Alteration of Medullary Dorsal Horn Neuronal Activity Following Inferior Alveolar Nerve Transection in Rats

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1 Department of Oral Physiology, Faculty of Dentistry, Osaka University, Osaka 565-0871; 2 Department of Physiology, School of Dentistry, Nihon University, Tokyo 101, Japan; and 3 Department of Oral Biology, Faculty of Dentistry, University of Toronto, Toronto, Ontario M5G 1G6, Canada

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Iwata, Koichi, Takao Imai, Yoshiyuki Tsuboi, Akimasa Tashiro, Akiko Ogawa, Toshifumi Morimoto, Yuji Masuda, Yoshihisa Tachibana, and James Hu. Alteration of medullary dorsal horn neuronal activity following inferior alveolar nerve transaction in rats. J Neurophysiol 86: 2868–2877, 2001. The effects of inferior alveolar nerve (IAN) transaction on escape behavior and MDH neuronal activity to noxious and nonnoxious stimulation of the face were precisely analyzed. Relative thresholds for escape from mechanical stimulation applied to the whisker pad area ipsilateral to the transection were significantly lower than that for the contralateral and sham-operated whisker pad until 28 days after the transection, then returned to the preoperative level at 40 days after transection. A total of 540 neurons were recorded from the medullary dorsal horn (MDH) of the nontreated naive rats [low-threshold mechanoreceptive (LTM), 27; wide dynamic range (WDR), 31; nociceptive specific (NS), 11] and sham-operated rats with skin incision (LTM, 34; WDR, 30; NS, 23) and from the ipsilateral (LTM, 82; WDR, 82; NS, 31) and contralateral MDH relative to the IAN transection (LTM, 77; WDR, 82; NS, 33). The electrophysiological properties of these neurons were precisely analyzed. Background activity of WDR neurons on the ipsilateral side relative to the transection was significantly increased at 2–14 days after the operation as compared with that of naive rats. Innocuous and noxious mechanical-evoked responses of LTM and WDR neurons were significantly enhanced at 2–14 days after IAN transection. The mean area of the receptive fields of WDR neurons was significantly larger on the ipsilateral MDH at 2–7 days after transection than that of naive rats. We could not observe any modulation of thermal responses of WDR and NS neurons following IAN transection. Also, no MDH neurons were significantly affected in the rats with sham operations. The present findings suggest that the increment of neuronal activity of WDR neurons in the MDH following IAN transection may play an important role in the development of the mecano-allodynia induced in the area adjacent to the area innervated by the injured nerve.

INTRODUCTION

It has been reported that peripheral-nerve injury induces a wide variety of changes in spinal dorsal horn neurons involved in the transmission of noxious information to the higher CNS (Bennett 1994; Bennett and Xie 1988; Burchiel 1984; Carlton et al. 1991; Doubell et al. 1997; Kim and Chung 1992; Laird and Bennett 1993; Lisney and Devor 1987; Molander et al. 1992; Palecek et al. 1992a, b; Seltzer et al. 1990; Woolf et al. 1992, 1995). Most of the papers studying nerve-injury-induced changes of the CNS were focused on the area innervated by the injured nerve. However, changes in noxious responses were observed at the area adjacent to the areas innervated by the injured nerve as well. Peripheral-nerve injury induces many different types of sensory changes that have been described as an abnormal sensation and decrement of pain threshold in both the center and surrounding areas innervated by the injured nerves. These sensory deficits have been attributed to mechanisms originating in the CNS or primary afferents neurons (Bennett 1994; Kajander and Bennett 1992; Laird and Bennett 1992, 1993; Miki et al. 1998). After inferior alveolar nerve transection, the primary afferent nerve fibers increased their spontaneous activity with abnormal firing and this firing lasted for 2–3 days (Bongeneihm and Robinson 1996, 1998; Chudler et al. 1997). This increased activity of the primary afferent fibers could lead to neuronal hyperactivity of the secondary order neurons in the medullary dorsal horn (MDH). Furthermore, Woolf et al. (1992, 1995) and Shortland and Woolf (1993) have reported that peripheral-nerve injury produces sprouting of large-diameter primary afferent nerve fibers from laminae III–IV to lamina II in the spinal cord at 7 days after nerve injury. These data suggest the possibility that peripheral-nerve injury produces increments of the primary afferent nerve fiber activity with an abnormal pattern at the early period after injury and reorganization of the large diameter nerve fiber terminals at the later period, which is manifested as “allodynia,” a phenomenon where nonnoxious touch stimuli induced pain sensation. This primary afferent discharge and reorganization of large-diameter nerve fiber terminals in the CNS following peripheral-nerve injury may occur in a wide area of the spinal cord, beyond the area innervated by the injured nerve.

Kim and Chung (1992) have reported that tight peripheral-nerve ligation produces hypersensitivity to mechanical stimulation of the area adjacent to the innervated regions. This is believed to be produced by a reorganization of primary afferent terminals in the CNS or hyperactivity of the primary afferent fibers. It is necessary to analyze the change in response properties of dorsal horn neurons driven by mechanical and/or thermal stimulation of the area adjacent to the area of nerve injury.
thermal stimulation of the adjacent regions of the area innervated by the injured nerves to clarify the central mechanism of the hypersensitivity in the adjacent areas as well as the areas innervated by the injured nerves. The trigeminal system can provide such an opportunity because the innervation territory of its three branches is well demarcated.

Furthermore, most of the previous papers studying nerve-injury pain described the data from the sciatic-nerve-lesion model (Bennett 1994; Bennett and Xie 1988; Burchiel 1984; Carlton et al. 1991; Doubell et al. 1997; Kim and Chung 1992; Laird and Bennett 1993; Lisney and Devor 1987; Molander et al. 1992; Palecek et al. 1992a,b; Seltzer et al. 1990; Woolf et al. 1992, 1995). The sciatic nerve includes motor nerves as well as sensory nerves. On the other hand, the trigeminal nerve is purely sensory without a motor component. It is very important to study the effect of pure sensory nerve lesion on nociception, without motor nerve damage, to clarify the underlying mechanism of the nerve-injury-induced neuropathic pain.

Given these findings, we decided first to establish a behavioral model and second to use the single-neuron recording technique to clarify the response properties of nociceptive and nonnociceptive neurons in the MDH of the rat with inferior alveolar nerve (IAN) transection.

METHODS

Behavioral test

Before the start of training, water was restricted to 100 ml · kg⁻¹ · d⁻¹ for 1 wk. First, rats were trained to stay in the plastic cage for 20 min and drink 10% sucrose in water through a hole made in the wall of the plastic cage. The experimenter was kept blind to the experimental conditions. In daily sessions, using sucrose water as reward, rats were trained to stay in the plastic cage and to keep drinking water through a hole on the wall during nociceptive mechanical stimulation of the whisker pad area. The criterion performance was when the rats could keep drinking sucrose water for 20 min without escape from the 40-g mechanical stimuli applied to the whisker pad. Forty grams was the maximum intensity used in the present study. The absence of an escape response to the 40-g stimulus was defined as a relative escape threshold of 1.0. The daily training sessions took place until criterion performance was reached. At this time, IAN transection was performed. The von Frey hair mechanical stimulation was applied to the whisker pad when the rats were drinking sucrose water and mechanical threshold of escape behavior was measured in IAN transected, sham-operated, and naive rats. When the relative threshold value 1.0 was obtained after surgery, the threshold value was defined as 1.0. Two, 7, 14, and 60 days after IAN transection or sham operation, rats were used for the acute recording experiments. The experimental conditions were similar for naive and experimental rats.

Inferior alveolar nerve transection

Sixty-six male Sprague-Dawley rats weighing 300–400 g (ipsilateral to the transection: n = 20, contralateral to transection: n = 15, sham operation: n = 11, naive: n = 20) were initially anesthetized with pentobarbital sodium (50 mg/kg ip). For the IAN transection, rats were placed on a warm mat, and a small incision was made on the surface of the facial skin over the maseteric muscle and to the alveolar bone through the maseteric muscle. The surface of the alveolar bone was exposed, and the bone surface covering the IAN was removed and the IAN was exposed. The IAN was tightly ligated at two points of the nerve trunk at just above the angle of the mandible and 1 mm proximal from the angle of the mandibular bone. For the sham-operated rats, the facial skin and the maseteric muscle were cut and the surface of the alveolar bone was removed. After surgery, penicillin G potassium (20,000 U im) was injected to prevent infection.

Rat preparation

In the present study, rats were classified into four groups according to their surgical preparation: ipsilateral to IAN transection group, contralateral to IAN transection group, sham-operated group (which received facial skin incision and lower jaw bone scraping), and naive group (which did not receive any surgical procedures). For neuronal recording, each group of rats was 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. Anesthesia was maintained with halothane (2–3%) mixed with oxygen during surgery. The rats were mounted in a stereotaxic frame, the medulla was exposed, and a mineral-oil pool was made with the skin flaps surrounding the laminectomy. A head holder was rigidly secured 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 receptive fields (RFs).

After surgery, anesthesia was maintained throughout the experiment by continuous inhalation of halothane (1–2%) mixed with oxygen. During recording sessions, the rats were immobilized with pancuronium bromide (1 mg · kg⁻¹ · h⁻¹ iv) and ventilated artificially. The expired CO₂ concentration was 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 about 2 mm caudal to the obex in 1-μm steps. Medullary dorsal horn neurons were searched by applying mechanical stimulation (pressure or brush) to the craniofacial 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.

Graded mechanical stimuli were applied to the most sensitive areas of the RFs. Mechanical stimuli consisted of brushing with a camel hair brush, pressure produced by a large arterial clip, and pinch produced by a small arterial clip. To avoid sensitization due to repeated stimulation, noxious mechanical stimuli were applied to only 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 wide dynamic range (WDR) neuron that responded to both nonnoxious and noxious mechanical stimuli and increased its firing frequency as stimulus intensity increased; or a nociceptive specific (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 medullary dorsal horn (MDH) neurons with mechanical stimuli, thermal stimuli (heating and cooling) were
applied to the most sensitive area of the cutaneous mechanical RF. When the WDR neurons or NS neurons were identified, heat and cold stimuli were applied to the most sensitive areas of the mechanical RFs. On the other hand, when LTM neurons were identified, a cold stimulus was applied to the most sensitive areas of the mechanical RFs. If these neurons responded to cooling of the RFs, they were then tested with heat stimulation. The thermal probe used in the present study was described 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 42–55°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. 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 the MDH neurons, lesions were made at the recording site by passing DC of 20 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 phosphate-buffered saline (PBS, pH 7.4) followed by 10% formalin in 0.1 M PB. 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 were first recorded for 10 s before application of mechanical or thermal stimulus, and they were subtracted from the neuronal responses during analysis. The peak firing frequency was calculated as the highest frequency that occurred during mechanical or thermal stimulation.

Stimulus-response (S-R) functions of each MDH neuron were obtained in response to the mechanical (brush, pressure, pinch) or thermal (42–50°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

The ANOVA was performed on the behavioral test at each time point after the operation. We used ANOVA on rank with post hoc Tukey or Dunn test where appropriate. To analyze the relationship between the firing frequency, days after operation and stimulus strength, we used three-way ANOVA. Similar tests were performed on the contralateral and sham-operated rats. Differences were considered significant at \( P < 0.05 \).

### RESULTS

#### Escape behavior from mechanical stimulation of the whisker pad

After completion of the training, in which the rats accepted the noxious mechanical stimulation applied to the whisker pad area, rats received the IAN transection. During mechanical stimulation of the whisker pad area, vocalization and autotomy was never observed. Figure 1 illustrates the relative threshold for escape behavior elicited by mechanical stimulation of the whisker pad. The decrement of threshold value was significantly greater in the rats with ipsilateral IAN transection than the contralateral and sham-operated rats. Furthermore, the threshold of rats on the contralateral side relative to the IAN transection was significantly lower than that of the sham-operated rats. One day after transection, the relative threshold on the ipsilateral side relative to IAN transection was decreased.

![FIG. 1. Change in the threshold intensity for eliciting escape behavior following inferior alveolar nerve (IAN) transection. The change in threshold intensity was plotted as relative values where escape threshold values are compared before transection and at different periods after IAN transection. A relative threshold of 1.0 was defined as the absence of a response to the highest stimulus intensity (40 g). Sham, sham-operated rats; contralateral, changes of escape threshold from the mechanical stimulation applied to the contralateral side relative to the IAN transection; ipsilateral, changes of escape threshold from the mechanical stimulation applied to the ipsilateral side relative to the IAN transection.](http://jn.physiology.org/abstract/2870)
to 10% of that before transection, that of the contralateral side decreased to 50% and sham-operated rats was reduced to 70%. The decrement of the relative escape threshold on the side ipsilateral to the transection was significantly lower than that for the contralateral and sham-operated whisker pad until 28 days after the transection. The relative threshold of the ipsilateral and contralateral whisker pad increased gradually with time after transection and reached a level similar to the sham-operated rats at 40 days after transection. In the present experiment, sham-operated rats showed slight hypersensitivity to mechanical stimulation just after the sham operation because sham-operated rats received skin surgery such as the cheek skin cut and approaching to the alveolar bone. This decrement of escape threshold returned to the preoperative level at 7 days after the operation.

**Spatial arrangement of neurons in the MDH**

A total of 540 nociceptive and nonnociceptive neurons were recorded from the medullary dorsal horn (MDH) (naive, 69; sham-operated rats, 84; ipsilateral to the IAN transection, 195; contralateral to the IAN transection, 192) and detailed electrophysiological properties were precisely analyzed (Tables 1 and 2). Figure 2 illustrates the intra-laminar distribution of LTM, WDR, and NS neurons recorded from the MDH of naive rats and that on the ipsilateral and contralateral side relative to the IAN transection. Basically, WDR and NS neurons were distributed both in the superficial (laminae I–II) and deep laminae (laminae III–IV) of the MDH in naive rats and rats with IAN transection. In naive rats, NS neurons were mainly distributed in the superficial laminae and LTM and WDR neurons were in the deeper laminae of the MDH.

**Background activity of MDH neurons**

Time course changes in background activity of MDH neurons following IAN transection are shown in Fig. 3. In IAN transectioned rats, MDH WDR neurons began increasing in background activity at 2 days after IAN transection on the ipsilateral side (ipsi/naive: 680% at 2 days, 556% at 7 days, and 419% at 14 days; ipsi/sham: 1249% at 14 days). The increase in background activity lasted for 14 days after transection then gradually decreased. A significant increment of the background activity on the ipsilateral side to IAN transection was observed at 2, 7, and 14 days after IAN transection as compared with that of naive rats ($P < 0.05$). On the other hand, when compared with the background activity of sham-operated rats, a significant increment was observed only at 14 days after surgery ($P < 0.05$). Background activity on the ipsilateral side to the IAN transection was also significantly increased in LTM neurons at 2 and 14 days after surgery as compared with naive rats (ipsi/naive: 422% at 2 days and 933% at 7 days). On the other hand, NS neurons did not show any significant increase in background activity after surgery. On the contralateral side relative to IAN transection and sham-operated rats, LTM and NS neurons did not show clear changes in their firing following IAN transection. On the other hand, WDR neurons slightly increased their background activity on the contralateral side relative to the transection, but this increase did not reach a significant level. We did not find any significant differences of background activities of LTM, WDR, and NS neurons between sham and naive rats ($P > 0.05$). These data reveal that only WDR neurons in the ipsilateral MDH relative to the transected side were affected by IAN transection in terms of their background activity.

**Table 1. Incidence of LTM, WDR, and NS neurons recorded from the MDH**

<table>
<thead>
<tr>
<th></th>
<th>Naive (days)</th>
<th>Sham (days)</th>
<th>Ipsilateral (days)</th>
<th>Contralateral (days)</th>
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</thead>
<tbody>
<tr>
<td>LTM</td>
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<td>2 7 14 60</td>
<td>2 7 14 60</td>
<td>2 7 14 60</td>
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<td>Heat</td>
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<tr>
<td>Cool</td>
<td>2 (14)</td>
<td>1 (10)</td>
<td>2 (9) 1 (4) 1 (7) 1 (7)</td>
<td>1 (6) 1 (5) 1 (6)</td>
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<tr>
<td>WDR</td>
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<tr>
<td>Heat</td>
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<tr>
<td>Heat + cool</td>
<td>7 (23)</td>
<td>4 (13)</td>
<td>3 (15) 4 (22) 3 (20) 2 (7)</td>
<td>3 (15) 1 (6) 3 (20) 2 (7)</td>
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<tr>
<td>Cool</td>
<td>2 (6) 2 (29) 2 (33) 2 (33) 2 (25)</td>
<td>2 (10) 3 (17) 2 (13) 5 (17)</td>
<td>4 (20) 4 (20) 2 (10) 3 (14)</td>
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<td>NS</td>
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<tr>
<td>Heat</td>
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<tr>
<td>Heat + cool</td>
<td>1 (9)</td>
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<tr>
<td>Cool</td>
<td>4 (36)</td>
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Number of neurons: naive, 69; sham operated (sham), 84; ipsilateral and contralateral inferior alveolar nerve transection 195 and 192, respectively. MDH, medullary dorsal horn; LTM, low-threshold mechanosensitive neurons; WDR, wide dynamic range neurons; NS, nociceptive specific neurons.

**Table 2. Incidence of LTM, WDR and NS neurons responded to thermal stimulation**

<table>
<thead>
<tr>
<th></th>
<th>Naive (days)</th>
<th>Sham (days)</th>
<th>Ipsilateral (days)</th>
<th>Contralateral (days)</th>
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<tbody>
<tr>
<td>LTM</td>
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<td>2 7 14 60</td>
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<td>Heat + cool</td>
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<td>2 (9) 1 (4) 1 (7) 1 (7)</td>
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<td>Cool</td>
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<td>WDR</td>
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<td>Heat</td>
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<td>Heat + cool</td>
<td>7 (23)</td>
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<td>3 (15) 4 (22) 3 (20) 2 (7)</td>
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<tr>
<td>Cool</td>
<td>2 (6) 2 (29) 2 (33) 2 (33) 2 (25)</td>
<td>2 (10) 3 (17) 2 (13) 5 (17)</td>
<td>4 (20) 4 (20) 2 (10) 3 (14)</td>
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<td>NS</td>
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<td>Cool</td>
<td>4 (36)</td>
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Cool: neurons responded to cooling of the receptive field (RF). heat: neurons responded to heating of the RF. The numbers in the parentheses indicate the percentage of thermally sensitive neurons.

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Evoked responses of MDH neurons

Figure 4 illustrates an example of responses of a WDR neuron recorded from lamina III of the ipsilateral MDH relative to the IAN transection (Fig. 4B). The IAN in this rat was transected 2 days before recording. The RF of this neuron was relatively large, extending from the eyelid to the whisker pad region as illustrated in Fig. 4A. In the present study, RFs with low (center)- and high-threshold areas (peripheral) were identified in the WDR neurons. Mechanical responses of WDR neurons increased their firing frequency following increases in stimulus intensity applied to both center and peripheral RFs. Mechanical responses in IAN transection rats were significantly larger on the ipsilateral side relative to the transection at 2–7 days after surgery, whereas responses to heat stimulation were not significantly affected by transection (Figs. 6A, C, E, and G) and cold stimuli (Fig. 7, B, D, F, and H) were constructed for each neuron and were averaged for each group. In the present study, we obtained the thermal responses of a relatively small number of nociceptive neurons (Table 2) in sham-operated rats (Fig. 7, C and D) and in the contralateral MDH (Fig. 7, G and H). WDR and NS neurons in the naive and operated rats that responded to heat stimulation increased their firing frequency following increases in stimulus temperature (Fig. 7, A, C, E, and G), and some of these neurons

FIG. 2. Intra-laminar distribution of low-threshold mechanosensitive (LTM), wide dynamic range (WDR), and nociceptive specific (NS) neurons in the medullary dorsal horn (MDH).

Evoked responses of MDH neurons

both heating and cooling of the RF. RF size and mechanical responses showed a similar pattern to that observed in a WDR neuron as shown in Fig. 4. Furthermore, cold responses were not clearly graded following graded decreases in stimulus temperature (Fig. 5F). In the LTM neurons recorded from the ipsilateral or contralateral side relative to the transection, significant increases in responses to innocuous brushing (contra/naïve: 192%) and pressure (contra/naïve: 212%, ipsi/naïve: 209%; Fig. 6) were observed at 2 days after nerve injury ($P < 0.05$) and in responses to noxious pinch at 14 days after transection (ipsi/naïve: 194%; $P < 0.05$). We could not observe significant changes in evoked responses of LTM, WDR, and NS neurons following the sham operation.

Stimulus-response functions of neuronal responses to graded heat (Fig. 7, A, C, E, and G) and cold stimuli (Fig. 7, B, D, F, and H) were constructed for each neuron and were averaged for each group. In the present study, we obtained the thermal responses of a relatively small number of nociceptive neurons (Table 2) in sham-operated rats (Fig. 7, C and D) and in the contralateral MDH (Fig. 7, G and H). WDR and NS neurons in the naive and operated rats that responded to heat stimulation increased their firing frequency following increases in stimulus temperature (Fig. 7, A, C, E, and G), and some of these neurons
responded to cooling of the RFs in a graded manner (Fig. 7, B, D, F, and H). In the present study, no significant differences of the slope of the S-R functions to thermal stimulation were observed at different periods after the IAN transection in WDR and NS neurons among the naive, sham, contralateral, and ipsilateral groups.

**RF size**

We investigated the RF size of MDH neurons only with RFs innervated by the first and the second branches or the second branch of the trigeminal nerve. Two to three penetrations were made into the dorsal part of the MDH (mandibular projection

**FIG. 4.** An example of a wide dynamic range (WDR) neuron in the rat at 2 days after IAN transection. A: the receptive field. The solid area indicates the low-threshold area and the hatched area shows the high-threshold area. B: the recording site is indicated. T2: electrode penetration track 2. C and D: the poststimulus time histograms (PSTHs) of responses to mechanical stimulation of the low- (C) and high-threshold area (D) of the receptive field. E: the PSTHs of responses to thermal stimulation of the low-threshold area of the receptive field. BR, brushing with camel brush; PR, pressure with large forceps; PI, pinch with small arterial clip. The same abbreviations are used in the subsequent figures.

**FIG. 5.** An example of a WDR neuron in the rat at 14 days after IAN transection. A: the receptive field. The solid area indicates the low-threshold area and the hatched area shows the high-threshold area. B: the recording site is indicated. T2: electrode penetration track 2. C and D: the PSTHs of responses to mechanical stimulation of the low-threshold area (C) and the high-threshold area (D) of the receptive field. E and F: the PSTHs of responses to thermal stimulation of the low-threshold area of the receptive field. Note that this neuron responded to both heating and cooling of the receptive field.
area) in each experiment. No neurons could be identified that responded to mechanical stimulation of the mandibular region of the facial skin on the ipsilateral side relative to the IAN transection.

The RF properties were much more strongly affected by the IAN transection as compared with the other electrophysiological properties. The low (ipsi/naive: 243% at 2 days and 224% at 7 days; contra/naive: 243% at 2 days) and high-threshold areas (ipsi/naive: 371% at 2 days, 241% at 7 days, and 175% at 60 days; ipsi/sham: 377% at 2 days, 377% at 7 days, and 370% at 60 days) of WDR neurons significantly increased their size at 2 to 7 and 60 days after operation on the ipsilateral sides relative to the IAN transection as illustrated in Fig. 8, A and B (P < 0.05). A significant increment in the RFs ipsilateral to IAN transection was observed only at the high-threshold areas at 2, 7, and 60 days after IAN transection as compared with those of sham-operated animals or contralateral side to IAN transection (Fig. 8C). On the other hand, in the low-threshold areas, no significant difference of the size of the RFs was observed between the ipsilateral side and sham-operated animals (Fig. 8B). LTM neurons also increased their RF size at 2 days after transection but this increment did not reach statistical significance (Fig. 8A). NS neurons increased their RF sizes at 2–7 days after transection on the ipsilateral side, but this increment also did not reach statistical significance (Fig. 8D).

We did not find any significant effect of sham surgery on the RF size in the LTM, WDR and NS neurons.

DISCUSSION

Effect of IAN transection on escape from noxious and nonnoxious stimuli

The present study demonstrated the time-dependent changes of escape behavior from mechanical stimulation of the whisker pad area (2nd branch of the trigeminal nerve) after IAN (the 3rd branch of the trigeminal nerve) transection. After IAN transection, the whisker pad area innervated by the second branch of the trigeminal nerve became hypersensitive to innocuous mechanical stimulation (Fig. 1). This hypersensitivity of the whisker pad area was observed at 2 days after IAN transection and lasted for 28 days as illustrated in Fig. 1. Thus IAN transection established sustained modifications in sensory processing that produced long-lasting mechano-allodynia in the area adjacent to the area innervated by the IAN. There are many reports describing that mechano-allodynia was induced in the hind paw region within several days after sciatic nerve injury (Bennett 1994; Bennett and Xie 1988; Kim and Chung 1992; Laird and Bennett 1993; Miki et al. 1998; Palecek et al. 1992a,b; Pitcher et al. 1999; Seltzer et al. 1990), and it has...
recently been reported that tight partial nerve ligation of the sciatic nerve induced hypersensitivity of the hind paw to innocuous mechanical stimulation. This hypersensitivity became obvious at 2 days after nerve injury and lasted for 145 days. Thus peripheral-nerve-injury-induced decrements of paw withdrawal threshold with an early onset that persisted for a long period. Furthermore, mechanical hypersensitivity of the whisker pad area was observed on the contralateral side relative to the IAN transection as well. A bilateral effect of nerve injury has also been reported by some researchers (Malan et al. 2000; Ossipov et al. 1999). The IAN is classified as a pure sensory nerve unlike the sciatic nerve. It is possible that this physiological difference between the IAN and sciatic nerve is responsible for the differences of behavioral responses in the IAN transected and CCI rats. However, the pattern of the escape behavior observed in the rats with IAN transection was similar to that in the CCI rats.

Ipsilateral effect of IAN transection on MDH neuronal activity

LTM and WDR neurons were distributed in the deep and superficial laminae of the MDH and NS neurons were exclusively found in the superficial laminae. Furthermore, distribution differences of these neurons in the MDH were not observed on the ipsilateral and contralateral sides relative to the transection, and in naive rats. The present data are consistent with the previous results showing that peripheral nerve injury does not affect the intraspinal distribution of nociceptive neurons in rats (Broton et al. 1988; Dubner and Bennett 1983; Dubner et al. 1978; Hu 1990; Iwata et al. 1999). It is likely that IAN transection does not alter the spatial arrangement of nociceptive and nonnociceptive neurons in the MDH.

It has been reported that the behavioral hypersensitivity to innocuous mechanical stimulation of the RF originated from the increased excitability of the peripheral and CNS. Numerous studies have shown that primary afferent nerve fibers have significantly higher spontaneous activity following peripheral nerve injury (Kajander and Bennett 1992; Lisney and Devor 1987; Tal and Eliav 1996; Wall and Devor 1983). Centrally, spinal and thalamic neurons have high spontaneous activity and hyperexcitability to mechanical and/or thermal stimulation of the RFs following nerve injury as well (Laird and Bennett 1992, 1993; Molander et al. 1992; Palecek et al. 1992a,b). Some reports indicate that peripheral-nerve-injury-induced changes in responses to both mechanical and thermal stimulation, whereas some demonstrated that only mechanical responses were affected by nerve injury (Catheline et al. 1999; Palecek et al. 1992a,b). Therefore it is still controversial whether peripheral-nerve injury contributes to the development of mecano-alldodynia and/or thermal hyperalgesia. These differences may be the result of technical differences in peripheral-nerve treatment in each study. To reduce the involvement of technical artifacts related to nerve treatment, the IAN was chosen because the IAN has only sensory afferent fibers. Thus its lesion would not produce impairment on motor functions. Furthermore, we used complete transection of the IAN to produce consistent damage on the IAN.

We observed hyperexcitability of MDH WDR neurons to innocuous and noxious mechanical stimulation of the RFs but
The effects of peripheral-nerve injury on the contralateral side have been reported in many previous studies (Kolzenburg et al. 1999; Malan et al. 2000; Ossipov et al. 1999). Some reports have shown that the effect of nerve injury on the contralateral side is similar to that of the ipsilateral side. Our behavioral data showed that a unilateral IAN transection induced hypersensitivity to innocuous mechanical stimulation of the whisker pad bilaterally even though the ipsilateral side was much more sensitive than the contralateral side. There have been many discussions about the mechanisms responsible for the bilateral effect of unilateral peripheral-nerve injury (Kolzenburg et al. 1999). It has been reported that plastic changes of the nervous system are frequently observed in the spinal cord after peripheral-nerve injury (Woolf et al. 1992, 1995). These changes are a possible mechanism underlying the hypersensitivity to innocuous mechanical stimulation on the contralateral side relative to the IAN transection. As observed in the present study, LTM neurons recorded from the contralateral side are similar to that of the ipsilateral side. Our behavioral data showed that a unilateral IAN transection induced hypersensitivity to innocuous mechanical stimulation of the whisker pad bilaterally even though the ipsilateral side was much more sensitive than the contralateral side. There have been many discussions about the mechanisms responsible for the bilateral effect of unilateral peripheral-nerve injury (Kolzenburg et al. 1999). It has been reported that plastic changes of the nervous system are frequently observed in the spinal cord after peripheral-nerve injury (Woolf et al. 1992, 1995). These changes are a possible mechanism underlying the hypersensitivity to innocuous mechanical stimulation on the contralateral side relative to the IAN transection. 

Contralateral effect of IAN transection on MDH neuronal activity

The effects of peripheral-nerve injury on the contralateral side have been reported in many previous studies (Kolzenburg et al. 1999; Malan et al. 2000; Ossipov et al. 1999). Some reports have shown that the effect of nerve injury on the contralateral side is similar to that of the ipsilateral side. Our behavioral data showed that a unilateral IAN transection induced hypersensitivity to innocuous mechanical stimulation of the whisker pad bilaterally even though the ipsilateral side was much more sensitive than the contralateral side. There have been many discussions about the mechanisms responsible for the bilateral effect of unilateral peripheral-nerve injury (Kolzenburg et al. 1999). It has been reported that plastic changes of the nervous system are frequently observed in the spinal cord after peripheral-nerve injury (Woolf et al. 1992, 1995). These changes are a possible mechanism underlying the hypersensitivity to innocuous mechanical stimulation on the contralateral side relative to the IAN transection. As observed in the present study, LTM neurons recorded from the contralateral side relative to the IAN transection were hypersensitive soon after nerve injury. It is probable that LTM neurons are
involved in shortening of withdrawal latency to innocuous mechanical stimulation of the whisker pad. Hyper-responsive-ness to innocuous mechanical stimulation of the MDH neurons recorded from the contralateral side relative to the transection may be induced by the mobilization of the immune system. Further studies may be necessary to clarify the central mech-anism for the contralateral effect of the unilateral IAN transec-tion.

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