

HARD-FOOD MASTICATION SUPPRESSES COMPLETE FREUND'S ADJUVANT-INDUCED NOCICEPTION

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Abstract—The effect of food hardness during mastication on nociceptive transmission in the spinal cord was studied by analyzing complete Freund's adjuvant (CFA) induced nociceptive behavior and Fos expression. The behavioral study showed that the shortening of the withdrawal latency following CFA injection into the hind paw was depressed after a change in the given food hardness from soft to hard. The depression of nociceptive behavior in the rats with hard food was reversed after i.v. injection of naloxone. Fos protein-like immunoreactive cells (Fos protein-LI cells) were expressed in the superficial and deep laminae of the L4–6 spinal dorsal horn after s.c. injection of CFA into the hind paw during soft food mastication. The number of Fos protein-LI cells was decreased in the rats with hard food mastication followed by soft food. This reduction of Fos protein-LI cells following change in food hardness was reversed after i.v. application of naloxone. Furthermore, the depression of Fos protein-LI cells following hard food intake was significantly inhibited after bilateral inferior alveolar nerve transection or bilateral ablation of the somatosensory cortex.

These findings suggest that the change in food hardness during mastication might drive an opioid descending system through the trigeminal sensory pathway and somatosensory cortex resulting in an antinociceptive effect on chronic pain. However, IAN transection and cortical ablation did not induce 100% reversal of Fos expression, suggesting other than trigeminal sensory system may be involved in this phenomena, such as the pathway through the brainstem reticular formation. © 2003 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: A, anterior; ABC, avidin–biotin complex; ANOVA, analysis of variance; CFA, complete Freund's adjuvant; DAB, 3,3'-diaminobenzidine-tetra HCl; DH, dorsal horn; Fos protein-LI, Fos protein-like immunoreactive; IAN, inferior alveolar nerve; NGS, normal goat serum; P, posterior; PBS, phosphate-buffered saline.

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Pain is a complex process, usually involving sensory–discriminatory, emotional–motivational, autonomic, motor and cognitive aspects. Most of our current knowledge is based on the sensory–discriminative aspect of pain. Little attention has been paid to the cognitive aspects of pain and its modulatory process.

Previous clinical studies indicated that the poor dentition status might lead to a poor health condition and mental impairment (Nakata, 1998; Miura et al., 1998; Locker et al., 2000, 2002). The poor dentition status might lead a poor health condition and mental impairment (Nakata, 1998; Miura et al., 1998; Locker et al., 2000, 2002). These data suggested that the characteristics of mastication might be important for the maintenance of our physical well being, especially for senescent people who retain their functional teeth. However, we know nothing concerning the correlations between the patients' well-being and mastication and the degree of pain suffering. Here we are reporting an animal study showing that the masticatory behavior can modulate pain processing with respect to sensory–motor integration via cortical mechanisms.

As pain is important to individual survival, mastication is a part of conscious food-consumption behavior. Food hardness can profoundly influence the masticatory process (Yamamura et al., 1998). The sensory information from teeth is known to be conducted in trigeminal nerve and ascending into higher levels for further sensory–motor integration in order to generate mastication (Komuro et al., 2001). It is also known that cortical control mechanisms appear to equate these with cognitive process (Yao et al., 2002); however, it is not clear either cortical process or motor behavior could contribute to antinociception. Recently, it has been reported that sweet solution intake induces antinociception in human newborns (Blass and Watt, 1999). These data reveal that the trigeminal sensory system is somehow involved in the modulation of chronic pain, but the exact neural pathways remain elusive. Despite our knowledge of the neural pathways involved in mastication, little is known concerning how the descending modulation system contributes to the modulation of the chronic pain through the trigeminal sensory afferents and their ascending neural paths to reach the modulatory circuit (Basbaum and Fields, 1979).

The aims of the present study were to determine how a change in food hardness modulates the descending inhibitory system and to explore the possible neural pathways involved with this effect. We also examined this

aspect of mastication to gain insight into the neural pathways involved with the mastication and pain modulation by studying the possible contributions from sensory input from the trigeminal system, and cortical involvement, as well as involvement of the opioid modulatory system.

EXPERIMENTAL PROCEDURES

Experimental animals

The present experiment was approved by the Animal Experimental Committee of Osaka University School of Dentistry and animals were treated according to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Adult male Sprague–Dawley rats (260–320 g, total number of rats: $n=55$) were used for all experiments. The rats were housed in plastic cages, whose walls and floor were smooth. Thus, they were not able to gnaw the wall of the cage. The soft food and hard food groups were classified according to the hardness of the food that was given. The soft food was made by grinding of the hard food (50 g/kg) with water (200 ml/kg).

The time schedule of each experiment group is illustrated in Fig. 1. Fig. 1A is the time schedule for the behavioral test and Fig. 1B is that for the Fos experiment. The soft-food-group rats were able to freely access the soft food (250 g/kg/day) through the whole experimental period. Fifty-gram (/kg) regular pellets (hard food) and 200 ml (/kg) water were given to the hard-food-group rats at every day after 10 days of exposure to the soft food.

Animal preparation

At 11 days after soft food intake, rats were anesthetized with ethyl ether. Complete Freund' adjuvant (CFA) suspended in an oil/saline (1:1) emulsion (0.05 ml) was spontaneously injected into the ventral surface of the left hind paw centered in the footpad. The rats were divided into six groups ($n=5$ in each group). Experimental groups were classified as soft-food group (behavior study: $n=5$, Fos study: $n=5$), hard-food group (behavior study: $n=5$, Fos study: $n=5$), hard food+saline (behavior study: $n=5$, Fos study: $n=5$), hard food+naloxone (behavior study: $n=5$, Fos study: $n=5$), hard food+inferior alveolar nerve (IAN) transection (Fos study: $n=5$), hard food+cortex ablation (Fos study: $n=5$) and control group in which rats were given hard food without change in food hardness (behavior study: $n=5$) as illustrated in Table 1. The rats were anesthetized with pentobarbital Na (50 mg/kg, i.p.) and surgeries were applied to the rats as follows. 1) Soft-food group: The rats subsequently had free access to soft food during the experimental period (Fig. 1). 2) Hard-food group: They had free access to hard food and water (200 ml/kg/day) following the soft food intake for 11 days (Fig. 1). 3) Naloxone-treated group: They were implanted with an osmotic mini-pump (Alza Corporation, Palo Alto, CA, USA) filled with naloxone. After the animals recovered from the anesthesia, they had free access to hard food and water (200 ml/kg/day) for 6 days (behavior experiments) or 3 days (Fos experiments) as illustrated in Fig. 1Ab and 1Bb. 4) Saline group: They were implanted with an osmotic mini-pump filled with saline similar to the naloxone group. After recovery from the anesthesia, they had free access to hard food and water (200 ml/kg/day) for 6 days (behavior experiments) or 3 days (Fos experiments) as illustrated in Fig. 1Ab and 1Bb. 5) The cortical lesion group: The primary somatosensory cortex was ablated bilaterally. After recovery, they had free access to the hard food and water (200 ml/kg/day for 3 days) as illustrated in Fig. 1Bc. 6) The IAN transection group: The bilateral IANs were transected. After the recovery from the anesthesia, they had free access to hard food and water (200 ml/kg/day) for 3 days as illustrated in Fig. 1Bc.

Behavioral assessment of nociception

The nociceptive threshold was quantitatively assessed as previously described by Hargreaves et al. (1988). Rats were placed on a glass surface of a thermal plantar testing apparatus (UGO BASILE, VA, Italy) and acclimated for 30 min on the glass plate before testing. The temperature of the glass surface was maintained at 25 °C. A high-intensity and movable radiant heat source was placed under the glass and focused at the mid-plantar surface of the paw. The paw-withdrawal latency was recorded by means of a digital timer. A cutoff time of 22 s was used to prevent tissue damage. The withdrawal latencies for the left and right paws were measured twice for each trial. The behavioral tests were done at >5-min intervals at the same time schedule every day. The average withdrawal latency of the ipsilateral hind paw was subtracted from the average latency of the contralateral untreated site to establish the difference score.

Implantation of the osmotic mini-pump

Animals were anesthetized with pentobarbital Na (50 mg/kg, i.p.) and the osmotic mini-pump was implanted subcutaneously in the interscapular region. It was connected to a 5-cm length of PE10 tubing and the end of the tubing was inserted into the left external jugular vein. This pump and tubing were filled with naloxone (7 mg/ml) or saline and the pump constantly delivered the naloxone or saline (0.001 ml/h) into the vein for 3 days. After the end of the experiment, the amount of the solution in the osmotic pump was measured to verify the continuous injection of the solution.

IAN transection

Five rats were anesthetized with pentobarbital Na (50 mg/kg, i.p.). The facial skin was cut and the alveolar bone was exposed. Both sides of the IAN were exposed and anesthetized with the topical application of lidocaine (0.05 ml) to prevent the afferent barrage due to nerve transection. The IANs were sutured at the proximal and distal portions with a length of 1.0 mm between them. Then a 0.5 mm length of IAN between the sutured regions was cut and removed under local anesthesia. After that, the facial skin was sutured and an antibiotic (penicillin G potassium, 20,000 units) was injected intraperitoneally for protection from infection.

Lesion of the cerebral cortex

The primary somatosensory cortex was removed bilaterally (rostro-caudal: A0.0–P3.2; medio-lateral: 4.0–7.0). Five rats were anesthetized with pentobarbital Na (50 mg/kg, i.p.) and mounted on a stereotaxic frame. The round holes (3 mm in diameter) which were located at 3 mm caudal from bregma and 5.5 mm lateral from the midline, were bilaterally drilled through the skull and the primary somatosensory cortex was aspirated using a blunted 22-gauge needle. After that, the head skin was sutured and an antibiotic (penicillin G potassium, 2000 units) was injected intraperitoneally for protection from infection.

Fos immunohistochemistry

At 14 days after soft-food and/or hard-food intake, animals were anesthetized with pentobarbital Na (80 mg/kg, i.p.) and perfused through the aorta with 500 ml of 0.02 M phosphate-buffered saline (PBS, pH 7.4) and followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The L4–6 spinal cord was removed, post-fixed in the same fixative for 3 days at 4 °C with agitation, and transferred to 30% sucrose (w/v) in phosphate buffer for several days for cryoprotection. Fifty-micron-thick sections were cut on a freezing microtome and every fourth section was collected in PBS for immunocytochemical processing. Free-floating tissue sections were rinsed in PBS, 3% normal goat serum

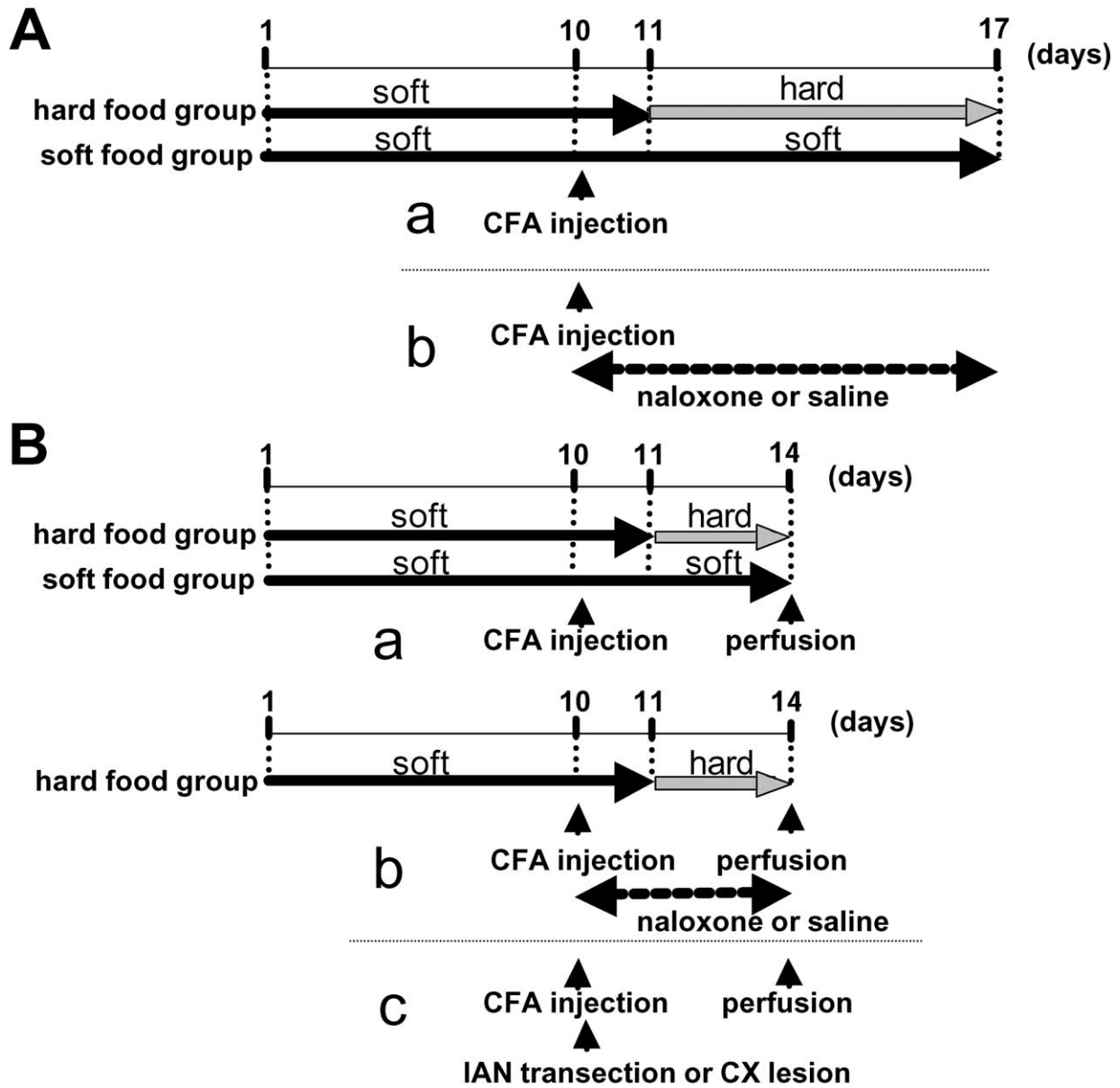


Fig. 1. A schematic illustration of the time course of the present experiment. Hard-food group. Rats were fed with soft food for 11 days and then the food hardness was changed from soft to hard after that. Soft-food group. Rats were fed with soft food through the experimental period, 14 or 17 days. (A) The time course for the behavioral experiments. (a) CFA was injected at 10 days after soft-food delivery and the food hardness was changed from soft to hard at 1 day after that. (b) CFA+naloxone or CFA+saline was injected with mini-pump at 10 days after soft-food delivery and the food hardness was changed from soft to hard at 1 day after that. (B) The time course for the Fos experiments. (a) CFA was injected at 10 days after soft-food delivery and the food hardness was changed from soft to hard or not changed after that and the rats were perfused at 14 days after soft-food delivery. (b) CFA+naloxone or CFA+saline was injected at 10 days after soft-food delivery and the food hardness was changed from soft to hard at 1 day after that, and the rats were perfused at 3 days after changing of the food hardness. (c) CFA injection+IAN transection or CFA injection+cortical lesion was applied at 10 days after soft-food delivery and the food hardness was changed from soft to hard at 1 day after that, and the rats were perfused at 3 days after changing of the food hardness.

(NGS) in PBS for 1 h, then incubated for 72 h with rabbit anti-c-fos (1:20,000: c-fos ab-5, Oncogene, MA, USA) in 3% NGS at 4 °C. After three washes in PBS with 0.75% Triton X-100 and in PBS, the sections were incubated with biotinylated secondary IgG (1:200; Vector Laboratories, Burlingame, CA, USA) for 24 h at 4 °C. Following rinses in PBS with Triton three times, the sections were incubated in peroxidase-conjugated avidin–biotin complex (1:100;

ABC, Vector Laboratories) for 2 h at room temperature. To develop the ABC reaction product, the sections were incubated in 0.035% 3,3'-diaminobenzidine-tetra HCl (Sigma), 0.2% nickel ammonium sulfate, and 0.05% peroxide in 0.05 M Tris buffer (pH 7.4). Finally the sections were rinsed in PBS, mounted on gelatin-coated slides, dehydrated in alcohols, cleared in xylems, and covered with Eukitt (O. Kindler, Germany).

Table 1. The experimental groups for the behavioral and Fos experiments

		Behavioral study			
		Drug administration or surgical treatment	Number of rats	CFA	Survival time (day)
Soft	—	—	5	+	17
Hard	—	—	5	+	17
Hard	Saline	—	5	+	17
Hard	Naloxone	—	5	+	17
Control	—	—	5	+	17
Fos Study					
Soft	—	—	5	+	14
Hard	—	—	5	+	14
Hard	Saline	—	5	+	14
Hard	Naloxone	—	5	+	14
Hard	IAN transection	—	5	+	14
Hard	Cortical ablation	—	5	+	14

Total: n=55

Data analysis

Cells with black deposits in the nuclei were considered as Fos protein-like immunoreactive (Fos protein-LI) cells. Three sections were chosen, the section with the most labeling, and the rostral and caudal sections from this section. The camera lucida drawings of these sections were done and the number of Fos-positive neurons was counted in each rat. Three sections (one most labeled and the two adjacent sections) were chosen from each rat and the number of labeled cells of individual nuclei per section was counted. The mean number of Fos-protein LI cells of these three sections (number of Fos protein-LI cells per section) was calculated from five rats. Data are presented as mean±S.E.M. Statistical comparisons were made by one of following methods: limited number of a priori Student's *t*-test where time point was compared within a time course study (Denenberg, 1984), or paired *t*-test, or one- or two-way analysis of variance (ANOVA) with repeated measures and post hoc Student-Newman-Keuls test where appropriate.

RESULTS

Nocifensive behavior

CFA-induced hyperalgesia was quantified by measuring withdrawal latencies to heat stimulation applied to the injected and untreated hind paws and difference score was calculated. Difference score on the injected side was significantly increased after CFA injection into the hind paw for 1–5 days and returned to the preoperative level at 6–7

days after injection as compared with the untreated side (Table 2 and Fig. 2A). We could not observe any differences between soft and control groups as illustrated in Table 2. The peak hyperalgesia was reached at 2 days following CFA injection (mean difference score±S.E.M., 1.45±0.3) as illustrated in Fig. 2A. When the food hardness was changed from soft to hard, difference score was significantly decreased at 2, 4 and 5 days during hard-food intake as illustrated in Fig. 2A (soft versus hard, mean±S.E.M., 2 days: 1.45±0.3 versus 0.79±0.24; 4 days: 1.35±0.28 versus 0.43±0.48; 5 days: 1.42±0.42 versus -0.1±0.13; *t*-test, *P*<0.05). This shortening of paw-withdrawal latency, which was indicated by an increase in difference score, was reversed after i.v. injection (via an osmotic mini-pump) of naloxone as compared with saline injection for 2–3 days following CFA injection (naloxone versus saline, 2 days: 2.08±0.32 versus 1.59±0.29, *t*-test, *P*<0.01; 3 days: 1.78±0.43 versus 0.75±0.5, *t*-test, *P*<0.01; 4 days: 1.16±0.31 versus 0.33±0.43, *t*-test, *P*<0.05) as illustrated in Fig. 2B. Thus, the naloxone effect lasted until the cessation of the experiment. A significant increase in the difference score was observed at 1–4 days after CFA injection as compared with that of pre-CFA during naloxone application and returned to the pre-CFA levels at 6–7 days after injection (solid squares, one-way ANOVA with repeated measures,

Table 2. Paw-withdrawal latency (ies) following heating of the hind paw

		Pre	1 day	2 days	4 days	7 days
Hard	Ipsi	3.3±0.44	3.2±0.31	3.6±0.46	3.4±0.34	3.9±0.17
	Cont	3.7±0.38	4.0±0.28	4.1±0.51	3.4±0.34	3.8±0.23
Soft	Ipsi	3.9±0.45	2.5±0.34	2.5±0.10	3.1±0.21	4.0±0.50
	Cont	3.8±0.39	3.4±0.53	3.5±0.26	4.1±0.30	4.3±0.64
Saline	Ipsi	4.0±0.55	3.6±0.38	3.7±0.23	3.8±0.20	4.5±0.52
	Cont	3.8±0.49	4.7±0.38	4.4±0.27	3.6±0.13	4.3±0.38
Naloxone	Ipsi	3.5±0.19	2.8±0.27	3.3±0.20	3.1±0.44	4.4±0.53
	Cont	3.5±0.19	4.4±0.12	5.2±0.34	4.2±0.51	4.5±0.54
Control	Ipsi	4.4±0.02	3.1±0.17	2.3±0.13	2.7±0.04	4.0±0.05
	Cont	4.5±0.09	4.3±0.10	4.5±0.20	4.1±0.21	4.0±0.06

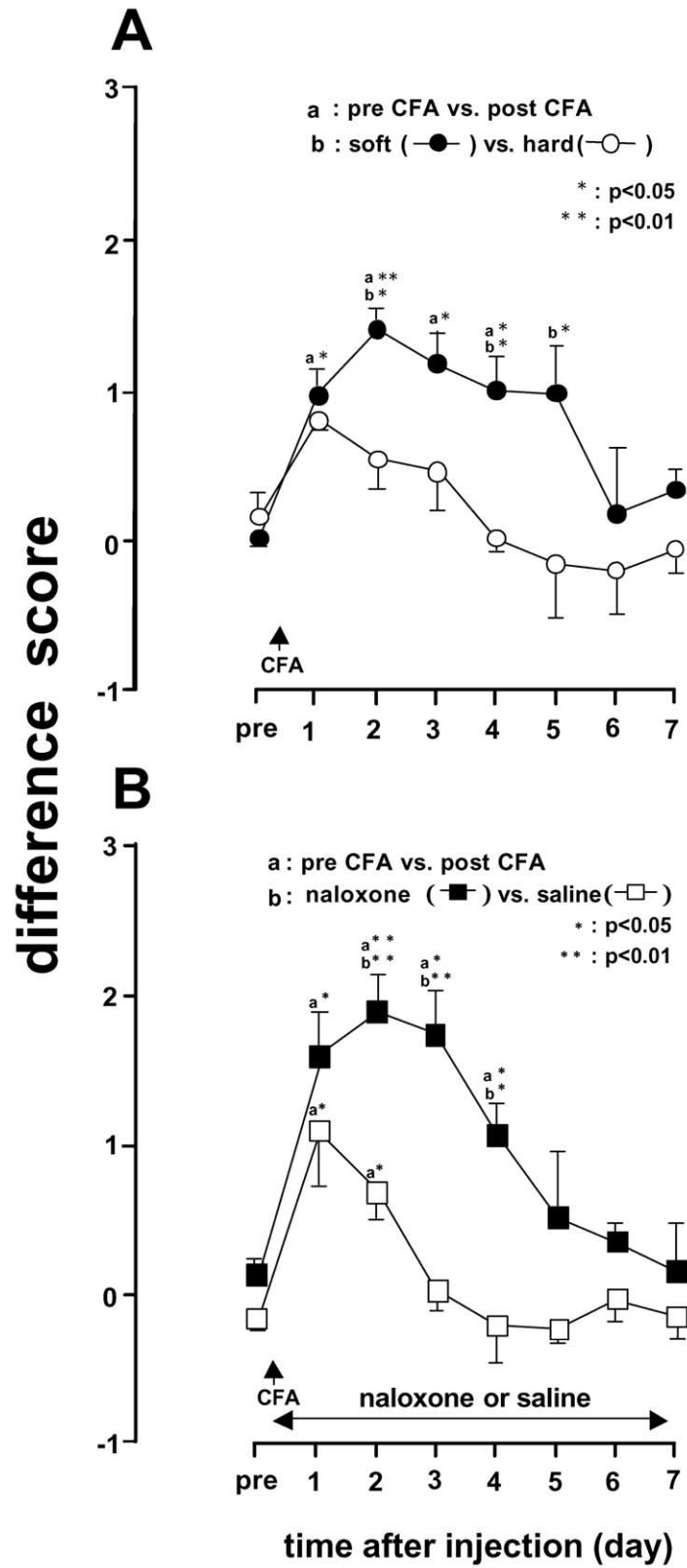


Fig. 2. Nocifensive behaviors following heat stimulation of the hind paw in the soft-food (solid circles) and hard-food groups (open circles) (A) and those with naloxone (solid squares) or saline (open squares) injection (B). pre-CFA: Mean paw-withdrawal latency before CFA injection. Post-CFA: Mean paw-withdrawal latency after CFA injection. Soft: Soft food group in this and following figures. Hard: Hard food group in this and following figures. Naloxone: Rats fed with hard food following CFA+naloxone injection. Saline: Rats fed hard food with CFA+saline injection.

Dunnett tests). In contrast, a significant increase in difference score was observed at 1–2 days during saline injection and no difference was observed after that as illustrated in Fig. 2B (open squares, one-way ANOVA with repeated measures, Dunnett tests).

These results show that CFA-induced hyperalgesia was significantly reduced in the hard-food group relative to the soft-food group and this inhibition of the hyperalgesia was reversed by naloxone administration.

Fos expression in the dorsal horn

Effect of hard-food mastication. Two days after CFA injection, many Fos protein-LI cells were expressed bilaterally in the superficial laminae of the dorsal horn (DH) of the L4–5 spinal cord as illustrated in Fig. 3A. Furthermore, we observed in soft-food group that significantly many more Fos protein-LI cells were present in the superficial laminae of the ipsilateral side to the CFA injection than the contralateral side (ipsilateral 100.7 ± 19.48 versus contralateral 50.2 ± 12.62 , paired *t*-test, $P < 0.05$). No significant differences were observed in the deeper laminae between ipsilateral and contralateral sides to the CFA injection (ipsilateral 51.1 ± 16.13 versus contralateral 49.9 ± 13.69 , paired *t*-test, $P > 0.05$). A significantly smaller number of Fos protein-LI cells was expressed in the ipsilateral side to CFA injection of the superficial DH of the rats with hard-food intake as compared with the soft-food group (soft 101.0 ± 21.73 versus hard 28.3 ± 3.33 , two-way ANOVA with repeated measures, Student-Newman-Keuls test, $P < 0.05$), but not for contralateral DH (soft 50.2 ± 12.62 versus hard 17.1 ± 3.26 , $P > 0.05$). We also observed a significant difference in the number of the Fos protein LI-cells in the ipsilateral side of the deeper DH between the soft- and hard-food groups (soft 51.1 ± 16.13 versus hard 20.2 ± 2.72 , $P < 0.05$ respectively), but not in the contralateral DH (soft 49.9 ± 13.69 versus hard 19.9 ± 5.31 , respectively; two-way ANOVA with repeated measures, Student-Newman-Keuls test $P > 0.05$). These observations indicate that the change in food hardness affects the expression of persistent pain induced by chronic inflammation.

Effect of naloxone injection. In order to test the involvement of opioid modulation on the food-hardness-induced antinociception, we studied the effect of the chronic infusion of naloxone on the CFA-induced Fos expression in the L4–5 DH neurons. As illustrated in Fig. 4, numerous Fos protein-LI cells were bilaterally expressed in both superficial and deep laminae and when the food hardness was changed from soft to hard, a significant depression of the number of Fos protein-LI cells was induced bilaterally. Although naloxone injection induced a significant increment of the expression of Fos protein-LI cells, naloxone injection did not completely reverse the hard-food effect to the preoperative level as shown in Fig. 3. As illustrated in Fig. 4, a significantly larger number of Fos protein-LI cells were expressed in the laminae I–II of the ipsilateral DH of the rats with naloxone administration (hard+naloxone 89.5 ± 26.66 versus hard 28.3 ± 3.33 and hard+saline

25.5 ± 3.91 , $P < 0.01$, two-way ANOVA with repeated measures, Student-Newman-Keuls tests) but no difference was found between hard and hard+saline groups ($P > 0.05$). On the other hand, we did not observe any significant differences between naloxone-treated and hard food or saline-injected rats in the contralateral DH and the deep laminae of the ipsilateral DH (two-way ANOVA with repeated measures, $P > 0.05$).

Effect of IAN transection. In order to evaluate the effect of trigeminal sensory input on the food-hardness-induced antinociception, we studied the effect of bilateral IAN transection on the CFA-induced Fos expression in the L4–5 DH neurons. As illustrated in Fig. 5 numerous Fos protein-LI cells were expressed bilaterally in the superficial laminae of the DH after IAN transection; a significantly larger number of Fos protein-LI cells were expressed in the ipsilateral DH of the rats after IAN transection (superficial: hard+transection 60.4 ± 4.53 versus hard 28.3 ± 3.33 , deep: hard+transection 39.9 ± 5.25 versus hard 20.2 ± 6.54), and both superficial (hard+transection 31.1 ± 3.85 versus hard 17.1 ± 3.26) and deep laminae (hard+transection 37.7 ± 5.25 versus hard 19.9 ± 5.31) of the contralateral side to IAN transection than the comparable hard-food-intake group without any treatments ($P < 0.01$, two-way ANOVA with repeated measures, Student-Newman-Keuls tests) as shown in Fig. 5B. Although the increment of Fos protein-LI cells in the IAN transection group was significantly larger as compared with the hard-food group, the number of Fos protein-LI cells in IAN transection group was not restored completely to the level found in the soft-food group (superficial laminae: 100.7 ± 19.48) as illustrated in Fig. 3B.

Effect of cortical lesion. As shown above, IAN transection produced a significant increment of CFA-induced Fos expression in the L4–5 DH neurons. It is known that the sensory information from the IAN relays to the somatosensory cortex via the trigeminal–thalamic–cortical pathway (Iwata et al., 1994, 1998; Sessle, 2000). Therefore, we tested the effect of somatosensory cortex lesion on CFA-induced Fos expression. Bilateral sensory cortices were ablated as shown in Fig. 6A. As we previously showed in Fig. 3, the hard-food intake induced significant depression of Fos protein-LI cells in the L4–5. This reduction of the number of Fos protein-LI cells was significantly reversed following cortical ablation as illustrated in Fig. 6C (superficial laminae, ipsilateral side: hard+cerebral cortex lesion 63.5 ± 6.18 versus hard 28.3 ± 3.33 , two-way ANOVA with repeated measures, Student-Newman-Keuls tests, $P < 0.01$, contralateral side: hard+Cr lesion 32.6 ± 3.37 versus hard 17.2 ± 3.26 , two-way ANOVA with repeated measures, Student-Newman-Keuls tests, $P < 0.05$). However, this reversal induced by cortical lesion was similar to that of the IAN transection (Fig. 5B). In the cortical lesion experiment, a small non-significant effect on the contralateral side to CFA injection was observed as well and the effect was much stronger in the superficial laminae than in the deeper laminae.

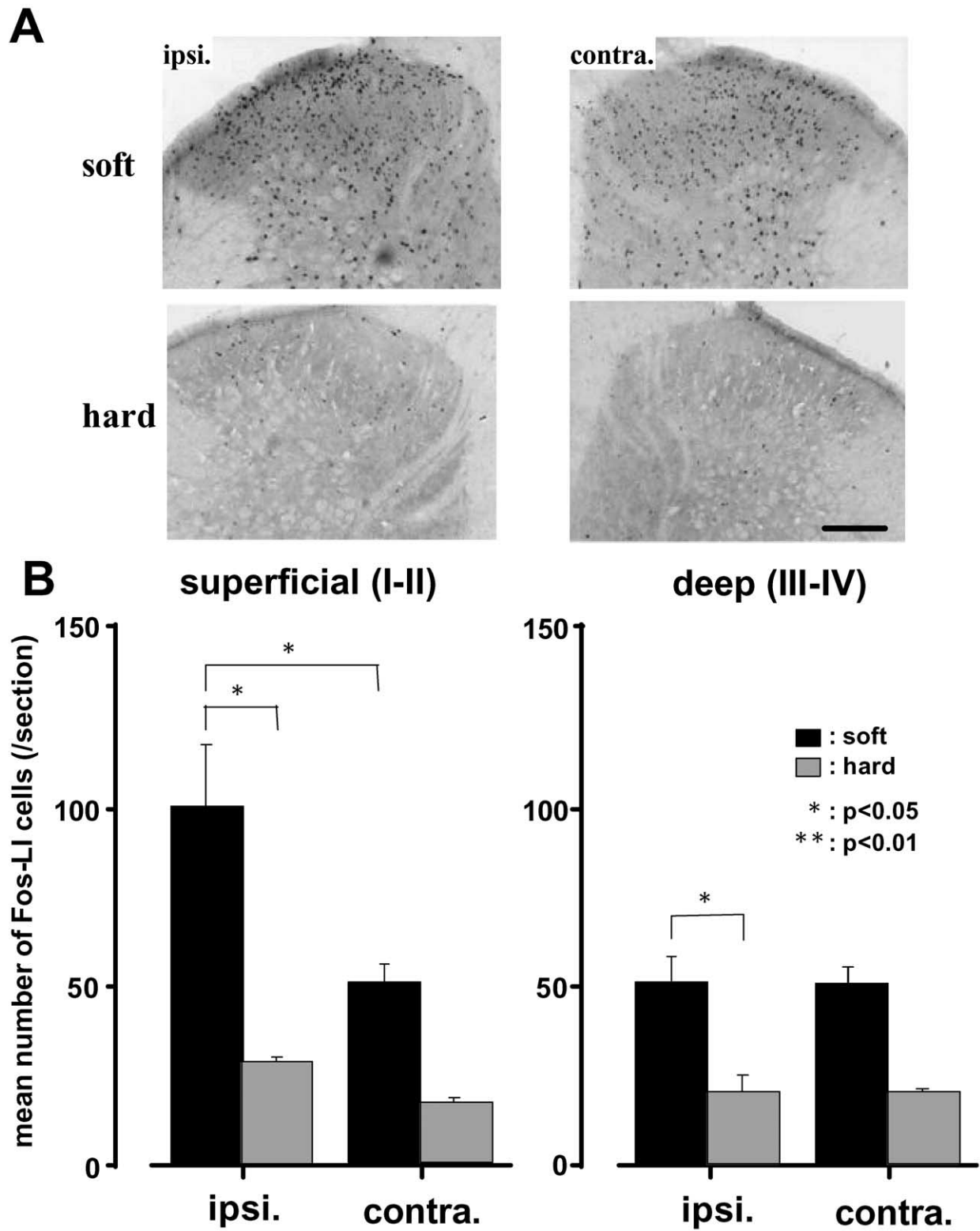


Fig. 3. Fos expression in the L4–5 spinal dorsal horn of the rats fed soft food or hard food following CFA injection. (A) Photomicrographs of L5 spinal dorsal horn of the rats fed soft food (upper panel) and hard food (lower panel) following CFA injection. Scale bar=150 μ m. (B) mean number of Fos protein-LI cells in the L4–5 dorsal horn of the rats fed soft food following CFA injection (soft, solid column) or hard food following CFA+injection (hard, shaded column). Three sections (the section with the largest number of Fos expression and other two sections with less Fos expression) were chosen from each rat and the number of Fos protein-LI cells was averaged. The averaged number of Fos protein-LI cells was collected from five rats and the mean number of Fos protein-LI was calculated.

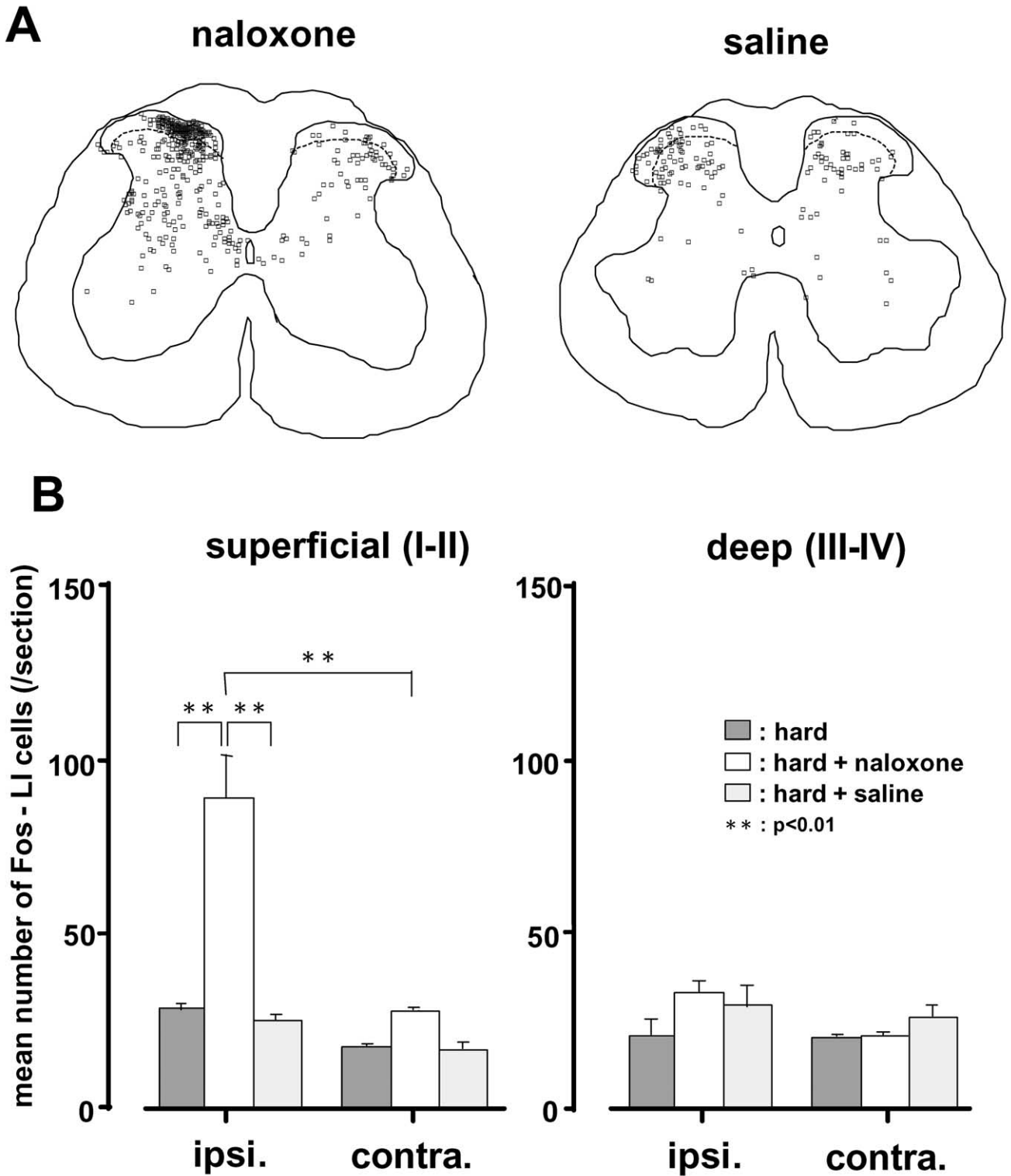
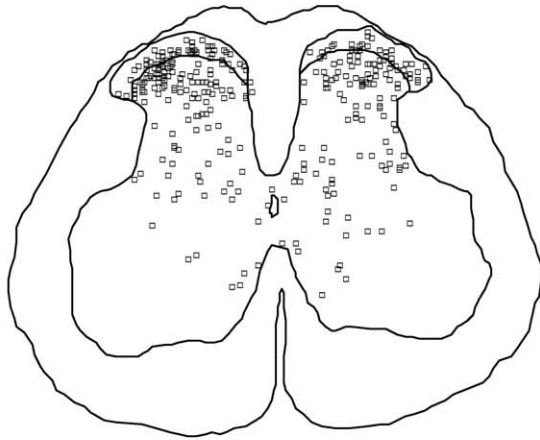


Fig. 4. Fos expression in the L4–5 dorsal horn of the rats fed hard food following CFA+naloxone (naloxone) or saline injection (saline). (A) Camera lucida drawings of L5 spinal dorsal horn of the rats fed hard food following CFA+naloxone injection (left side) and CFA+saline injection (right side). (B) Mean number of Fos protein-LI cells in the L4–5 dorsal horn of the rats fed hard food following CFA injection (hard, shaded column), CFA+naloxone injection (naloxone, open column) or CFA+saline injection (saline, dotted column).

A

IAN transection



B

superficial (I-II)

deep (III-IV)

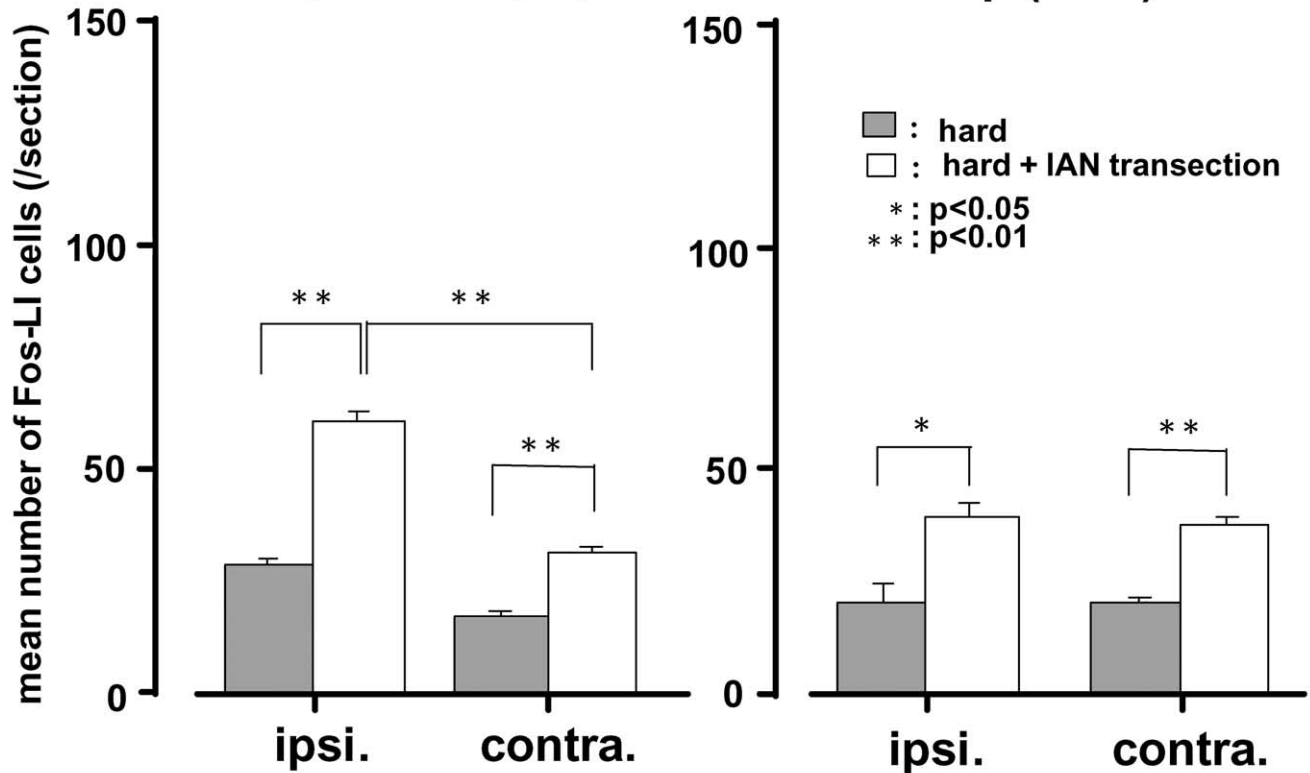


Fig. 5. Fos expression in the L4–5 dorsal horn of the rats fed hard food following CFA+IAN transection. (A) Camera lucida drawings of L5 spinal dorsal horn of the rats fed hard food following CFA+IAN transection. (B) Mean number of Fos protein-LI cells in the L4–5 dorsal horn of the rats fed hard food following CFA injection (hard, shaded column) or CFA+IAN transection (hard+IAN transection, open column).

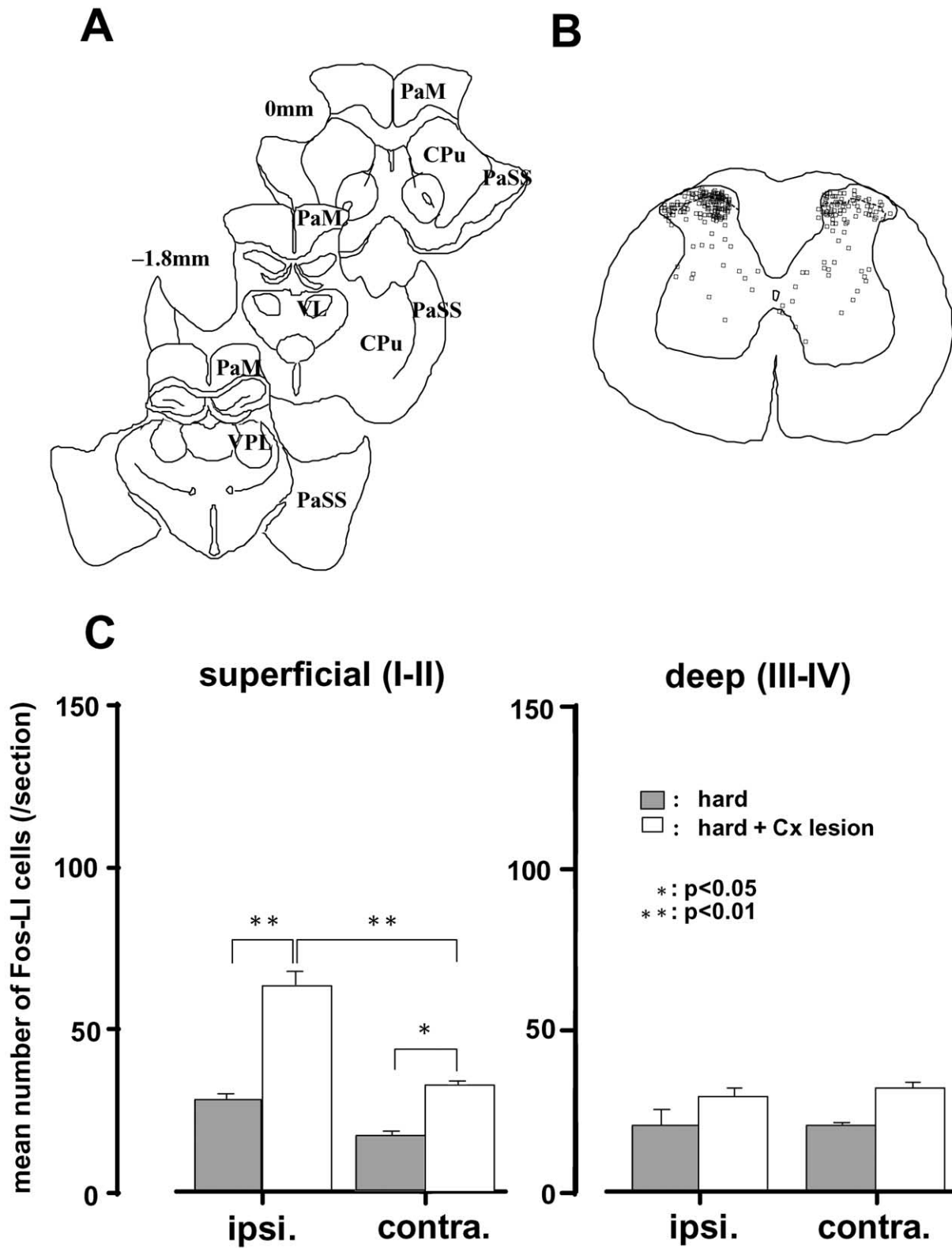


Fig. 6. Fos expression in the L4–5 dorsal horn of the rats fed hard food following CFA+cortical ablation. (A) The camera lucida drawings of ablated cortical areas. (B) The camera lucida drawings of L5 spinal dorsal horn of the rats fed hard food following CFA+bilateral cortical ablation. (C) Mean number of Fos protein-LI cells in the L4–5 dorsal horn of the rats fed hard food following CFA injection (hard, shaded column) or CFA+cortical ablation (hard+cerebral cortex lesion, open column). PaM, parietal motor cortex; PaSS, parietal somatosensory cortex; CPu, caudate putamen; VL, ventrolateral thalamic nucleus; VPL, ventral posterolateral thalamic nucleus.

DISCUSSION

We observed that a significant decrease in the escape threshold for thermal stimulation of the hind paw was induced at 1–5 days after CFA injection into the hind paw during soft-food intake. This decrement in escape threshold following CFA injection was reversed after a change in food hardness from soft to hard. Furthermore, the Fos-expression pattern also illustrated a similar reduction of dorsal horn neurons after the change in given food hardness from soft to hard. These data reveal that change in food hardness induced antinociceptive effect on CFA-induced persistent pain. The systemic administration of naloxone reversed this escape-threshold increment induced by hard-food intake. This observation indicates that the opioid system is somehow involved in the antinociception induced by hard-food intake. The number of Fos protein-LI cells was decreased in the rats with hard-food mastication following soft food. This reduction of Fos protein-LI cells following change in food hardness was also reversed after i.v. administration of naloxone. Furthermore, the depression of Fos protein-LI cells following hard-food intake was significantly inhibited after bilateral IAN transection or bilateral ablation of the somatosensory cortex.

It is likely that this antinociceptive effect induced by hard-food intake has a similar neuronal mechanism to that responsible for the antinociception observed in infant rats (Blass and Fitzgerald, 1988; Blass et al., 1991, 1995) or human infants (Blass and Hoffmeyer, 1991; Blass and Shah, 1995; Blass, 1997), during sweet-food or milk intake. Although they did not study beyond the neuronal pathway involved, they suggested that trigeminal sensory input somehow contribute to the some opioid descending pathways. Together with previous data, the present results suggest that the activation of trigeminal sensory and/or motor systems in a particular state, i.e. chewing the “hard” food, activates the descending pain modulation system, which induced antinociception in the hind-paw region. It has been reported that the extensive regions of the spinal cord were affected by the diffuse inhibitory system activated by peripheral inflammation (Miki et al., 2002; Terayama et al., 2002). It is probable that this antinociceptive effect induced by chewing hard food or ingesting sweet food is produced by an activation of this diffuse inhibitory system.

It is not known which neuronal pathways are involved in the activation of the opioid-related descending modulation system following hard-food intake. Two possible pathways are thought to be involved in this descending system. One includes the sensory afferents pathway from the intra-oral structures. The other system is the efferent pathway producing jaw movement. In order to clarify which pathways are dominantly involved in this antinociception, we arranged two lesion experiments. First, the effect of the bilateral IAN transection on Fos expression following change in food hardness was studied to elucidate how intra-oral sensory information contributes to this antinociception on CFA-induced hyperalgesia. As illustrated in Fig. 4A, the depression of Fos expression induced by hard-

food intake was significantly reversed following IAN transection. However, the increment of Fos LI cells after IAN transection was not completely reversed as compared with untreated rats that had soft food or the rats with naloxone injection (superficial laminae of the ipsilateral DH, CFA+soft food: 100.7 ± 19.5 ; CFA+hard food: 28.3 ± 4.0 , CFA+hard food+IAN: 60.4 ± 4.5). The reason for this incomplete reversal may be that other mechanisms may be involved, such as the masticatory motor system or muscular afferent activation to interact with the cortical loop, as we studied in the cortical lesion experiment (see below) before finally reaching the opioid pain modulatory system.

Recently, it has been reported that the primary and second sensory cortical areas or parietal cortex are involved in endogenous antinociceptive function (Kharkevich and Churukanov, 1999; Kuroda et al., 2001, 2002). These studies suggest that the somatosensory cortex may be involved in descending modulation system for pain perception. Therefore, we designed the lesion study of the somatosensory cortex in our animal model, in order to clarify the possible involvement of the somatosensory cortex in the antinociceptive effect induced by “hard food” mastication. The present study demonstrated that the bilateral ablation of the somatosensory cortex produced a significant increment of the number of the Fos protein-LI cells in the L5 spinal dorsal horn. This suggests that the antinociceptive effect of hard food was reversed by cortical lesion. It is of interest that the effect of cortical ablation and IAN transection on Fos expression is similar each other as illustrated in Figs. 5 and 6. As described above, we did not observe a 100% reversal of the antinociception in the rats with hard-food intake following IAN transection or cortical ablation. It is known that there are many ascending pathways from the intra-oral structures to the CNS, such as the pathways through the parabrachial nucleus, hypothalamus and medial thalamic nuclei (Craig et al., 1989; Burstein et al., 1990; Cliffer et al., 1991; Iwata et al., 1992; Craig, 1995). It is probable that other systems may be involved in hard-food-induced antinociception as well as the thalamo-cortical pathway described in the present study. It was interesting that following cortical ablation or IAN transection, almost same number of Fos protein LI cells was expressed on both sides after CFA injection into the hind paw. These data sharing of common neural substrates suggest a possible direct connection that the afferent information from the intra-oral structures comes up to the somatosensory cortex and activates the descending modulation system, resulting in an antinociceptive effect induced by peripheral inflammation. However, we could not observe a 100% reversal of antinociception after INA transection or cortical ablation. Thus, we cannot deny the involvement of other neuronal pathways contributing to this antinociceptive effect instead of somatosensory pathway.

CONCLUSIONS

The possible antinociceptive pathway suggested in the present study is as follows. The sensory receptors in the intra-oral structures are activated by jaw movement during

hard-food intake. The somatosensory information from the intra-oral structures is sent to the higher CNS through the ascending sensory pathways and reaches the somatosensory cortex. The activation of the somatosensory cortex facilitates the descending inhibitory system, resulting the antinociceptive effect on CFA-induced nociception. Although the naloxone injection produced almost a 100% reversal of Fos expression as compared with control rats, IAN transection or cerebral cortex ablation did induce a partial, but significant, reduction of Fos expression.

These partial reversible effects may indicate the involvement of other neural pathways in this antinociceptive effect in addition to the pathways we have suggested in the present study.

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