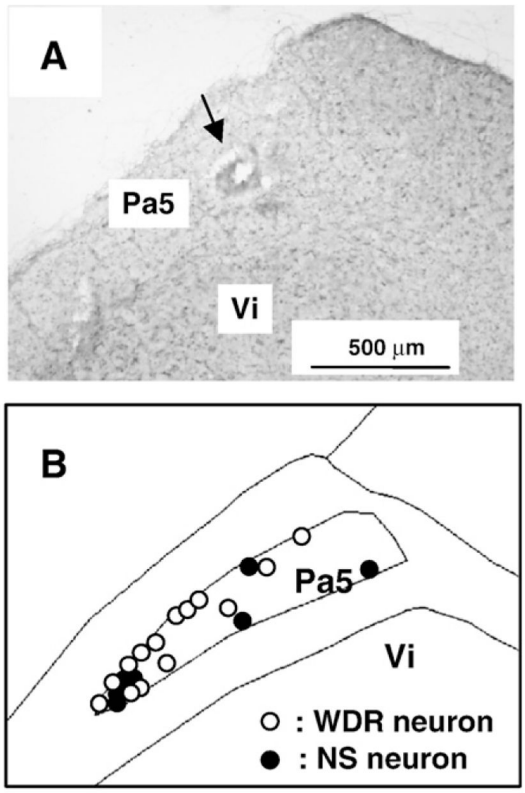

Fig. 3.
Location of the recording sites in Pa5 ipsilateral to the CFA injection side. (A) A photomicrograph of the Pa5 with a lesion indicated by an arrow, (B) the camera lucida drawings of recording sites of WDR and NS neurons in Pa5.

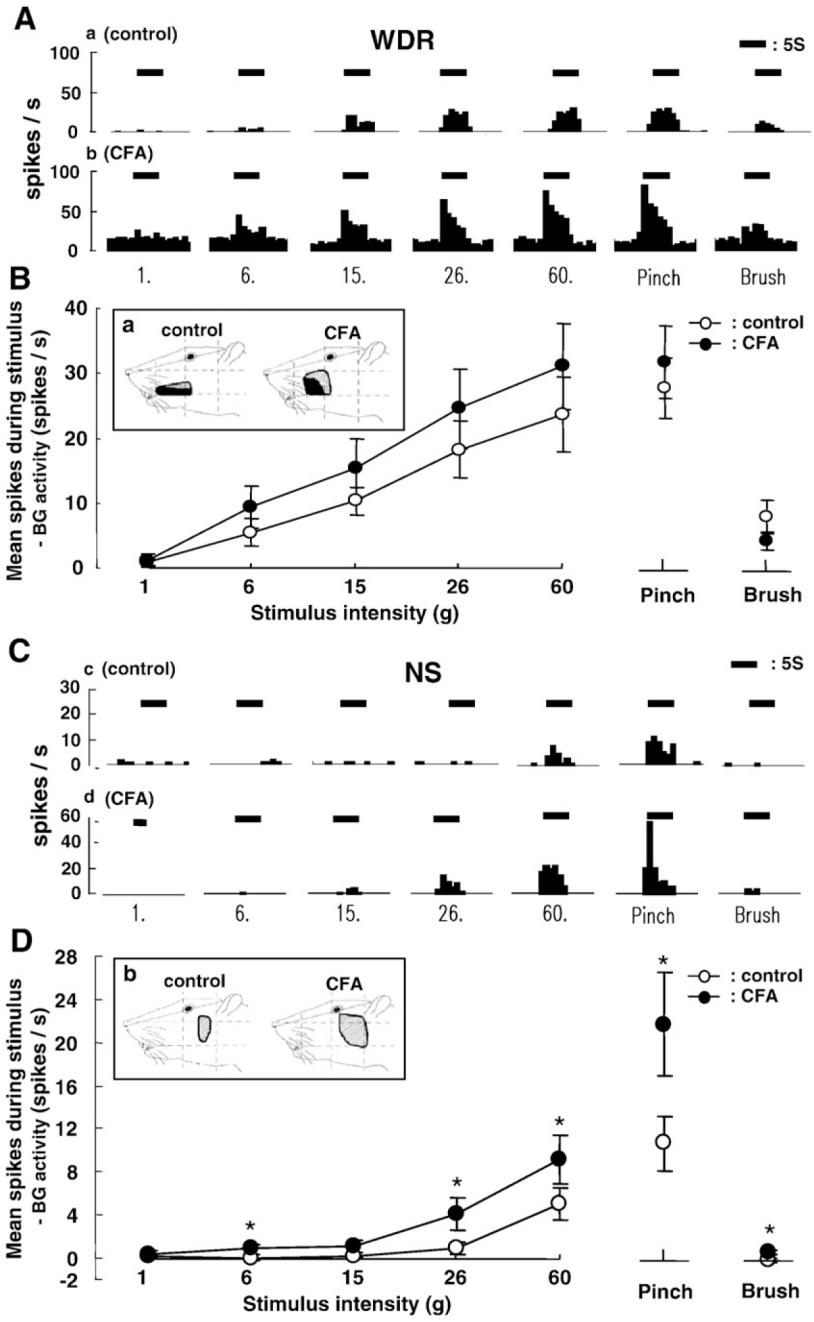

Fig. 4.

Mechanically evoked responses of WDR (A and B) and NS (C and D) neurons recorded from control (open circles) and TMJ-inflamed rats (solid circles). A and C: Typical mechanical stimulus-evoked responses of WDR and NS neurons in control (a) and TMJ-inflamed rats (b). B and D: Stimulus–response functions of WDR and NS neurons to graded mechanical stimulation in naive and TMJ-inflamed rats. Ba and Db: typical examples of RFs of WDR and NS neurons. *: $p<0.05$ (control vs. CFA).

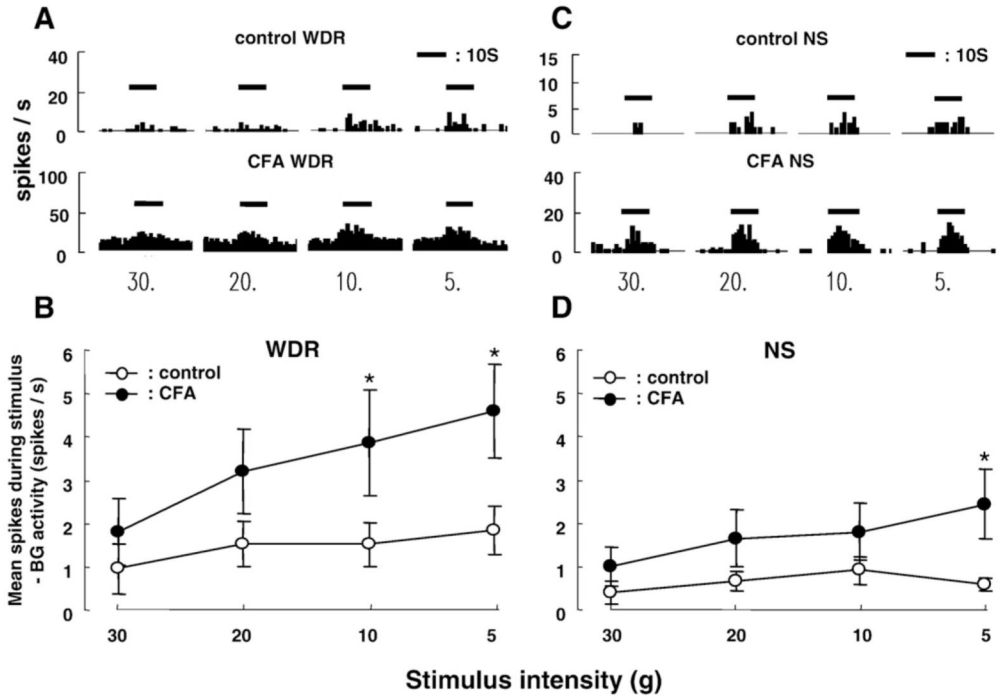

Fig. 5.

Cold-evoked responses of WDR (A and B) and NS (C and D) neurons recorded from control (open circles) and TMJ-inflamed rats (solid circles). A and C: Typical responses of cold WDR (A) and NS (B) units following graded cold stimuli. B and D: Stimulus–response functions of cold WDR (B) and NS (D) units. *: $p<0.05$ (control vs. CFA).

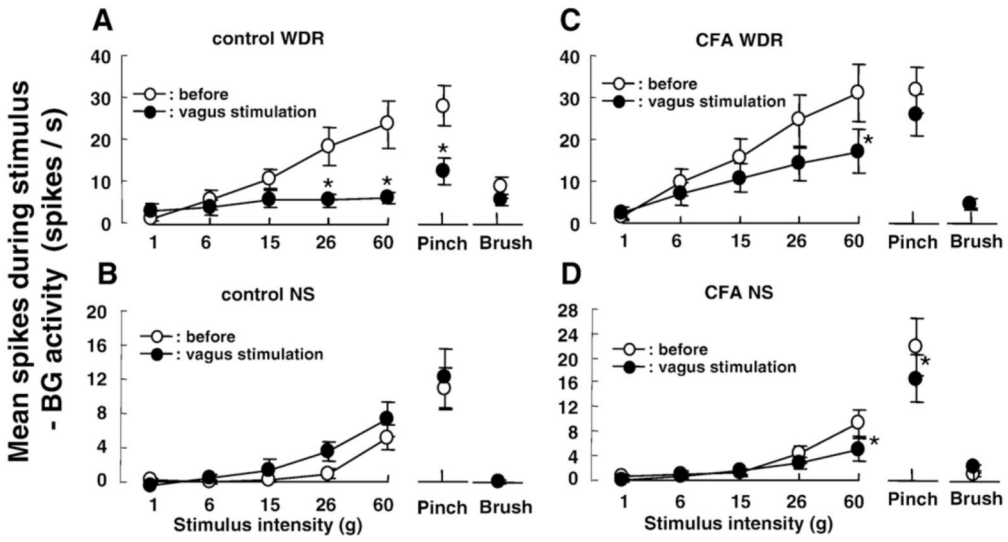

Fig. 6.

Effect of vagus nerve stimulation on mechanically evoked responses. Mechanically evoked responses of WDR (A and C) and NS (B and D) neurons following graded mechanical stimulation of the receptive field in control (A and B) and TMJ-inflamed rats (C and D). A and B: S–R functions from control rats. C and D: S–R functions from TMJ-inflamed rats. *: $p<0.05$ (before vs. during vagus nerve stimulation).

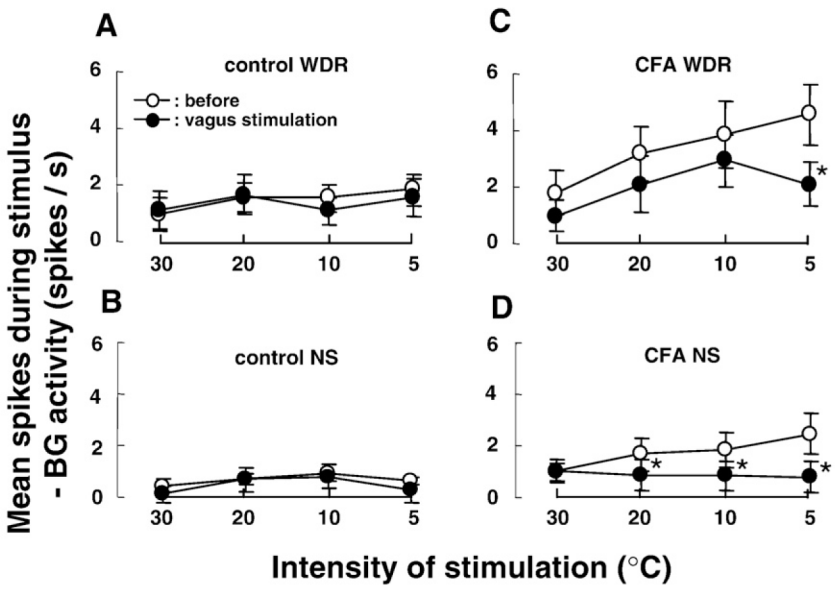

Fig. 7.

Effect of vagus nerve stimulation on cold-evoked responses. Stimulus–response functions of WDR (A and C) and NS (B and D) neurons following graded cold stimulation of the receptive field in control (A and B) and TMJ-inflamed rats (C and D). A and B: Stimulus–response functions from naive rats. C and D: Stimulus–response functions from TMJ-inflamed rats. *: $p<0.05$ (before vs. during vagus nerve stimulation).

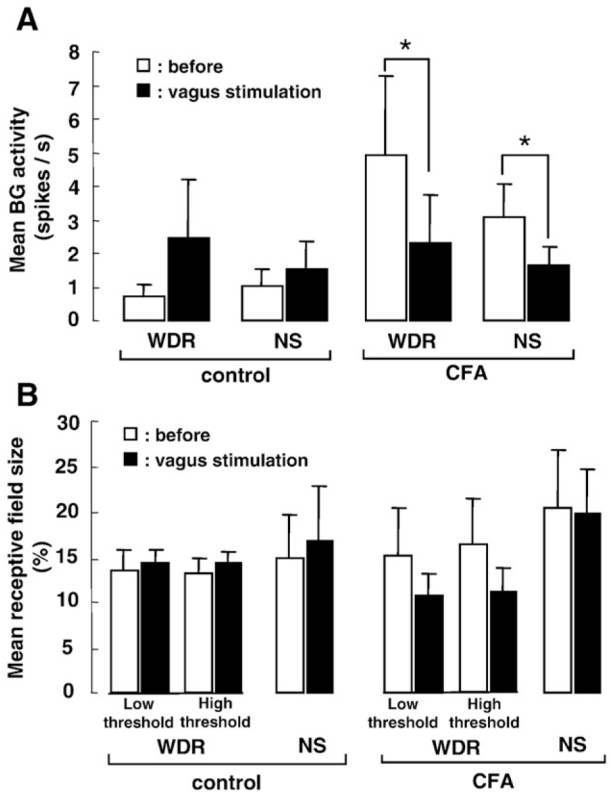

Fig. 8.

Effect of vagus nerve stimulation on background activity (A) and receptive field size (B). Note that the background activity in WDR and NS neurons was significantly depressed after vagus nerve stimulation in CFA-injected rats but not in control rats (A). *: $p<0.05$ (before vs. during vagus nerve stimulation).