TMJ Inflammation Increases Fos Expression in the Nucleus Raphe Magnus Induced by Subsequent Formalin Injection of the Masseter or Hindpaw of Rats

By

Sang-Hoon OH, Hiroki IMBE and Yasutomo IWAI-LIAO

Department of Oral Anatomy, Osaka Dental University, Kuzuhahanazono-cho 8-1, Hirakata-shi, 573-1121, Japan

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Summary: The study was designed to examine the effect of persistent temporomandibular joint (TMJ) inflammation on neuronal activation in the descending pain modulatory system in response to noxious stimulus. Formalin was injected into the left masseter muscle or hindpaw of rats 10 days after injection of the left TMJ with saline or complete Freund's adjuvant (CFA). The results showed that 10-day persistent TMJ inflammation (induced by CFA) alone did not induce a significant increase in Fos-like immunoreactive (Fos-LI) neurons in the rostral ventromedial medulla (RVM) or locus coeruleus (LC), but that formalin injection of the masseter muscle or hindpaw induced a significant increase in Fos-LI neurons in the RVM and LC of rats with and without TMJ inflammation ($P < 0.05$). However, persistent TMJ inflammation significantly increased Fos-LI neurons in the nucleus raphe magnus (NRM) induced by subsequent formalin injection of the masseter muscle and hindpaw (70.2% increase and 53.8% increase, respectively, over the control TMJ-saline-injected rats; $P < 0.05$). The results suggest that persistent TMJ inflammation increases neuronal activity, in particularly in the NRM, by the plastic change of the descending pain modulatory system after ipsilateral application of a noxious stimulus to either orofacial area or a spatially remote body area.

Temporomandibular disorders (TMDs) cause various types of pain involving the masticatory muscles and temporomandibular joint¹. Clinical studies have described TMD patients having generalized hyperalgesia beyond the orofacial and neck regions^{2–4}. Not only a significantly lower pain threshold in the masticatory muscles has been found in TMD patients than in healthy persons, but dysfunction in the endogenous pain control system as well^{5,6}. A recent study has reported that TMD induces widespread neural changes in supraspinal structures involved in the central nociceptive pathways⁷.

Inflammation of the TMJ and masticatory muscles is likely to contribute to the induction and/or maintenance of persistent TMD pain^{8,9}. In earlier studies the authors injected complete Freund's adjuvant (CFA) into the rat TMJ and observed thermal and mechanical hyperalgesia of the facial skin ipsilateral to the inflamed TMJ that persisted for 2 weeks, and enhancement of orofacial nociceptive behavioural activity induced by subsequent nox-

ious stimulus was observed in the same TMJ inflammation model^{10–13}. There have been several studies on persistent TMJ inflammation, and they have shown an increase in expression of Fos, a protein product of the *c-fos* immediate early gene (IEG), and opioid gene accompanied by neuronal hyperexcitability in the spinal trigeminal nucleus (Vsp)^{10,11,14–16}. Thus, the results of these studies have suggested that central neuronal changes play a critical role in the induction and maintenance of persistent orofacial pain in TMJ inflammation.

A study on chronic TMJ monoarthritis of rats described a subsequent mustard oil injection of the TMJ contralateral to inflammation led to increased excitability of trigeminal brainstem neurons¹⁷. Another study reported that nociceptive behaviours evoked by formalin injection of the hindpaw of rats were exaggerated by chronic TMJ inflammation¹⁸. Although the studies indicated that the facilitation of nociceptive responses in spatially remote body areas by chronic TMJ inflammation could be explained by a mechanism of sensitization

of supraspinal structures in the descending pain modulatory system, how the inflammation affects modulatory neuronal activity following a noxious stimulus was not elucidated.

The rostral ventromedial medulla (RVM), which contains the nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis pars alpha (GiA), and the locus coeruleus (LC) have been found to play crucial roles in the descending pain modulatory system^{19–21}. Moreover, one study indicated that electrical or chemical stimulation of the RVM arouses biphasic (facilitatory and inhibitory) modulation of spinal nociceptive transmission²². Since the RVM has been demonstrated to contribute to the maintenance of inflammatory pain and neuropathic pain, a great deal of attention has been focused on the RVM^{23,24}.

Fos expression excited by various stimuli has been widely used to assess neuronal activation, and is known to contribute to the establishment of long-term functional changes in the central nervous system^{25,26}. Some previous studies have presented histological evidence that formalin injection of the hindpaw or face of rats induces Fos expression in the RVM and LC^{27,28}. Furthermore, Fos expression in NRM serotonergic neurons was assumed to activate the descending pathways involved in behavioural hyperalgesia²⁹.

In the present study we investigated the effects of persistent TMJ inflammation on Fos expression in supraspinal structures evoked by formalin injection of the masseter muscle or hindpaw, in order to study plastic neuronal changes in the descending pain modulatory system in TMD patients.

Materials and Methods

1. Animal preparation

Male Sprague-Dawley rats (150–250 g body weight; Japan SLC, Shizuoka, Japan) were used in this study. The protocol (No. 06-02016: Identification of the mechanism of secondary hyperalgesia in TMJ chronic inflammation model) was approved by the Ethics Review Board of Osaka Dental University and implemented in accordance with the ethical guidelines for the treatment of animals of the International Association for the Study of Pain³⁰.

2. TMJ inflammation

Complete Freund's adjuvant (*Mycobacterium tuberculosis*, Calbiochem, CA, USA) suspended in an oil:saline (1:1) emulsion was used as the inflammatory agent. Orofacial thermal hyperalgesia and mechanical allodynia, peaking at 1 day and persisting for 2 weeks after CFA injection into the

rat TMJ, were observed as described in previous studies^{10,11}. Histologically, acute inflammation occurred in soft tissues around the TMJ at 6 hours after the CFA injection and shifted to a chronic inflammation phase at day 10³¹. We also observed that Fos expression in the Vsp peaked between day 1 and day 3 and gradually recovered by day 10 after the CFA injection, as reported in a previous study¹⁵. The increased nocifensive effects evoked by formalin injection of the masseter muscle and hindpaw were observed at 7–10 days after CFA injection^{12,13,18,32}.

3. Experimental groups

Rats were divided into the following 6 groups (Fig. 1):

- 1) A TMJ-saline group (n = 4), anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and in which the left TMJ was injected with saline (0.05 mL) 10 days before sacrifice.
- 2) A TMJ-CFA group (n = 4), anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and in which the left TMJ was injected with CFA (0.05 mL, 0.025 mg) 10 days before sacrifice.
- 3) A TMJ-saline + Masseter-formalin group (n = 4), in which the mid-region of the left masseter muscle was injected with 5% formalin (0.05 mL) 10 days after injection of the left TMJ with saline.
- 4) A TMJ-CFA + Masseter-formalin group (n = 5), in which the left masseter muscle was injected with formalin 10 days after injection of the left TMJ with CFA.
- 5) A TMJ-saline + Hindpaw-formalin group (n = 4), in which the left hindpaw was injected with formalin 10 days after saline injection of the left TMJ.
- 6) A TMJ-CFA + Hindpaw-formalin group (n = 4), in which the left hindpaw was injected with formalin 10 days after CFA injection of the left TMJ.

4. Tissue preparation and immunohistochemical analysis

The rats were euthanized with CO₂ gas 2 h after formalin injection of the masseter or hindpaw and 10 days after the saline or CFA injection. They were then transcardially perfused with 100 mL of saline solution followed by 500 mL of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB) at pH 7.4. The brainstems were removed, placed in the same fixative overnight at 4°C, and then transferred to 30% sucrose (w/v) in 0.1 M PB for 3 days for tissue cryoprotection. The brainstem were cut into 30 µm sections at –20°C with a cryostat, and then serially transferred to multi-well tissue culture

Fig. 1. Experimental design of the TMJ-saline, TMJ-CFA, TMJ-saline + Masseter-formalin, TMJ-CFA + Masseter-formalin, TMJ-saline + Hindpaw-formalin and TMJ-CFA + Hindpaw-formalin groups. Saline or CFA was injected into the TMJ 10 days prior to formalin injection (5%, 0.05 mL) of the masseter muscle or hindpaw.

plates containing 0.1 M Tris-buffered saline (TBS). Every sixth section (between -8.96 mm and -11.30 mm from the bregma) of each brainstem was collected and processed for immunohistochemical study and analysis.

Free-floating sections were washed for 30 min with TBS containing 0.1% Triton X-100 (TBST), and after incubation in 1% hydrogen peroxide in TBST for 30 min followed by another three washes with TBST, they were incubated for 1 h at room temperature (RT) in TBST containing 3% bovine serum albumin (BSA). The sections were incubated with the primary antibody (rabbit polyclonal antibody against c-Fos, Ab-5, dilution 1:20000, Oncogene Research products, CA, USA) in TBST with 3% BSA for 24 h at 4°C . After incubation, sections were washed again in TBST and then incubated for 1 h at RT with the biotinylated secondary antibody (goat anti-rabbit, Vector Laboratories, CA, USA). The sections were then washed twice in TBST, incubated with avidin-biotin-peroxidase complex (Elite ABC kit, Vector Laboratories) for 1 h at RT, and washed again in TBST. The horseradish peroxidase reaction was developed in TBS (pH 7.5) containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride, 0.2% nickel sulfate and 0.01% hydrogen peroxide. The control sections processed without primary antibody incubation were not stained. The brainstem sections were mounted on slides, air-dried, dehydrated in a graded ethanol series, cleared in xylene, and cover-slipped.

The number of Fos-like immunoreactive (Fos-LI) neurons in the nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis pars alpha (GiA), ventral nucleus reticularis gigantocellularis (Gi), and nucleus raphe pallidus (NRP) in 10 sections (between -9.68 mm and -11.30 mm from the bregma) of each brainstem was counted under a light microscope. Fos-LI neurons in the locus coeruleus (LC) in 10 sections (between -8.96 mm and -10.58 mm from the bregma) of each brainstem were also counted. Nuclear and laminar boundaries were defined according to a cytoarchitectonic atlas and other previous studies^{33,34}. The total number of Fos-LI neurons within the designated rostrocaudal levels (by means $\pm$ S.E.M.) in the brainstem nuclei of each experimental group were statistically analyzed by ANOVA with Fisher's PLSD for inter-group comparisons ($p < 0.05$).

Results

1. Fos-LI expression

We observed very few Fos-LI neurons in the RVM or LC at day 10 after injection of the TMJ with saline or CFA (Fig. 2: B, G and L; Fig. 2: C, H and M). Formalin injection of the masseter muscle in both the presence and absence of chronic TMJ inflammation induced an increase in Fos-LI neurons, particularly in the NRM of the RVM (Fig. 2:

Fig. 2. Schematic illustrations of the rostral VLM (A; bregma -10.30 mm), caudal VLM (F; bregma -11.00 mm) and LC (K; bregma -10.04 mm). Photomicrographs showing Fos-LI neurons in the rostral VLM (B, C, D and E corresponding to the boxed area in A), caudal VLM (G, H, I and J corresponding to the boxed area in F) and LC (L, M, N and O corresponding to the boxed area in K). Histological findings in the TMJ-saline group (B, G and L), TMJ-CFA group (C, H and M), TMJ-saline + Masseter-formalin group (D, I and N) and TMJ-CFA + Masseter-formalin group (E, J and O) (7: facial nucleus, 7n: facial nerve, Gi: nucleus reticularis gigantocellularis, GiA: nucleus reticularis gigantocellularis pars alpha, LPGi: nucleus lateralis paragigantocellularis, NRM: nucleus raphe magnus, NRP: nucleus raphe pallidus, LC: locus coeruleus; Scale bars = $100\ \mu\text{m}$) indicate the presence of few Fos-LI neurons in the VLM at day 10 after saline (B, G and L) or CFA (C, H and M) injection of the TMJ. However, formalin injection of masseter muscle induces a significant increase in Fos-LI neurons, in particular in the NRM, in the rats with TMJ inflammation (E and J) compared to the rats without TMJ inflammation (D and I).

D and I; Fig. 2: E and J). More Fos-LI neurons were observed in the NRM of the TMJ-CFA + Masseter-formalin group (Fig. 2: E and J) than in the TMJ-saline + Masseter-formalin group (Fig. 2: D and I). On the other hand, there was a marked increase in Fos-LI neurons in the LC of the groups without and

with chronic TMJ inflammation after formalin injection of masseter muscle (Fig. 2: N and O). However, no significant difference in numbers of Fos-LI neurons in the LC was observed between the TMJ-CFA + Masseter-formalin group and the TMJ-saline + Masseter-formalin group.

Fig. 3. Effect of chronic TMJ inflammation on Fos expression in the RVM and LC induced by formalin injection of masseter muscle (A: NRM, B: GiA, C: Gi, D: NRP, E: LC; values shown are means $\pm$ S.E.M.). The letter "a" indicates a significant difference between the TMJ-saline group and TMJ-saline + Masseter-formalin group or between the TMJ-CFA group and TMJ-CFA + Masseter-formalin group ($p < 0.05$). The letter "b" indicates a significant difference between the TMJ-saline + Masseter-formalin group and the TMJ-CFA + Masseter-formalin group ($p < 0.05$).

We observed that the histological findings in the TMJ-saline + Hindpaw-formalin group and TMJ-CFA + Hindpaw-formalin group (not shown) were similar to the histological findings in the TMJ-saline + Masseter-formalin group (Fig. 2: D, I and N) and TMJ-CFA + Masseter-formalin group (Fig. 2: E, J and O), respectively.

2. Analysis of the effect of TMJ-CFA inflammation on Fos-LI in the brainstem

Comparison of the Fos-LI counts in brainstem nuclei revealed that CFA or saline injection of the TMJ induced similar Fos-LI in the TMJ-CFA group (Figs. 3A–E: dotted bars; Table 1) and TMJ-saline group (Fig. 3: A–E: open bars; Table 1). By contrast,

Table 1. Effect of persistent TMJ inflammation on induction of Fos expression in nuclei by formalin injection of the masseter muscle

	TMJ-saline group	TMJ-CFA group	TMJ-saline + Masseter-formalin group	TMJ-CFA + Masseter-formalin group
NRM	17.5 ± 7.1	22.0 ± 3.8	145.8 ± 25.1 ^a	248.2 ± 31.8 ^{ab}
GiA	22.0 ± 3.0	22.5 ± 1.6	84.0 ± 21.0 ^a	113.0 ± 22.9 ^a
Gi	4.8 ± 3.8	3.5 ± 1.4	11.0 ± 4.7	32.4 ± 8.0 ^{ab}
NRP	16.8 ± 1.9	17.0 ± 4.1	71.8 ± 14.0 ^a	90.8 ± 24.8 ^a
LC	19.3 ± 5.2	41.5 ± 10.7	152.8 ± 33.2 ^a	173.2 ± 28.7 ^a

Total numbers of Fos-LI neurons in the TMJ-saline group, TMJ-CFA group, TMJ-saline + Masseter-formalin group and TMJ-CFA + Masseter-formalin group are expressed as means ± S.E.M. The letter “a” indicates a significant difference between the TMJ-saline + Masseter-formalin group and TMJ-saline group or between the TMJ-CFA + Masseter-formalin group and TMJ-CFA group ($p < 0.05$). The letter “b” indicates a significant difference between the TMJ-CFA + Masseter-formalin group and TMJ-saline + Masseter-formalin group ($p < 0.05$).

an increase in Fos-LI counts of 733.1% in the NRM, 281.8% in GiA, 327.4% in NRP and 691.7% in the LC was observed in the TMJ-saline + Masseter-formalin group compared to the TMJ-saline group (Table 1). However, no significant differences in total number of Fos-LI neurons in the Gi were found between the TMJ-saline + Masseter-formalin group (Fig. 3C: meshed bar; Table 1) and TMJ-saline group (Fig. 3C: open bar; Table 1). Comparison of the Fos-LI neuron counts revealed significantly more Fos-LI neurons in the NRM of the TMJ-CFA + Masseter-formalin group than that of the TMJ-saline + Masseter-formalin group (Fig. 3A; Table 1: 248.2 ± 31.8 vs. 145.8 ± 25.1), and the Gi contained significantly more Fos-LI neurons in the TMJ-CFA + Masseter-formalin group than in the TMJ-saline + Masseter-formalin group (Fig. 3C; Table 1: 32.4 ± 8.0 vs. 11.0 ± 4.7). The results indicated that persistent TMJ inflammation induced an increase in Fos-LI neurons in the NRM and Gi, 70.2% and 194.5%, respectively, in rats exposed to second noxious stimulus of the masseter muscle (Fig. 3: A and C; closed bars vs. meshed bars; Table 1).

There were 874.3%, 369.5%, 340.5% and 550.3% increase in Fos-LI counts in the NRM, GiA, NRP and the LC, respectively, of the TMJ-saline + Hindpaw-formalin group than that of the TMJ-saline control group (Fig. 4: A, B, D and E; meshed bars vs. open bars; Table 2). No significant difference in Fos-LI counts in the Gi was found between the TMJ-saline + Hindpaw-formalin group and TMJ-saline group (Fig. 4C; meshed bar vs. open bar; Table 2). On the other hand, comparison of the Fos-LI neuron counts revealed a significant difference in Fos-LI neurons in the NRM between the TMJ-CFA + Hindpaw-formalin group (262.3 ± 43.0) and TMJ-saline + Hindpaw-formalin groups (170.5 ± 25.2). There was a 53.8% increase in Fos-LI neurons in the NRM of the rats with persistent TMJ inflammation and exposed to a sec-

ond noxious stimulus of the hindpaw, but no significant increase was found in the Fos-LI neurons in the Gi (Fig. 4: A and C; closed bars vs. meshed bars; Table 2).

Discussion

Studies on inflammation have demonstrated that deep craniofacial tissue inflammation caused neuronal plastic changes in both the PNS and CNS^{14,15,35–37}. Peripheral and central sensitization increases the responsiveness and reduces the response threshold of peripheral and central sensory neurons, and contribute to inflammatory hyperalgesia and allodynia. Since the TMJ is innervated by branches of the masseteric nerve, which originates from the mandibular division of the trigeminal nerve, persistent TMJ inflammation causes peripheral sensitization of nerve fibres distributed to the masseter muscle^{12,13,38}. A recent study reported that TMJ inflammation increases nociceptive behaviours induced by formalin injection of the hindpaw of rats, and suggested that inflammation induces central sensitization of supraspinal structures¹⁸. Consistent with the results of the behaviour studies, the present study found that persistent TMJ inflammation increased the number of Fos-LI neurons induced by subsequent formalin injection of the masseter muscle or the hindpaw, especially in the NRM. The results of this study suggested that chronic TMJ inflammation gave rise to central sensitization in the descending pain modulatory system as what have been described in some previous clinical studies^{4,6,7}.

Although some studies have demonstrated that Fos expression in the dorsal horn induced by noxious stimulus was modulated by another stimulus previously applied to a spatially remote body areas, how the supraspinal structures being affected was not mentioned^{17,39–41}. However, re-application of

Fig. 4. Effect of persistent TMJ inflammation on Fos expression in the RVM and LC induced by formalin injection of the hindpaw (A: NRM, B: GiA, C: Gi, D: NRP, E: LC; values shown are means $\pm$ S.E.M.). The letter "a" indicates a significant difference between the TMJ-saline group and the TMJ-saline + Hindpaw-formalin group or between the TMJ-CFA group and the TMJ-CFA + Hindpaw-formalin group ($p < 0.05$). The letter "b" indicates a significant difference between the TMJ-saline + Hindpaw-formalin group and the TMJ-CFA + Hindpaw-formalin group ($p < 0.05$).

a same stimulus 2 weeks after inescapable foot-shock induced a significant increase in Fos expression in the agranular insular cortex, frontal cortex, basolateral amygdala, CA1 area of the hippocampus, paraventricular hypothalamic nucleus and LC, which are supraspinal structures involved in nociceptive transmission, fear/anxiety, neuroendocrine and autonomic responses⁴²).

The neurons in the RVM were classified into three types in a tail flick reflex (TFL) study: on-cells, which discharge just prior to the TFL, off-cells, which shut off just prior to TFL, and neutral-cells, which do not show consistent changes in activity when TFL occurs. On-cell activity facilitates nociceptive behaviours, whereas off-cell activity inhibits them⁴³). On the other hand, a

Table 2. Effect of persistent TMJ inflammation on induction of Fos expression in nuclei by formalin injection of the hindpaw

	TMJ-saline group	TMJ-CFA group	TMJ-saline + Hindpaw-formalin group	TMJ-CFA + Hindpaw-formalin group
NRM	17.5 ± 7.1	22.0 ± 3.8	170.5 ± 25.2 ^a	262.3 ± 43.0 ^{ab}
GiA	22.0 ± 3.0	22.5 ± 1.6	103.3 ± 23.3 ^a	121.3 ± 19.3 ^a
Gi	4.8 ± 3.8	3.5 ± 1.4	19.5 ± 6.6	23.5 ± 6.1 ^a
NRP	16.8 ± 1.9	17.0 ± 4.1	74.0 ± 32.4 ^a	69.3 ± 6.2 ^a
LC	19.3 ± 5.2	41.5 ± 10.7	125.5 ± 32.9 ^a	139.0 ± 23.0 ^a

Total numbers of Fos-LI neurons in the TMJ-saline group, TMJ-CFA group, TMJ-saline + Hindpaw-formalin group and TMJ-CFA + Hindpaw-formalin group are expressed as means ± S.E.M. The letter "a" indicates a significant difference between the TMJ-saline + Hindpaw-formalin group and TMJ-saline group or between the TMJ-CFA + Hindpaw-formalin group and TMJ-CFA group ($p < 0.05$). The letter "b" indicates a significant difference between the TMJ-CFA + Hindpaw-formalin group and TMJ-saline + Hindpaw-formalin group ($p < 0.05$).

previous study that related the responses with paw-withdrawal-behaviour test after CFA-induced inflammation identified a significant increase (in percentage) in on-like and off-like cells but a decrease (in percentage) in neutral-like cells in the RVM neurons⁴⁴. Some studies have reported that formalin injection of the hindpaw and face of rats induces Fos expression in the RVM and LC^{27,28}. A study of CNS neuronal activity based on glucose utilization showed an increase in neuronal activity in the NRM, GiA, and LC in rats during CFA-induced monoarthritis⁴⁵. A recent study revealed that peripheral inflammation induces activation of extracellular signal-regulated kinases (ERKs), serine/threonine protein kinases play important roles in synaptic plasticity, and suggested that inflammation affects RVM neuronal activity⁴⁶. The results of the present study suggested that formalin injection of the masseter muscle or hindpaw increased the Fos expression in the NRM, in which increased neuronal reactivity may have been maintained by persistent TMJ inflammation.

It has been reported that Fos expression in NRM serotonergic neurons seems to be implicated in behavioural hyperalgesia, since 5HT released from the descending neurons in the NRM facilitates spinal nociception by activating 5HT₃ receptors^{29,47}. The results of the present study showed that a second noxious stimulus of the rat masseter or hindpaw differentially induced increase in Fos-LI expression in the RVM nuclei and indicated that Fos-LI expression, particularly in the NRM and Gi, was significantly enhanced by chronic TMJ inflammation. We therefore postulate that increased Fos expression in the NRM is involved in induction and maintenance of the hyperalgesia by spinal 5HT₃ receptors. In addition, a previous study on formalin injection of the rat hindpaw demonstrated that 5HT facilitated aversive responses via 5HT₃ receptors⁴⁸.

There is a large group of GiA neurons that in-

nervates and modulates NRM activity⁴⁹. The GiA contains on-cells that are specifically activated by peripheral noxious stimuli and triggered to release GABA onto off-cells to eliminate the tonic inhibition of dorsal horn neurons⁵⁰. There has another study demonstrated that microinjection of cholecystokinin (CCK-8) into the Gi of naïve rats caused allodynia and hyperalgesia⁵¹. By contrast, a chemical lesion of the Gi has been found to lead to attenuation of hyperalgesia and reduce inflammation-evoked spinal Fos expression²⁰. Furthermore, the Gi was assumed to be mainly involved in descending facilitation, because a Gi lesion restored a decreased descending inhibition of inflammation⁵². The results of the present study also showed an increased masseter-formalin-induced Fos-LI in the Gi of TMJ-inflamed rats, and we speculated that it was related to hyperalgesia via descending facilitation of inflammation. However, most of the RVM nuclei seemed to exert a biphasic modulatory effect of nociception, and the increase in formalin-evoked Fos-LI may be a secondary response that counteracts the behavioural hyperalgesia²².

The LC, and A5- and A7-group supraspinal structures contain major noradrenergic neurons that project to the dorsal horn. Electrical stimulation of the structures inhibits dorsal horn nociceptive neurons⁴³. A previous study that used the formalin test reported that long-term removal of spinal noradrenergic afferent axons had no significant effect on nocifensive behaviours⁵³. On the other hand, another study found that stressful stimuli increased Fos-LI neurons in the NRP and LC. The same study demonstrated that NRP neurons project to the intermediolateral cell column and eventually activate the adrenal medulla, whereas LC neurons project to the paraventricular hypothalamic nucleus and eventually activate the adrenal cortex²⁷. We speculated that formalin-evoked Fos-LI, in particular in the NRP and LC, of the

TMJ-inflamed and non-inflamed rats, might be closely related to the secondary autonomic response counteracting hyperalgesia.

In summary, based on the results of the present study it was concluded that persistent TMJ inflammation increases Fos-LI in the NRM induced by formalin injection of either the ipsilateral trigeminal system (masseter muscle) or a spatially remote body area (hindpaw). The results suggest that chronic TMJ inflammation increases neuronal activity, in particular in the NRM, after exposed to a second noxious stimulus by neuronal plastic change of the descending pain modulatory system.

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