Medullary Dorsal Horn Neuronal Activity in Rats with Persistent Temporomandibular Joint and Perioral Inflammation

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Iwata, Koichi, Akimasa Tashiro, Yoshiyuki Tsuboi, Takao Imai, Rhyuji Sumino, Toshifumi Morimoto, Ronald Dubner, and Ke Ren. Medullary dorsal horn neuronal activity in rats with persistent temporomandibular joint and perioral inflammation. J. Neurophysiol. 82: 1244–1253, 1999. Studies at spinal levels indicate that peripheral tissue or nerve injury induces a state of hyperexcitability of spinal dorsal horn neurons that participates in the development of persistent pain and hyperalgesia. It has not been demonstrated that persistent injury in the orofacial region leads to a similar state of central hyperexcitability in the trigeminal system. The purpose of the present study was to conduct a parametric analysis of the response properties of nociceptive and nonnociceptive neurons in trigeminal nucleus caudalis (medullary dorsal horn, MDH) in a rat model of persistent orofacial inflammation. Neurons were recorded extracellularly and classified as low-threshold mechanoreceptive (LTM), wide dynamic range (WDR, n = 82), and nociceptive-specific (NS, n = 11) neurons according to their response properties to mechanical stimuli applied to their cutaneous receptive fields (RFs). The inflammation was induced 24 h before the recordings by injecting complete Freund’s adjuvant (CFA) into the temporomandibular joint (TMJ) capsule or the perioral (PO) skin. The mean areas of the high-threshold RFs of WDR neurons in TMJ (8.66 ± 0.61 cm², n = 25) and PO (5.61 ± 2.07 cm², n = 25) inflamed rats were significantly larger than those in naive rats (1.10 ± 0.16 cm², n = 32). The mean RF size in TMJ-inflamed rats also was significantly larger than that in PO-inflamed rats (P < 0.01). Furthermore the mean area of the RFs of NS neurons (3.74 ± 1.44 cm², n = 5) was significantly larger in TMJ inflamed rats as compared with naive rats (0.4 ± 0.09 cm², n = 3) (P < 0.05). The background activity in the TMJ- and PO-inflamed rats was generally greater in WDR and NS neurons, but less in LTM neurons, when compared with naive rats. The responses of WDR neurons to noxious mechanical stimuli were increased significantly in TMJ-inflamed rats (P < 0.05) as compared with naive rats. WDR neuronal responses to mechanical stimulation also were increased in PO-inflamed rats but to a lesser extent than in TMJ-inflamed rats. The injection of CFA into the TMJ or PO skin resulted in reduced responses of LTM neurons to mechanical stimuli. The responses of MDH nociceptive neurons to 48–55°C heating were greater in inflamed rats as compared with naive rats. A subpopulation of WDR neurons recorded from TMJ (n = 4 of 10)- or PO (n = 3 of 13)-injected rats responded to cooling in addition to heating of the RFs but did not grade their responses with changes in stimulus intensity. These results indicate that persistent orofacial inflammation produced hyperexcitability of MDH nociceptive neurons. TMJ inflammation resulted in more robust changes in MDH nociceptive neurons as compared with PO inflammation, consistent with previous studies of increased inflammation, increased MDH Fos-protein expression, and increased MDH preprodynorphin mRNA expression in this deep tissue orofacial model of pain and hyperalgesia. The inflammation-induced MDH hyperexcitability may contribute to mechanisms of persistent pain associated with orofacial deep tissue painful conditions.

INTRODUCTION

Disorders of the temporomandibular joint (TMJ) result in a variety of symptoms including persistent pain in areas of the jaw and orofacial region. This severe pain may result from long-term changes in the CNS after peripheral tissue injury (Dubner 1991a,b). Studies in animal models of persistent pain at spinal levels indicate that peripheral tissue or nerve injury induces a state of hyperexcitability that participates in the development of persistent pain and hyperalgesia (Dubner 1991; Woolf 1983). It has not been demonstrated that persistent pain in the orofacial region leads to a similar state of central hyperexcitability in the trigeminal system. The purpose of the present study was to conduct a parametric analysis of the response properties of neurons in trigeminal nucleus caudalis in a model of persistent inflammation of the TMJ and perioral (PO) tissues.

The trigeminal nucleus caudalis is the rostral extension of the upper cervical spinal dorsal horn and also is referred to as the medullary dorsal horn (MDH). There are anatomic and functional similarities between medullary and spinal dorsal horns (Gobel et al. 1981; Price et al. 1976). Similar to the spinal dorsal horn, nociceptive-specific (NS) neurons and some wide dynamic range (WDR) neurons are distributed in the superficial laminae, and most WDR neurons are found in the deep laminae of the MDH (Dubner and Bennett 1983). Neurons excited by TMJ input also are classified as WDR and NS neurons, and the RFs of those neurons extend to wide areas of the facial skin and intraoral structures (Broton et al. 1988).

At the spinal level, persistent input after inflammation leads to increased responsiveness to thermal and mechanical stimuli, an expansion of peripheral RFs, and an increase in background activity (Calvino et al. 1987; Hylden et al. 1989; Ren et al. 1992; Schaible and Schmidt 1988; Woolf and King 1990). Although MDH neuronal activity has been extensively examined (Broton et al. 1988; Chiang et al. 1994; Hu 1990; Kojima...
1990; Price et al. 1976; Wang et al. 1996), previous studies have focused almost exclusively on neuronal responses to transient noxious stimuli. Recently, the responses to longer lasting stimuli have been examined (Hu et al. 1992; Meng et al. 1997; Raboisson et al. 1995; Yu et al. 1993). After the application of mustard oil into the deep masseter, the RFs of MDH nociceptive neurons gradually expand, sometimes extending across the midline, and background activity also is increased significantly (Hu et al. 1992). The facilitatory effects of mustard oil on MDH neurons typically resolve within an hour (Hu et al. 1994; Yu et al. 1993). In contrast, the injection of complete Freund’s adjuvant (CFA), into the rat hindpaw, results in behavioral hyperalgesia that peaks within 4–6 h and can persist for ≥2 wk. A CFA model of inflammation of the orofacial region recently has been developed in which there is persistent behavioral hyperalgesia, an increase in Fos-protein expression, and selective upregulation of preprodynorphin mRNA (Imbe et al. 1999; Ren 1999; Ren and Dubner 1996; Zhou et al. 1997). In the present study, we extended this analysis of adjuvant-induced persistent orofacial inflammation to determine the long-term effects of such inflammation on MDH neuronal activity.

Pain arising from deep tissues such as the TMJ have characteristics different from pain originating from cutaneous and subcutaneous orofacial tissues. We directly compared the effects of persistent deep versus cutaneous orofacial inflammation on nociceptive and nonnociceptive MDH neurons in the trigeminal system. Our findings indicate that CFA-induced inflammation originating in both the TMJ and PO tissues leads to robust hyperexcitability of MDH neurons. A portion of these results has been reported previously (Iwata et al. 1997).

METHODS

Inflammation

Thirty-five male Sprague Dawley rats weighing 300–400 g (Harlan; TMJ CFA: n = 10, PO CFA: n = 9, naive: n = 16) initially were anesthetized with pentobarbital sodium (50 mg/kg ip). For injection of CFA (0.05 ml 1:1 oil/saline suspension) into the TMJ, rats were placed on the stereotaxic frame, and a small incision was made on the scalp far from the cutaneous tissue surrounding the TMJ. The dorsal surface of the zygomatic arch was exposed by a reflection of masseteric muscle, and CFA was injected directly into the TMJ capsule under an operating microscope. For injection of CFA into the PO skin, the same amount of CFA was injected subcutaneously into a spot ~2 mm supralateral to the labial commissure. The TMJ and PO inflammation was indicated by intense edema and redness around their injection sites. One day after CFA injection, animals were checked for abnormal pain sensations by probing the injected sites and surrounding orofacial skin with von Frey filaments or forceps. Rats receiving the CFA injection exhibited lower mechanical threshold and stronger responses to mechanical stimuli, suggesting the presence of hyperalgesia or allodynia. For all TMJ- and PO-injected rats, CFA was injected into one side. Naive animals that did not receive any surgical treatment were used as a control. After surgery, penicillin G potassium (20,000 U im) was injected to prevent infection.

Animal preparation

For neuronal recording, rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and the trachea and left jugular veins were cannulated to allow artificial respiration and intravenous administration of drugs. Bipolar enamel coated platinum wire electrodes (inter-electrode distance: 1 mm) were placed on both sides of the TMJ capsule under a microscope. Anesthesia was maintained with halothane (2–3%) mixed with air during surgery. The rats were mounted on a stereotaxic frame, the medulla and the C1 spinal cord were exposed, and a mineral oil pool was made with the skin flaps surrounding the laminctomy. A head holder was secured rigidly to the skull by stainless steel screws and dental acrylic resin, and the ear bars and nose holder were removed. This setup allowed convenient access to orofacial RFs.

After surgery, anesthesia was maintained throughout the experiment by continuous inhalation of halothane (1–2%) mixed with air. During recording sessions, the rats were immobilized with pancuronium bromide (1 mg · kg⁻¹ · h⁻¹ iv) and ventilated artificially. Expired CO₂ concentration was monitored and maintained between 3.0 and 4.0%. Rectal temperature was maintained at 37–38°C by a thermostatically controlled heating pad (FHC) and the electrocardiogram was monitored. Blood pressure was measured every 30 min indirectly from the tail and kept at 90–120 mmHg during the experiments.

Stimulation and recording

Enamel-coated tungsten microelectrodes (impedance = 10–12 MΩ, 1,000 Hz) were advanced into the MDH at the levels ~2 mm caudal to the obex in 1-μm steps. Medullary dorsal horn neurons were searched for by applying mechanical stimulation (pressure or brush) to the craniocerebral region. When a single neuron was isolated, the responses to mechanical stimulation of the facial skin were carefully examined and the RFs were mapped. Only cutaneous RFs were mapped in the present study. Electrical stimuli (duration = 0.2 ms, intensity <0.5 mA) then were applied to the TMJ capsule. The response latencies of the first spike to electrical stimulation were measured on a storage oscilloscope.

Graded mechanical stimuli were applied to the most sensitive areas of the RFs. Mechanical stimuli consisted of brushing with a camel brush, pressure produced by a large arterial clip, pinch produced by a small arterial clip, and squeezing produced with a rat tooth forceps. To avoid sensitization due to repeated stimulation, noxious mechanical stimuli were applied to small areas of the RFs of each neuron. If the nonnoxious 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 as a low-threshold mechanoreceptive (LTM) neuron that had only transient firing at the onset and termination of the mechanical stimulus or had tonic responses during mechanical stimulation of the RFs but decreased its firing frequency after noxious mechanical stimulation; a WDR neuron that responded to both nonnoxious and noxious stimuli and increased its firing frequency as stimulus intensity increased; or a NS 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 MDH neurons to mechanical stimuli, thermal stimuli (heating and cooling) were applied to the most sensitive area of the cutaneous mechanical RF. The thermal probe used in the present study was illustrated in our previous study (Iwata et al. 1990). The tip of the thermal probe was 5 mm in diameter, and the rate of temperature change was set at 10°C/s. Before application of the thermal stimulus to the RF, the surface temperature was adapted to 38°C for 180 s. Skin heating ranged from 42 to 55°C and lasted for 30 s. Cold stimuli consisted of cooling of the skin to 10–30°C. The thermal stimuli were applied to the RFs at 210-s intervals (adaptation time: 180 s, stimulus time: 30 s) to avoid sensitization of peripheral nociceptors (Beitel and Dubner 1976). Neuronal responses were fed into a tape recorder (bandwidth DC to 20 kHz) for subsequent analysis of the signals. After recording of the response properties of MDH.
neurons, lesions were made at the recording site by passing DC of 10 µA for 10 s.

Histology

At the end of the experiment, the rats were overdosed with pentobarbital sodium and perfused transcardially with 50 ml 0.01 M PBS (pH 7.4) followed by 10% formalin in 0.1-M phosphate buffer. The brains were removed and placed in cold fixative for a few days, then transferred to cold phosphate-buffered 30% sucrose for 48 h. Serial sections (50-µm-thick) were cut along the path of the electrode penetration. The sections were counterstained with thionin for identification of recording sites. Precise camera lucida tracings of the recording sites were drawn at ×400 magnification with a drawing tube.

Data analysis

The waveform of single or multiple neuronal activity was analyzed off-line. The waveform of each neuron was identified using Spike 2 software (CED). Peristimulus time histograms (binwidth = 1 s) were generated in response to each stimulus. Background discharges first were recorded for 10 s before application of the mechanical or thermal stimulus, and they were subtracted from the neuronal responses during analysis. Stimulus-response (S-R) functions of each MDH neuron were obtained in response to the mechanical (brush, pressure, pinch) or thermal (42–45°C) stimuli. The combined mechanical responses of WDR neurons were calculated as mean value of peak firing frequency minus background activity at each stimulus intensity (brush, pressure, and pinch). 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 30 s (1 trial for each neuron with 180-s intervals) after thermal stimulation differed from the mean background discharge rate by ±2 SD. Results are presented as means ± SE. The RFs of all neurons were drawn to scale on standard diagrams of a rat head. Areas of the RFs were calculated using image analysis software (NIH image 1.60).

Statistical analysis

Statistical analysis was performed by using ANOVA followed by post hoc Fisher’s protected least significant difference (PLSD) or Scheffe F tests. Differences were considered significant at \( P < 0.05 \).

RESULTS

Neuron sample

Ninety neurons were recorded from the MDH 24 h after CFA injection into the TMJ capsule or PO skin. Fifty-two neurons were recorded from rats that did not receive CFA treatment (naive group consisted of naive rats without any surgical treatment). The classification of the sample is summarized in Table 1. WDR neurons (82/142, 57.7%) were encountered most frequently in the superficial and deep laminae of the MDH. Only a small number of NS neurons were encountered (11/142, 7.7%). The search method (pressure or brush of the RFs) may have recruited neurons responding to low-threshold stimuli so that NS neurons were overlooked. As shown in Fig. 1, NS neurons mainly were located in the superficial laminae (laminae I and II) of the MDH ~2 mm caudal to the obex, and some were in the deep laminae (laminae III–V), whereas most WDR and LTM neurons were in the deep laminae.

An example of a WDR neuron recorded from a TMJ-injected rat is illustrated in Fig. 2. This neuron was located in the deep laminae of the MDH ipsilateral to the CFA injection (Fig. 2A). The orofacial RF of this neuron was unusually large when compared with naive animals. The RFs of WDR neurons can be subdivided into low-threshold (center of the RF in general) and high-threshold (peripheral region of the RF) areas. These two areas were tested separately for mechanical stimulation in the present study. For this neuron, the low-threshold portion of the RF covered the ophthalmic and maxillary territories of the trigeminal nerve (Fig. 2B, ■); the high-threshold (Fig. 2B, □) area spread over the whole facial skin involving all three branches of the trigeminal nerve. This neuron responded to electrical stimulation of the TMJ capsule with a latency of 12.0 ms (Fig. 2C). After graded mechanical stimulation of the low-threshold area of the RF, the firing frequency was increased as the stimulus intensity increased from brushing to squeezing (Fig. 2D). No clear response was found to brushing the high-threshold area of the RF (Fig. 2E). The magnitude of the responses to noxious mechanical stimulation of the high-threshold area was approximately the same as that of the low-threshold area (Fig. 2E). This neuron had a graded response to noxious heating (Fig. 2F). There was also a response to cooling that did not grade with stimulus intensity (Fig. 2G).

An example of a WDR neuron ipsilateral to CFA injection into the PO region is illustrated in Fig. 3. This neuron was...
located in the superficial laminae of the MDH (Fig. 3A). The RF was relatively large and covered most facial areas (Fig. 3B). This neuron also responded to electrical stimulation of the TMJ capsule with a latency of 4.5 ms (Fig. 3C). Responses to graded mechanical stimulation of the RF after PO CFA injection was similar to that observed in WDR neurons in TMJ CFA-injected rats (Fig. 3, D and E). Heat responses were graded after increases in stimulus intensity (Fig. 3F).

**Receptive fields**

The locations of the RFs for different groups of animals were similarly distributed in the proximal and distal orofacial region (Fig. 4). The RFs of WDR neurons in naive rats were typically small. The largest RF possessed by a WDR neuron in naive rats was less than a quarter of the total orofacial region (Fig. 4). In contrast, the RFs of WDR and NS neurons in inflamed rats often included the complete orofacial area ipsilateral to CFA injection. The mean area of the RFs of MDH neurons are quantified in Fig. 5. For LTM neurons, the RF size was similar in all groups. There were also no significant differences in the low-threshold portion of the RFs of WDR neurons between all groups. For the high-threshold portion of the RFs of WDR and NS neurons, however, CFA injection produced a significant enlargement of the RFs. The mean areas of the high-threshold RFs of WDR neurons in TMJ (8.66 ± 0.61 cm², n = 25)- and PO (5.61 ± 1.0 cm², n = 25)-inflamed rats were 787 and 510% of that in naive rats (1.10 ± 0.16 cm², n = 32), respectively (Fig. 5). The mean RF size in TMJ-inflamed rats was significantly larger than that in PO-inflamed rats (P < 0.01). It is noteworthy that in PO-inflamed animals, the enlarged RFs were always associated with the injured site (Fig. 4). Neurons with RFs located outside the injured region were not affected by CFA injection and had RF sizes that were not significantly different from naive animals (compare Figs. 5 and 6). Receptive field size of the peripheral region of WDR neurons with RFs located within the CFA injection site was significantly larger than those located outside of the injection site (Fig. 6). In contrast, neurons with RFs located beyond the facial skin over the TMJ also had enlarged RFs in TMJ-inflamed rats (Fig. 4). The enlargement of the RFs of NS neurons were significantly larger in TMJ-inflamed animals as compared with NAIVE animals (P < 0.05) (Fig. 5). Although the RFs of NS neurons in PO-inflamed rats also were enlarged when compared with naive rats, the difference didn’t reach statistical significance (Fig. 5), probably due to the small sample size in this group of animals (n = 3).

**Background activity**

The differences in background activity in naive, TMJ- and PO-inflamed animals are shown in Fig. 7. The background activity of WDR neurons in the TMJ-inflamed rats was significantly higher than that in naive rats (P < 0.05); a trend toward a difference was seen in the PO-inflamed rats as compared with naive rats. There were no significant differences in background activity in NS neurons among the three groups. Interestingly, the background activity of LTM neurons in CFA-injected rats
FIG. 3. Example of a WDR neuron in the rat after PO CFA injection. A: recording site is indicated. T2, electrode penetration track 2. B: receptive field. ■, low-threshold area; □, high-threshold area. C: response to electrical stimulation (0.18 mA) of the TMJ. D and E: PSTHs of responses to mechanical stimulation of the low-threshold area (D) and the high-threshold area (E) of the receptive field. F: PSTHs of responses to noxious heating of the low-threshold area of the receptive field.

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FIG. 4. Receptive fields of MDH nociceptive (WDR and NS) and nonnociceptive (LTM) neurons in rats receiving different treatments. Largest and smallest receptive fields are shown for each category. ■, low-threshold areas; □, high-threshold areas. →, receptive fields.
Responses to thermal stimuli

Stimulus-response functions of WDR neurons to graded heating or cooling of the low-threshold areas are illustrated in Fig. 8. The responses to heating of the skin increased linearly in the 45–55°C range in all groups (Fig. 8A). The thermal threshold of all units was 45°C. Although there was no difference in heating response threshold among groups, the responses to 48–55°C heating were greater in inflamed rats as compared with naive rats. In TMJ-inflamed rats, the neuronal response to 55°C heating was 78.68 ± 19.28 spikes/s (n = 13), which was significantly greater than that in naive rats (40.52 ± 19.28 spikes/s, n = 7, P < 0.05, Fig. 8A). The noxious heat-evoked responses were slightly higher in TMJ- versus PO-inflamed rats, but the differences did not reach statistical significance.

Some WDR neurons recorded from the MDH of the TMJ- or PO-injected rats responded to cooling stimuli as well as heating of the RFs (Table 1). Unlike responses to heating, there was not a clear relationship between the cooling temperature and the magnitude of the neuronal responses (Fig. 8B, also see Fig. 2G). The firing levels were maintained in the cooling temperature range of 10–25°C. However, these heat/cool units were very sensitive to decreases in stimulus temperature (Fig. 2G).

Responses to mechanical stimuli

The responses of nociceptive neurons to mechanical stimuli increased as the stimulus modality changed from brush to pressure to pinch to squeeze (Figs. 2 and 3). In general, for WDR neurons, the responses to all stimuli were greater in TMJ- and PO-inflamed rats as compared with naive rats. The combined (brush, pressure, and pinch) mechanical responses (peak response frequency−background activity) of WDR neurons were significantly greater in TMJ-inflamed (center: 85.84 ± 19.08, peripheral: 86.60 ± 22.02) as compared with PO-inflamed (center: 43.45 ± 7.01, P < 0.01 vs. TMJ-inflamed).
and subnucleus caudalis of 15 mm in a 400 g rat, the minimum conduction velocities were in the C- and A-delta range in TMJ-inflamed (~1.0 m/s) and naive (~5.4 m/s) rats.

**DISCUSSION**

The purpose of the present study was to compare the responsivity of MDH neurons after CFA-induced deep or cutaneous orofacial inflammation. A parametric analysis was performed that included the examination of RF size, responses to mechanical and thermal stimuli, background activity, and minimal response latencies. We found that orofacial inflammation induced hyperexcitability of MDH nociceptive neurons as compared with noninflamed animals. Robust increases were found in RF sizes of WDR neurons as compared with LTM neurons after inflammation and the increases in RF size of WDR neurons after TMJ inflammation were greater than after PO inflammation. Neurons with enlarged RFs were found outside the zone of injury after TMJ inflammation but not after PO inflammation. Responses to thermal stimuli were significantly greater in inflamed animals than in naive animals in the 48–55°C range but there were no differences in threshold. Similarly, responses to nonnoxious and noxious mechanical stimuli were greater in inflamed as compared with naive rats and generally were greater after TMJ than PO inflammation. Finally, background activity was greater after inflammation in WDR neurons but, interestingly, was greater in naive rats in LTM neurons. It is unlikely that other factors such as penicillin injection contributed to changes in neuronal activity in inflamed animals as indicated by differential effects of CFA injection in TMJ- and PO-inflamed rats. Thus similar to findings at the spinal dorsal horn level (Hylden et al. 1989; Ren et al. 1992), TMJ and PO inflammation lead to hyperexcitability of MDH neurons. Although we focused on a limited segment of the MDH in the present study, these findings may help to understand chronic craniofacial pain mechanisms in general. The inflammation-induced changes in nociceptive neuronal activity are most likely related to long-term alterations in dorsal horn nociceptive processing commonly referred to as central sensitization (Dubner 1991b; Woolf 1983).

The robust increases in RF sizes after TMJ and PO inflammation cannot explain changes in RF size of peripheral nociceptors for the following reasons: peripheral nociceptors show minimal increases in size after hindpaw inflammation (Hylden et al. 1989; Kocher et al. 1987), local anesthetization of peripheral nociceptors innervating hindpaw tissue does not affect RFs outside the anesthetized zone (Hylden et al. 1989), N-methyl-D-aspartate receptor antagonists applied intrathecally or systemically dramatically reduce RF size (Chiang et al. 1998; Ren et al. 1992), and in the present study, after TMJ inflammation, the RFs expanded into noninjured zones. These findings support the hypothesis that the increased peripheral barrage originating from peripheral nociceptors leads to development of a central sensitization at the level of the MDH, which is manifested as an increase in responsivity including enlargement of RFs (Dubner 1991b, 1992). These enlarged RFs contribute to hyperalgesia produced by CFA due to greater overlap of dorsal horn neuron RFs and their enhanced activation (Dubner 1991b).

The expansion of the RFs and increase in background activity of nociceptive neurons in the TMJ- and PO-inflamed

**Response latency**

Thirty-nine of 142 MDH neurons responded to electrical stimulation of the TMJ capsule (TMJ CFA: n = 15, PO CFA: n = 4, NAIVE: n = 20). The mean first spike latency of WDR neurons recorded from the ipsilateral side of the TMJ-inflamed rats was significantly longer than that of naive animals (TMJ CFA: 13.5 ± 2.8 ms, naive: 2.8 ± 0.4 ms, P < 0.01). Considering a distance between the stimulating site in the TMJ and

**FIG. 8.** Stimulus-response (S-R) functions of WDR neurons to graded thermal stimulation of the low-threshold areas of the receptive fields. A: S-R functions to graded heating of the receptive fields (TMJ CFA: n = 13, PO CFA: n = 10, naive: n = 7). B: S-R functions to graded cooling of the receptive fields (TMJ CFA: n = 4, PO CFA: n = 3).

flamed; peripheral: 43.21 ± 7.09, P < 0.05 vs. TMJ-inflamed) and naive (center: 49.46 ± 4.54, peripheral: 36.28 ± 6.48, center: P < 0.01 vs. TMJ-inflamed; peripheral: P < 0.01, vs. TMJ-inflamed) rats. There were no significant effects of TMJ and PO inflammation on NS neuronal responses to mechanical stimuli (not shown). When compared with naive animals, the injection of CFA into the TMJ or PO skin generally resulted in reduced responses of LTM neurons to mechanical stimuli (not shown).

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animals were similar to that observed in other animal models of spinal dorsal horn hyperexcitability (Calvino et al. 1987; Hu et al. 1992; Hylden et al. 1989; Laird and Cervero 1989; McMahon and Wall 1984; Ren et al. 1992). The present findings on enlarged RFs and responses to mechanical stimulation further revealed that the TMJ inflammation produced greater facilitatory effects on MDH nociceptive neurons as compared with PO cutaneous and subcutaneous inflammation. These results are consistent with previous findings that the injection of CFA into the TMJ capsule resulted in a more intense inflammation (even though the same amount of CFA was administered), greater induction of Fos-protein expression, and stronger preprodynorphin mRNA upregulation (Imbe et al. 1999; Yu et al. 1993; Zhou et al. 1997). Two related mechanisms may explain this increased responsiveness of MDH neurons during persistent TMJ inflammation as compared with PO inflammation: higher frequency primary afferent activity originating from the TMJ than from PO tissues consistent with the finding that there is heavy C fiber innervation of the TMJ (Broton et al. 1988; Sessle and Hu 1991); silent C-fibers that were not active in the absence of inflammation became active after CFA injection. In addition, at spinal levels, C-fiber inputs produce more robust and longer-lasting reflex hyperexcitability after muscle than after cutaneous nerve stimulation (Wall and Woolf 1984). This has not been shown at trigeminal levels with the exception of a greater expansion of the deep mechanical receptive fields of trigeminal nociceptive neurons after mustard oil application into the tongue muscle versus application into the facial skin (Yu et al. 1993). The present results support the hypothesis that central sensitization is more robust after deep than after superficial tissue inflammation at both trigeminal and spinal levels.

The comparison of the RF properties of MDH neurons in TMJ- and PO-inflamed rats demonstrated that TMJ inflammation resulted in more widespread excitation of MDH nociceptive neurons. In TMJ-inflamed animals, neurons that did not receive direct input from the TMJ, as indicated by a lack of the RFs associated with the injured joint, exhibited enlarged RFs. This is in sharp contrast with PO inflammation where neurons with enlarged RFs always received direct input from the injection site; i.e., the enlarged RFs always overlapped with the injured site. In PO-inflamed rats, neurons that did not receive direct input from the PO region had normal-sized RFs. These results further support the hypothesis that the inflammation of orofacial deep tissue produces profound central sensitization and is consistent with clinical observations that inflammation of deep tissues, such as muscle and joints, causes more severe and persistent pain than inflammation of the superficial cutaneous tissues (Schellhas et al. 1989). The present findings also provide a possible mechanism for the clinical observation that deep pain is frequently referred to cutaneous sites (Dubner 1991a).

The enlargement of RFs seen in the present study was limited to the more peripheral RF zone that responds to both low- and high-threshold mechanoreceptive input. Changes in size were not seen in the low-threshold portion of the RF. It has been reported that cutaneous nociceptors have no enlarged RFs after CFA injection, whereas RFs of second-order neurons in the spinal dorsal horn are expanded dramatically (Hylden et al. 1989). Other studies also suggest a central component responsible for changes in the RFs in different situations (Kocher et al. 1987; Laird and Cervero 1989; Ren et al. 1992). The present data suggest that the threshold for activation of the peripheral high-threshold area of the spinal dorsal horn WDR neurons became lower after inflammation. This may have contributed to expansion of peripheral RFs after CFA injection. It is not clear, however, whether similar changes occurred in deep RFs of MDH nociceptive neurons (see Yu et al. 1993).

After the injection of mustard oil, a small fiber irritant, into the masseter muscle, Hu and colleagues (1992) demonstrated facilitation of the low- and high-threshold portions of the RF of WDR neurons. These contrasting findings may be related to possible damage of low-threshold mechanoreceptive afferents associated with persistent CFA inflammation. Consistent with this explanation is our unique finding that orofacial inflammation resulted in a suppression of the responses of LTM neurons. This is in contrast to other reports where no changes in LTM activity have been noted after minor injury. The injection of mustard oil into the deep masseter produced no effect on LTM neurons (Hu et al. 1992). LTM neuronal activity recorded extensively from the trigeminal spinal nucleus in cats with pulp deafferentation, also showed no significant changes in activity (Hu et al. 1986). LTM neurons also are not responsive to algesic chemical injections into the TMJ (Broton et al. 1988).

In the present study, the background activity of LTM neurons was suppressed dramatically in inflamed rats when compared with naive rats. Our findings on reduction in background activity and mechanical responsivity of LTM neurons suggest that persistent inflammation may have produced changes in peripheral nerve activation. LTM neurons primarily receive input from large myelinated low-threshold afferents (Hu et al. 1981; Price et al. 1976), which may have been damaged by the inflammatory process. The significant increase in response latency to electrical stimulation of the TMJ capsule found in the present study also suggests that damage of large diameter primary afferents after TMJ inflammation occurred. A similar increase in response latency of dorsal column nuclei neurons has been reported in nerve-injured rats (Miki et al. 1998). Such reduction in low-threshold primary afferent input may result in a decrease in primary afferent depolarization and an attenuation of presynaptic inhibition, further contributing to the development of hyperexcitability of MDH nociceptive neurons (Laird and Bennett 1992).

The response of MDH nociceptive neurons after inflammation to thermal stimulation of their RFs has not been studied previously. Compared with naive rats, the responses of WDR neurons to 48–55°C skin heating were significantly greater in TMJ- and PO-inflamed rats. However, the response threshold of these neurons was not changed after inflammation. This result is at first difficult to reconcile with behavioral studies where the head withdrawal latency to a graded thermal stimulus was reduced after orofacial inflammation (Ren 1999; Ren and Dubner 1996), suggesting a decrease in thermal response threshold. However, the expansion of RFs results in a greater number of neurons activated after inflammation (Hylden et al. 1989); this may provide a neural signal to account for lower behavioral thresholds to heat. In addition, the present studies did not include many NS neurons, and in hindpaw inflamed rats, the thermal response threshold of spinal NS neurons was reduced significantly (Hylden et al. 1989).

In the present study, some MDH nociceptive neurons exhibited responses to both noxious heating and cooling of the skin. Cooling-responsive spinothalamic neurons have been identi-
fied in other species (Craig and Kniffki 1985; Dostrovsky and Craig 1996). Han et al. (1998) reported recently that some rat nociceptive neurons in the superficial laminae of the spinal dorsal horn respond to both thermal and cold stimuli. The heat-cold responsive neurons found in the present study had a maintained response to 10–25°C cooling; this resembles the Type 2 cells described by Hutchison et al. (1997). However, most Type 2 cold cells do not respond to noxious heating of the skin (Hutchison et al. 1997). In the trigeminal system, a few heat-cold responsive neurons also activated by corneal input have been reported but not described in detail (Meng et al. 1997). It appears that the heat-cold responsive neurons in the present study may represent a novel population of MDH nociceptive neurons that undergo changes in their response properties after persistent inflammation. Primary afferent “cold” fibers become sensitive to heat stimulation after injury and represent (Dubner et al. 1975) a candidate neuronal population responsible for these changes. Orofacial inflammation and resulting central sensitization may facilitate the convergence of this input on MDH nociceptive neurons.

In summary, our findings indicate that persistent orofacial inflammation results in hyperexcitability of MDH nociceptive neurons. TMJ inflammation resulted in more robust changes in MDH nociceptive neurons as compared with PO inflammation, consistent with previous studies of increased inflammation, increased Fos-protein expression, and increased preprodynorphin mRNA expression in this orofacial model. (Imbe et al. 1999; Ren 1999; Ren and Dubner 1996; Zhou et al. 1997). The inflammation-induced MDH hyperexcitability may contribute to mechanisms of persistent pain associated with jaw and craniofacial painful conditions.

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