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Dorsal horn neurons having input from low back structures in rats

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Abstract

The mechanisms of nociception in the low back are poorly understood, partly because systematic recordings from dorsal horn neurons with input from the low back are largely missing. The purpose of this investigation was to (1) identify spinal segments and dorsal horn neurons receiving input from the low back, (2) test the effect of nerve growth factor (NGF) injected into the multifidus muscle (MF) on the neurons' responsiveness, and (3) study the influence of a chronic MF inflammation on the responses. In rats, microelectrode recordings were made in the segments L2, L3, and L5 to find dorsal horn neurons having input from the low back (LB neurons). In control animals, the proportion of LB neurons in L2 was larger than in L3 and L5. Most LB neurons had a convergent input from several tissues. Injections of NGF into MF increased the proportion of LB neurons significantly. A chronic MF inflammation likewise increased the proportion of LB neurons and the input convergence. The centers of the neurons' receptive fields (RFs) were consistently located 2–3 segments caudally relative to their recording site. The results show that (1) input convergence from various tissues is common for LB neurons, (2) the input from structures of the low back is processed 2–3 segments cranially relative to the vertebral level of the RFs, and (3) the responsiveness of LB neurons is increased during a pathologic alteration of the MF. The above findings may be relevant for some cases of chronic low back pain in patients.

Keywords: Low back pain mechanisms; Multifidus muscle; Dorsal horn neurons; Nerve growth factor (NGF); Complete Freund's adjuvant (CFA); Hypertonic saline

1. Introduction

Low back pain (LBP) can originate from many tissues in the low back [17]. Irritation of nerve roots by a dislocated vertebral disc has long been known to cause LBP [15]. Moreover, substances released from nucleus pulposus tissue may sensitize or excite dorsal roots [19,21,22]. Recently, muscles and fascia of the low back as sources of LBP are being more and more appreciated [4,16].

The basic mechanisms of LBP have not been thoroughly studied so far. The activity of primary afferents from rat paraspinal tissues has been investigated by Yamashita and colleagues [28] and Bove and Light [5]. Spinal neurons having input from the cat lumbar skin were described by Devor and Wall [6]. Later, Gillette and colleagues [7] characterized neurons that had input also from deep tissues of the low back. The topography of receptive fields (RFs) and neuronal sensitization were not addressed, however. The authors also reported the modulatory influence of sympathetic activity [8] and somatosensory input [9]. This overview shows that in comparison to other types of pain, little is known about LBP.

Traditionally, genuine low back muscles are assumed to receive their innervation from the same spinal segment [3]. However, several studies showed that – in the rat – primary afferent fibers supplying low back muscles originated 2–3 segments cranially relative to the location of the muscle segment studied [20,25,26].

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Therefore, for a better understanding of LBP, a systematic study on the topography of RFs and the response behavior of dorsal horn neurons having input from low back structures was necessary.

LBP patients often exhibit symptoms of peripheral or central sensitization (allodynia, hyperalgesia and spread of pain). However, the behavior of sensitized dorsal horn neurons with input from structures in the low back (called LB neurons in this article) is unknown. Central sensitization is assumed to be essential for the development of chronic LBP [2].

In this study, two techniques were used to study the sensitization of LB neurons:

1. Nerve growth factor (NGF). In humans, intramuscular injection of NGF evoked mechanical allodynia and hyperalgesia one day later [24]. We used NGF to study acute sensitization.
2. Experimental myositis. Infiltration of a muscle with complete Freund's adjuvant (CFA) induces a sensitization for more than 2 weeks, a period considered chronic for rats [23]. The CFA myositis was used to study effects of chronic sensitization.

The present study aimed at answering three questions: (1) in which spinal segment(s) is the input from low back structures processed, and what are the response properties of LB neurons? (2) What is the acute effect of NGF injected into the multifidus muscle (MF, a genuine low back muscle) on these neurons? and (3) how do LB neurons react to a chronic CFA-induced inflammation of MF?

2. Materials and methods

2.1. Animals

Forty-six male Sprague–Dawley rats (290–500 g) were used in this study. Free access to food and water was available throughout the experiment. The animals received special care in accordance with the German law on the protection of animals and with the ethical guidelines of the International Association for the Study of Pain (IASP, [29]). All experimental procedures were reviewed and approved by the Animal Care and Use Committee of the University of Heidelberg prior to the onset of experiments.

2.2. Experimental groups

The experimental animals were assigned to one of the following groups.

Groups 1–3: control (CTR) groups in which recordings were made in the segments L2, L3, and L5 in rats with intact (no NGF, no CFA) MF muscle.
Group 1: recordings in L2 (CTR:L2, $n = 38$ neurons, 6 rats)
Group 2: recordings in L3 (CTR:L3, $n = 38$ neurons, 5 rats)
Group 3: recordings in L5 (CTR:L5, $n = 38$ neurons, 6 rats)
Groups 4 and 5: rats in which the left MF was injected with NGF (concentration 0.8 μM, injection volume 2×25 μl) one day prior to recording. The injections were made at the vertebral level L4 and L5, i.e. two segments of the left MF were injected.
Group 4: recordings in L3 (NGF:L3, $n = 38$ neurons, 6 rats)
Group 5: recordings in L5 (NGF:L5, $n = 38$ neurons, 6 rats)
Groups 6–8: animals in which an experimental inflammation of 6 and 12 days duration was induced with bilateral CFA injections (4×50 μl) into the MF at the vertebral level L4 and L5.
Group 6: CFA 6 days, recordings in L2 (CFA:L2 (6d), $n = 33$ neurons, 6 rats)
Group 7: CFA 6 days, recordings in L3 (CFA:L3 (6d), $n = 28$ neurons, 6 rats)
Group 8: CFA 12 days, recordings in L2 (CFA:L2 (12d), $n = 28$ neurons, 5 rats)

2.3. Intramuscular injection of NGF and CFA

To see if NGF had a sensitizing effect on LB neurons, the animals in the NGF group (groups 4 and 5) were given intramuscular injections of a concentration of NGF identical to that used by Svensson and colleagues ([24]; β-nerve growth factor, human recombinant, Calbiochem, concentration 0.8 μM, 2×25 μl) into the left MF at the vertebral level L4 and L5 one day prior to the experiment. This acute sensitizing action of NGF was compared to the effect of a chronic muscle inflammation on the dorsal horn neurons. The chronic inflammation was induced by bilateral injections of CFA (Difco Laboratories, USA) into the MF at the vertebral level L4 and L5, 6 or 12 days prior to the experiment. The injections of NGF and CFA were made over 3 s with a 27-gauge needle under ultra-short anesthesia with isoflurane (Baxter Germany, GmbH). The needle was advanced into the muscle just beside the spinous process until it contacted the bone of the vertebral arches. Then the cannula was withdrawn 1 mm and the material deposited close to the bone to make sure that the MF was injected. After the injections of NGF and CFA, the animals were returned to their cages, fed and drunk normally until the day of the final experiment.

2.4. General surgical procedures

In the final experiment, the animals were deeply anesthetized with sodium thiopental (Trapanal®, Altana Pharma, Germany, 100 mg/kg i.p. initially), followed by an i.v. infusion of the same anesthetic at a constant rate of 2 mg/h using an infusion pump to maintain a deep and constant level of anesthesia during the experiment. A catheter was inserted into the right external jugular vein for administration of the anesthetic and a muscle relaxant. The anesthesia was deep enough to abolish flexor reflexes and marked blood pressure changes

(exceeding 10 mmHg) to noxious pinching of the tail and to electrical stimulation of dorsal roots at an intensity supramaximal for C-fiber excitation. The muscle relaxant (pancuronium bromide, Pancuronium®, Organon, Germany, 0.6–0.8 mg/h/rat i.v.) was used to immobilize the animals. Another catheter was inserted into the right common carotid artery for measuring blood pressure. Mean arterial blood pressure and body core temperature were continuously monitored and kept at physiological levels (above 80 mmHg and between 37 and 38 °C, respectively). The electrocardiogram was monitored, and the depth of the anesthesia adjusted so that the heart rate did not exceed 430 bpm. The animals were artificially ventilated via a tracheal cannula with a gas mixture of 47.5% O_2, 2.5% CO_2, and 50% N_2 (arterial pO_2 above 100 mmHg, pCO_2 between 32 and 40 mmHg, pH close to 7.4). The rats were mounted on a spinal frame and fixed in prone position.

2.5. Laminectomy

Under deep anesthesia a small laminectomy was performed to expose the spinal segments L2, L3, and L5 for recording and the dorsal roots L3–L5 for electrical stimulation to identify dorsal horn neurons. The laminectomy did not affect the caudal back muscles, because the spinal segments for recording were located about 3–5.5 cm cranially from these muscles. Following the laminectomy, a pool was made with silicon oil to cover the recording sites and the exposed low back muscles. The dura mater was then opened with fine scissors and glass microelectrodes were inserted into the spinal dorsal horn (Fig. 1A).

2.6. Extracellular recordings

Microelectrodes were made from glass filaments (GB150F-10, Science Products GmbH, Germany) using a horizontal puller (Brown-Flaming Micropipette Puller, Model P-80, Sutter Instrument Co., San Francisco, CA, USA). The microelectrodes were filled with 5% NaCl solution (DC resistance 8–33 MΩ) for extracellular recording of dorsal horn neurons. Vertical microelectrode tracks were made aiming at the lateral one-third of the dorsal horn up to a depth of about 1000 μm from the dorsal surface of the spinal cord. The filtered (Neurolog 125) and amplified (Neurolog 106) action potentials were monitored on an oscilloscope and stored on a computer using Spike2 software (Cambridge Electronic Design, Ltd., version 3.21, data sampled at 20 kHz). The action potentials of the neurons were captured later by an off-line analysis using the same software.

2.7. Electrical stimulation

As a search stimulus during microelectrode tracking, the dorsal roots L3–L5 were electrically stimulated with a bipolar electrode (square pulses of 0.3 ms width, once per 3 s, Fig. 1B). Once a neuron was identified, its electrical threshold was determined with gradually decreasing stimulus intensities. The electrical threshold was defined as an intensity that induced neuronal excitation at a probability of approximately 50%. The latency of the action potential was measured at 1.2 times electrical threshold. Dorsal horn neurons were identified by the criteria that their action potentials showed a clear jitter and/or did not follow high frequency stimulation (333–500 Hz).

2.8. Classification of dorsal horn neurons

The receptive fields (RFs) of the electrically identified dorsal horn neurons were primarily identified with mechanical stimuli applied to the ipsilateral hindlimb, hip, abdominal wall, and tail, as well as low back structures (e.g. muscle, fascia, and periosteum) bilaterally. Light touch with an artist's brush was used as an innocuous stimulus for the skin, pinching of the epidermis with a watchmaker's forceps as a noxious one. When the neuron did not respond to brushing and pinching of the skin, the RF was considered to be deep. The deep tissues of the low back could be tested directly, because the skin of that area was opened to make the pool. In the low back, moderate (innocuous deformation) and noxious (strong squeezing) pressure stimuli were applied with the blunt back of the artist's brush. The noxious pressure stimulus was painful when applied to the experimenter's hand. If the RF was located in the superficial tissues of the low back, pinching of the thoracolumbar fascia with watchmaker's forceps was used to check if the RF was in the fascia.

The dorsal horn neurons were functionally classified according to a previous study of our group [12]. The following neuron types were distinguished: (1) LTM cutaneous

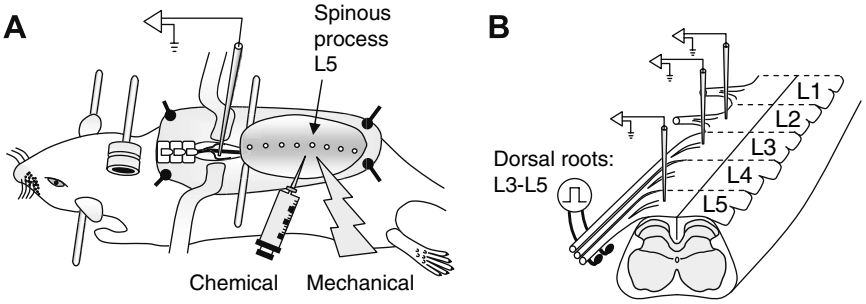

Fig. 1. Experimental set-up. (A) Schematic drawing of the set-up. The animal was mounted on a spinal frame with clamps. The back skin was opened to expose the lumbar spinal cord and the low back structures, then a pool was made of skin flaps and filled with silicon oil. The distance between the recording segment and the low back structures searched for RFs was about 3–5.5 cm. (B) The spinal segments recorded from in this study. Extracellular recordings were made from the segments L2, L3, and L5. Single dorsal horn neurons were identified by electrical stimulation of the dorsal roots (L3–L5).

(low-threshold mechanosensitive), having a low mechanical threshold and maximal response to innocuous stimuli, (2) MR cutaneous or WDR (multi-receptive or wide dynamic range), having a low mechanical threshold but maximal response to noxious stimuli, (3) HTM cutaneous (high-threshold mechanosensitive), having a high mechanical threshold, response only to noxious stimuli, (4) LTM deep (low-threshold mechanosensitive), having response to weak innocuous pressure, and (5) HTM deep (high-threshold mechanosensitive), having response only to noxious stimuli.

2.9. Chemical stimulation

An intramuscular injection of hypertonic saline (5%, 50 μl) into the MF beside the spinous process L5 or directly into the RF was given to examine the sensitivity of the dorsal horn neurons to chemical stimuli. In some cases when a neuron had a RF in the low back fascia, a cotton ball soaked with 5% NaCl was put on the RF. In a previous study of our group, hypertonic saline was the most effective stimulus for group IV primary afferent units [13].

2.10. Effect of CFA on the mechanical withdrawal threshold

In the CFA groups, the mechanical pain threshold of the MF was measured in behavioral experiments before, 6 days, and 12 days after CFA injection to test for mechanical hyperalgesia. The threshold was also determined in the GS to see if the CFA injection into the MF had a systemic sensitizing effect. An electronic von Frey anesthesiometer (IITC Inc., USA) was used to measure the threshold (Tip area: 3.46 mm^2). The animals were lightly constrained under a towel. The tip of the anesthesiometer was applied to the MF muscle beside the spinous process L5 or on the center of the GS (between the medial and the lateral head) through the skin. Therefore, an influence of skin input on the measured threshold cannot be excluded. However, with the blunt tip used for stimulation, the contribution of cutaneous receptors to our results is probably small, cf. [27]. Measurements were made bilaterally. Any pain-related reaction of the animal (local muscle twitching, withdrawal of hindlimb, escape movements, vocalization) was considered as threshold. Measurements were repeated three times, and the average of the three trials was taken as the mechanical withdrawal threshold.

2.11. Statistical analysis

Data are shown as means ± standard error of the mean (SEM). Comparison between the different groups was made using the Mann–Whitney *U*-test. Between the different experimental groups, the proportion of neurons was compared with Fisher's exact probability test. The paired *t*-test was used to compare the baseline mechanical withdrawal threshold between the GS and the MF before CFA injection. Comparison of the mechanical pain threshold after CFA injection was made with one-way ANOVA followed by Bonferroni's multiple comparison test. $p < 0.05$ was considered significant.

3. Results

3.1. Depth of the recorded cells

The mean depth of the recorded neurons ranged from 560 to 610 μm at the spinal segment L2. There was no significant difference in the recording depth between the groups 1, 6, and 8 (Fig. 2A and Table 1). At the spinal segment L3, the mean depth of the recorded neurons ranged from 590 to 730 μm. There was a significant difference between groups 2 and 7 (CFA duration 6d), $p < 0.05$, Mann–Whitney *U*-test (Fig. 2B and Table 1). The mean depth of the neurons recorded in L5 ranged from 600 to 650 μm. There was no difference between the two groups (group 3 vs. group 5, Fig. 2C and Table 1).

The reason for the significant difference in recording depths between groups 2 and 7, and for the trend in the same direction in the other CFA injected groups, is not clear. Possibly the main input area from the low

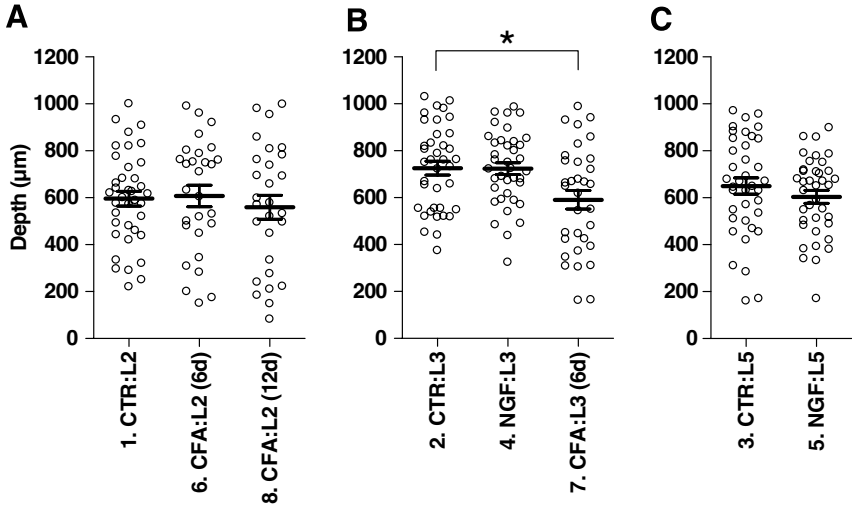

Fig. 2. Depth of the dorsal horn neurons recorded from. (A) Data from segment L2, (B) L3, and (C) L5. Note the significant difference in recording depth in L3 between control group 2 and the CFA group 7 (6d). $^{*}p < 0.05$, Mann–Whitney *U*-test.

Table 1
Characteristics of LB neurons

	Experimental group	Dorsal horn neurons (total)			No. of LB neurons						Center of RFs of LB neurons (vertebral level)
		No. of neurons tested	Depth (μm)	Background activity (imp/s)	No. and (%)	With convergent input	With bilateral RFs	With muscle input			
								Total	LTM	HTM	
1	CTR:L2	38	596.0 ± 31.9	0.08 ± 0.04	7 (18.4)	6	1	6	5	1	L5
2	CTR:L3	38	725.7 ± 29.4	0.44 ± 0.38	2 (5.3)	0	0	1	1	0	L5–L6
3	CTR:L5	38	649.5 ± 34.5	0.27 ± 0.15	1 (2.6)	1	0	1	1	0	S2
4	NGF:L3	38	723.5 ± 25.5	0.56 ± 0.31	9 (23.7)[b]	2	1	8	5	3	L5–L6
5	NGF:L5	38	603.9 ± 27.2	0.78 ± 0.71	5 (13.2)	4	0	5	1	4	S1–S2
6	CFA:L2 (6d)	28	607.6 ± 45.9	1.31 ± 0.75	9 (32.1)	9	1	7	4	3	L4–L5
7	CFA:L3 (6d)	33	590.7 ± 39.6[a]	2.36 ± 0.99	7 (21.2)	7	1	4	3	1	L5–L6
8	CFA:L2 (12d)	28	559.6 ± 50.7	1.89 ± 0.96	9 (32.1)	7	1	9	3	6	L5

[a] Significant difference (CTR:L3 group vs. CFA:L3 (6d) group), $p < 0.05$, Mann–Whitney *U*-test.
[b] Significant difference (CTR:L3 group vs. NGF:L3 group), $p < 0.05$, Fisher's exact probability test.

back shifted to more superficial laminae due to myositis-induced neuroplastic changes in the dorsal horn [11].

3.2. Proportion of LB neurons

In the non-treated control animals, the proportion of LB neurons was 18.4% (7/38 neurons) in segment L2, 5.3% (2/38) in L3, and 2.6% (1/38) in L5 (Fig. 3A–C and Table 1). So, the proportion of neurons with LB input was lowest in that segment (L5) that is traditionally assumed to process the input from the MF portion at the vertebral level L4/L5. In the NGF-injected animals of group 4, nine out of 38 (23.7%) in L3 were LB neurons. This proportion was significantly higher than that of the control animals in the same segment (group 2, $p < 0.05$, Fisher's exact probability test, Fig. 3B and Table 1). All the other data obtained from animals that had received injections of NGF or CFA, respectively, showed an increase in the proportion of LB neurons, but the effect was not significant. These results show that the LB neurons can be easily sensitized by a pathophysiological alteration of the multifidus muscle, but the effects were not very marked. The data from groups 1 and 2 (CTR) as well as groups 6 and 7 (CFA 6d) in the segments L2 and L3 were not statistically different. Therefore, the results from these groups were pooled. The pooled data yielded a significantly higher proportion of LB neurons in the CFA (6 days) groups compared to the CTR groups ($p < 0.05$, Fisher's exact probability test, Fig. 3D).

3.3. Background activity

The mean background (resting) activity during a 60-s period before any intentional stimulation is shown in Table 1. There were no significant differences between the groups. In the control groups at all segments (L2, L3, and L5), the proportion of the LB neurons having

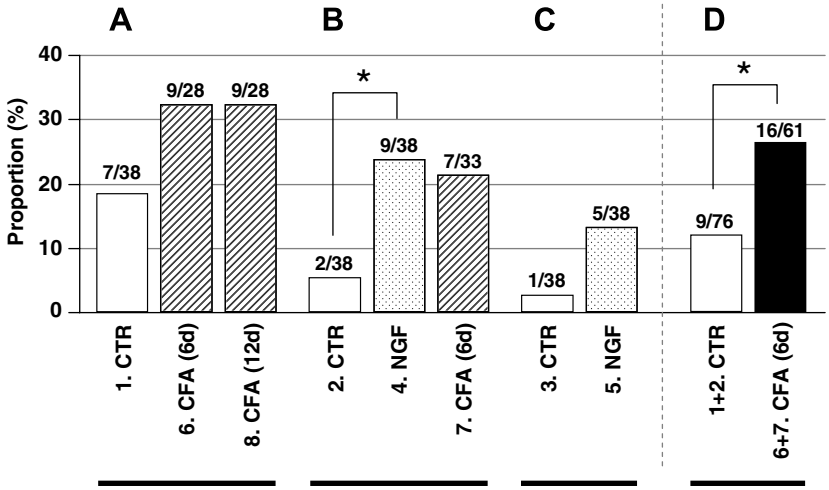

Fig. 3. Proportion of neurons with low back input in the experimental groups. (A) Data from spinal segment L2, (B) L3, (C) L5. (D) Data from segments L2 and L3 were pooled. The differences in the proportion of LB neurons between the CTR:L3 group (group 2) and the NGF:L3 group (group 4, in B), as well as between the pooled CTR:L2/L3 group and the pooled CFA:L2/L3 group in (D), were significant. $^{*}p < 0.05$, Fisher's exact probability test.

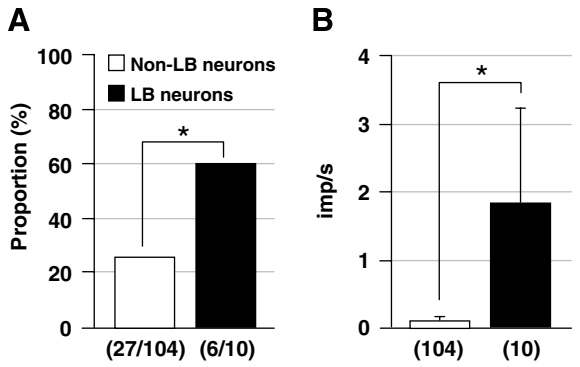

Fig. 4. Background activity of dorsal horn neurons in the control groups. Data from the segments L2, L3, and L5 in the control groups (groups 1–3) were pooled. (A) Proportion of dorsal horn neurons showing background activity. (B) Mean background activity of the same neurons. The proportion and mean activity of the dorsal horn neurons were significantly higher in the LB neurons compared to that of the non-LB neurons ($^{*}p < 0.05$, Fisher's exact probability test).

background activity was 60% (6/10 neurons). In contrast, the proportion of the non-LB neurons showing background activity was 26% (27/104 neurons). The proportion of the LB neurons with background activity was significantly higher than that of the non-LB neurons ($p < 0.05$, Fisher's exact probability test, Fig. 4A). The mean background activity of the LB neurons was likewise significantly higher than that of the non-LB neurons (0.12 ± 0.05 imp/s ($n = 104$ neurons) vs. 1.82 ± 1.42 imp/s ($n = 10$ neurons), $p < 0.05$, Mann–Whitney U-test, Fig. 4B).

3.4. Receptive fields of LB neurons, and types of LB neurons with input from muscle

Fig. 5 shows an example of a neuron from a control animal. The neuron was quite typical in that it exhibited a convergent input from muscle, fascia and skin. Whereas the cutaneous RF had a low mechanical threshold, the deep RFs required noxious stimuli for activation. In Fig. 6, a neuron from a CFA-injected animal (6d) is shown. It likewise had convergent input from muscle, fascia and skin, but in this case the nociceptive input from the fascia was bilateral (Fig. 6A, D and Table 1). The neuron also responded to hypertonic saline put on the surface of the fascia with a small cotton ball (Fig. 6B). The neuron in Fig. 7 was dominated by input from the deep LB muscles at the vertebral level

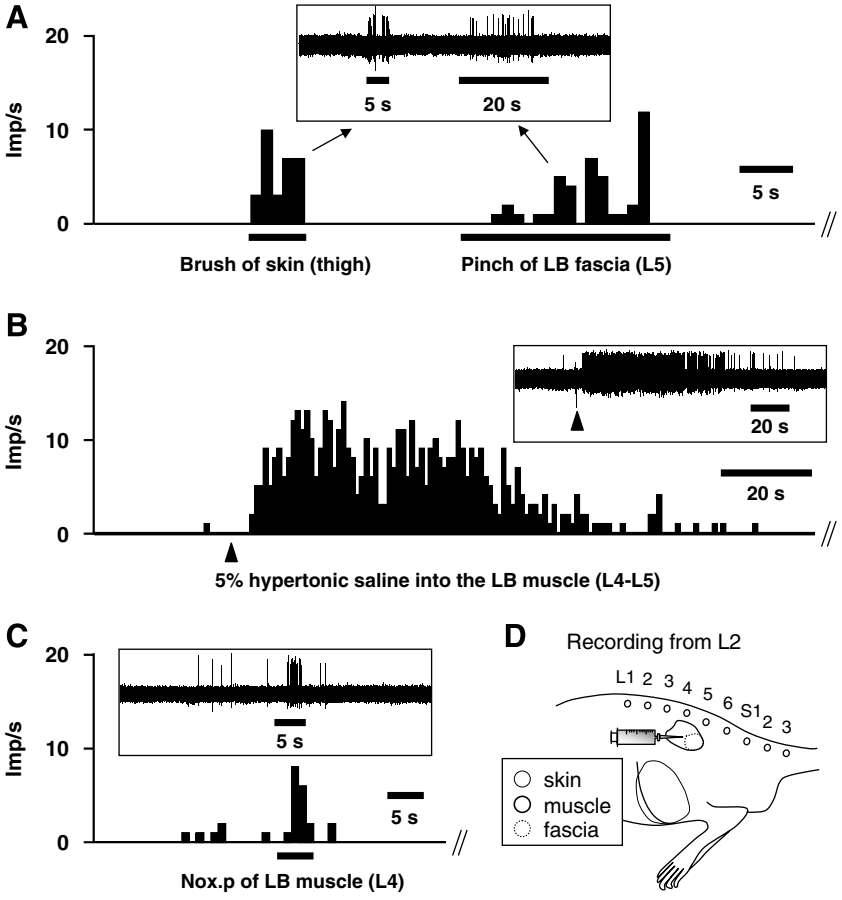

Fig. 5. Recording from a single dorsal horn neuron in segment L2 in a control animal (CTR:L2, group 1). (A) Responses to brushing of the skin (thigh) followed by pinching of the LB fascia (L5). (B) Response to hypertonic saline (5%, 50 μl) injected into an LB muscle lateral to the MF (L4–L5). (C) Response to Nox.p of the LB muscle (L4). This neuron also responded to Mod.p. Continuous recording (A–C). (D) Approximate location and size of the RFs of this neuron. Recording depth 932 μm.

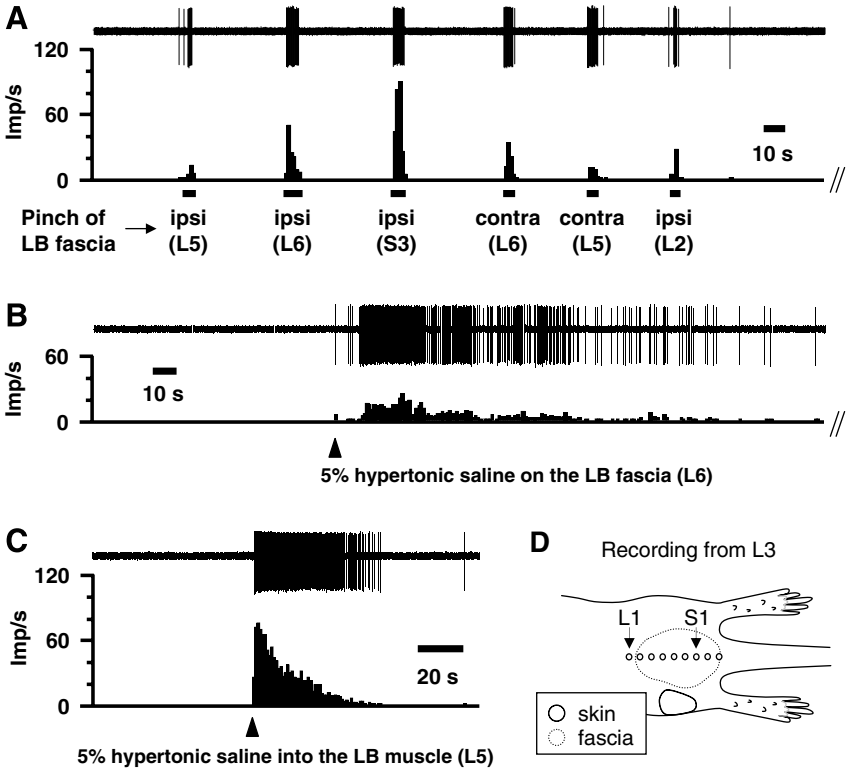

Fig. 6. Recording from a neuron in segment L3 in an animal injected with CFA 6 days prior to the experiment (CFA:L3 (6d), group 7). (A) Responses to pinching of the thoracolumbar fascia bilaterally. (B) Response to 5% hypertonic saline applied on the LB fascia (ipsilateral L6). (C) Response to 5% hypertonic saline injected into the deep MF muscle (ipsilateral L5). Continuous recording (A–C). (D) Approximate location and size of the RFs of this neuron. Recording depth 678 μm. The location and size of the RF in the muscle is not indicated in the figure because it was located underneath the fascia, and the borders of the RFs could not be determined.

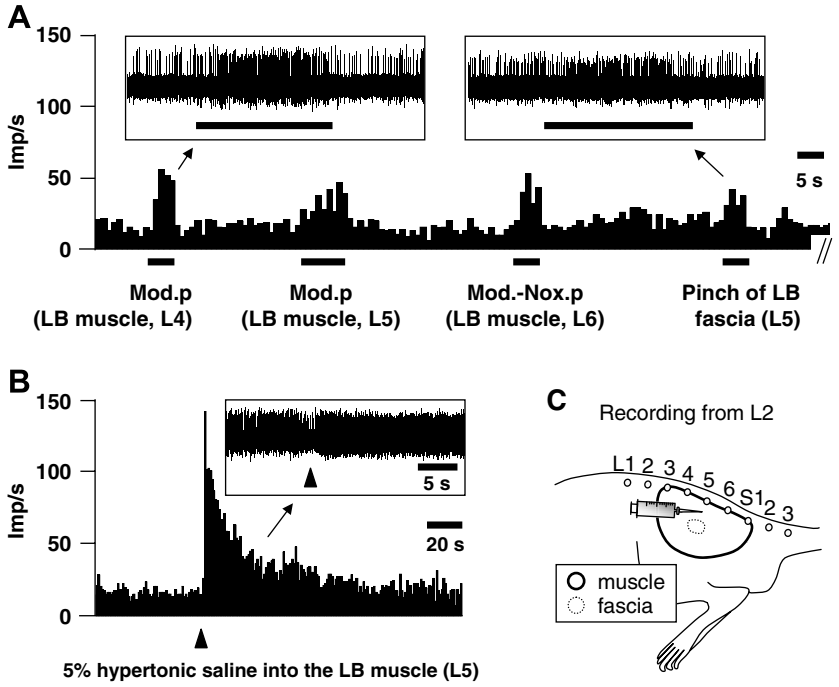

Fig. 7. Recording from a neuron in segment L2 in an animal injected with CFA 12 days prior to the experiment (CFA:L2 (12d), group 8). (A) Responses to Mod.p (L4), Mod.p (L5), Mod.-Nox.p (L6), and pinching of the LB fascia (L5). (B) Response to hypertonic saline (5%, 50 μl) injected into the LB muscle RF (L5). The neuron was vigorously activated by 5% saline at a short latency. Continuous recording (A–B). (C) Approximate location and size of the RFs of this neuron. Recording depth 808 μm.

L3–S1 and had additional input from the lateral part of the thoracolumbar fascia overlying part of those muscles. The neuron was special in that it had a high background activity, probably due to the fact that the MF was chronically inflamed.

The receptive fields of the LB neurons in each group are summarized in Fig. 8, G1–G8. A consistent finding was that the RFs in the skin were located laterally to the deep ones, and sometimes were found even on the distal hindlimb (Fig. 8, G5). The skin of the low back was cut in the midline and reflected to build the pool. This skin region was not tested for cutaneous RFs. The approximate center of the RFs of the LB neurons was always shifted 2–3 vertebral segments caudad relative to the spinal segment the neurons were recorded in (Fig. 8 and Table 1). This finding was consistent and independent of the prior treatment (NGF or CFA) and the spinal segment studied (L2, L3, and L5).

In the CTR groups, 70 % of the LB neurons (7/10 neurons) had convergent input from different regions of the body – such as hindlimb, hip, abdominal wall, and tail – or from different tissues (Table 1). In segment L2, the proportion of LB neurons with convergent input was unchanged 6 days after CFA injection into the MF (group 1 vs. group 6, Fig. 9A). In L3, Fisher's exact probability test yielded a significant increase in the proportion of convergent neurons 6 days after CFA injection (group 2 vs. group 7, $p < 0.05$, Fig. 9B), but the result of the test is questionable, because the CTR group included two cells only. When the data from the segments L2 and L3 were pooled, the proportion of convergent cells was significantly higher after CFA injection (group $1 + 2$ vs. group $6 + 7$, $p < 0.05$, Fisher's exact probability test, Fig. 9C).

Among the LB neurons with input from muscle, both LTM-deep and HTM-deep ones were found. There were no recognizable trends towards a change of the response type after injection of NGF or CFA, or between the spinal segments recorded from (Table 1).

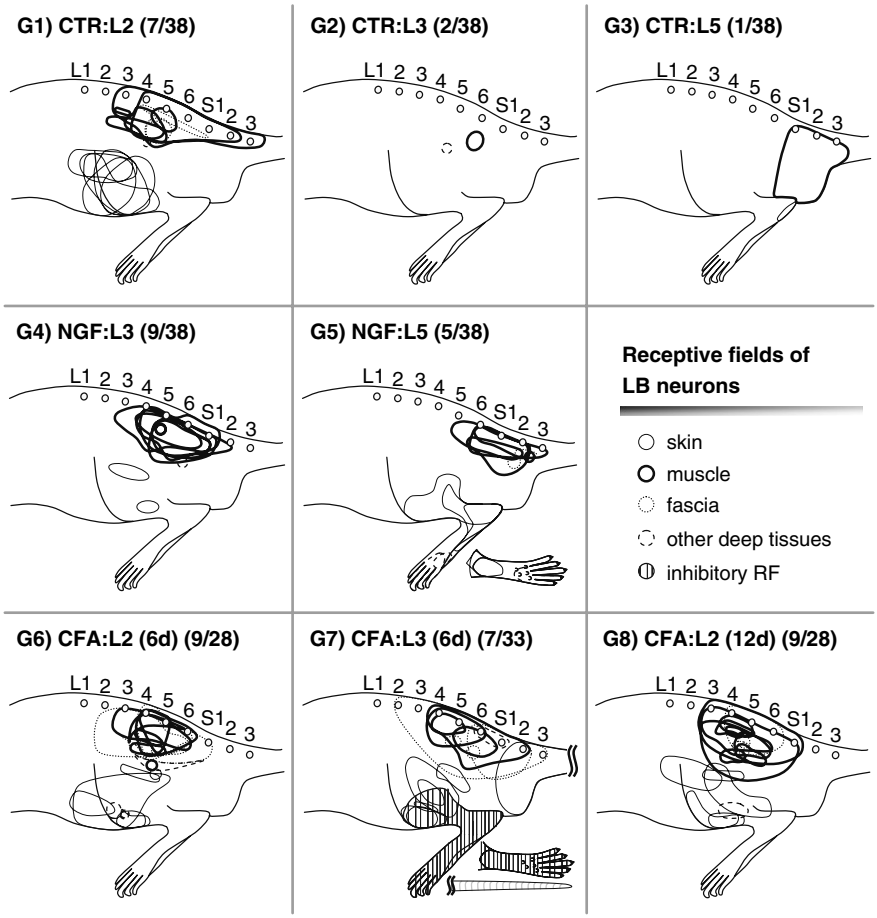

Fig. 8. Summary of the receptive fields of LB neurons in the lumbar segments studied. G1 (group 1): CTR:L2 group. The number in parentheses shows the number of LB neurons in relation to the number of the dorsal horn neurons recorded from in that segment. G2: CTR:L3 group, G3: CTR:L5 group, G4: NGF:L3 group, G5: NGF:L5 group, G6: CFA:L2(6d) group, G7: CFA:L3(6d) group, and G8: CFA:L2(12d) group. Note that the approximate centers of the receptive fields of the LB neurons were consistently shifted 2–3 segments caudad relative to the recorded segments. The shift was likewise present in the groups treated with NGF or CFA.

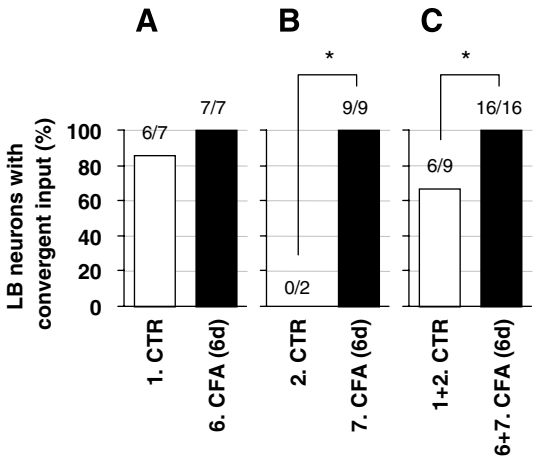

Fig. 9. Proportion of LB neurons with convergent input 6 days after CFA injection into the MF. (A) Proportion of LB neurons with convergent input at L2 and (B) L3. (C) The data from segments L2 and L3 were pooled. The differences in the proportion between the CTR:L3 group (group 2) and the CFA:L3(6d) group (group 7, in B), and between the pooled data from the groups CTR:L2/L3 and the CFA:L2/L3 groups in (C), were significant. $^{*}p < 0.05$, Fisher's exact probability test.

3.5. Effect of CFA on the mechanical withdrawal threshold

In the control animals (no CFA injection), the mechanical threshold upon stimulation of the MF was significantly higher than that of the GS (on both the left and right side; $p < 0.001$, paired t-test, Fig. 10A and B). The pain threshold upon mechanical stimulation of the MF was significantly decreased on the left side 12 days after bilateral CFA injection into the MF, and on the right side 6 and 12 days after CFA when compared to baseline ($p < 0.05$, one-way ANOVA followed by Bonferroni's multiple comparison test, Fig. 10B). The mechanical withdrawal threshold of the GS on both sides was unchanged 6 and 12 days after CFA injection into the MF (Fig. 10A), i.e. under our experimental conditions, no systemic CFA effect on the withdrawal threshold could be detected. Moreover, the results show that there is a significant difference in the mechanical withdrawal threshold between the MF and GS.

4. Discussion

4.1. Deep low back structures do not receive their sensory innervation from the segment they topically belong to

In a previous study of our group, dorsal root ganglion (DRG) cells retrogradely labeled from the MF at the vertebral level L5 were found in all lumbar DRGs on the ipsilateral side with a bell-shaped distribution peaking at L3 [25]. Unexpectedly, there were few labeled DRG cells at the vertebral level of true blue injection (L5). A similar result was obtained by another group with the tracer DiI injected into the MF at the L4 level [20]. The same group also reported that the DiI-labeled neurons were present predominantly in DRGs L2 and L3 when the tracer was applied to the fascia of the longissimus muscle at the vertebral level L5 [26].

In electrophysiological experiments on rat primary afferents by Bove and Light [5], the majority of receptive fields of unmyelinated nociceptors were located in the tail or base of the tail when the recordings were made from the dorsal roots L6 and S1. Other low back structures, such as the intervertebral disc, facet joint, spinous process, skin, and the sacroiliac joint, likewise do not appear to be segmentally innervated at the primary afferent level (for a review, see [1]). These results and ours confirm that the primary afferent fibers from low back structures project to DRGs that are located several segments cranially relative to the location of the tissues. These findings contradict (at least in the rat) the traditional assumption that the genuine low back muscles are supplied by the same spinal segment [3].

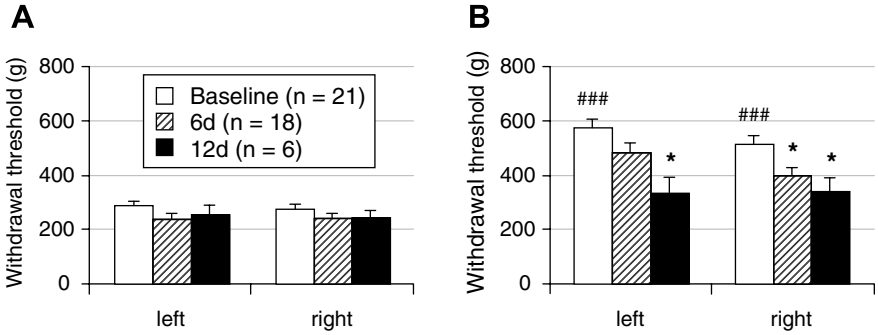

Fig. 10. Data from behavioral experiments showing the mechanical withdrawal threshold of the GS and MF after CFA injection. The animals were injected with CFA into the MF muscle bilaterally at the vertebral level L4 and L5 (4×50 μl). (A) Threshold upon stimulation of the GS, (B) threshold in MF. Note that (1) the baseline threshold in the MF was significantly higher than that in the GS ($^{\#\#\#}p < 0.001$, paired t-test), and that (2) the withdrawal threshold in the MF was significantly decreased after CFA injection ($^{*}p < 0.05$, one-way ANOVA followed by Bonferroni's multiple comparison test), whereas the threshold in the GS remained unchanged 6 and 12 days after CFA injection into the MF.

4.2. Spinal segments receiving input from low back structures

In the control animals, the number of neurons having RFs in the low back was higher in the segment L2 than in L3 and L5. In addition, a convergent input (with at least one RF in the low back) was more frequent at the segment L2. These results suggest that the spinal segment L2 is a major site for the processing of input from low back structures at the vertebral level L4/L5.

At the level L3, the depth of the recorded dorsal horn neurons was more superficial in the CFA(6d) group than in the control group. Particularly, the input from a depth of 300–500 μm, mainly corresponding to the laminae III and IV, appeared to be increased (Fig. 2B). A possible explanation for this finding is that in the sensitized state formerly subthreshold potentials in the superficial dorsal horn have become suprathreshold [14], and therefore, the connections now became visible in the extracellular recordings of the present study.

4.3. Location of receptive fields in the low back

This is the first systematic study describing dorsal horn neurons with RFs in the deep tissues of the low back. The approximate center of the RFs in the low back was consistently shifted 2–3 segments caudad relative to the spinal segments recorded from. The location of the RFs relative to the recording site was not affected by treatment with NGF one day prior to the experiment nor by CFA 6 or 12 days prior to the experiment. In their study on dorsal horn neurons in the cat, Gillette and colleagues [7] did not focus on the location of RFs in the low back, but they presented data showing that RFs in muscle were located at the vertebral level L5–L7 when recordings were made from the segments L4/L5. Taken together, these results from rat and cat suggest that the spinal processing of the input from low back structures is shifted 2–3 segments craniad relative to the location of the tissues, probably mainly due to the cranial shift at the primary afferent level. As in our previous experiments, primary afferents from the MF at the level L5 projected mainly to the DRG L3, and the spinal processing of the input took place mainly in segment L2, a further cranial shift of one segment appears to be present.

4.4. Background activity of LB neurons

In the control groups, 60 % of the LB neurons exhibited background activity, and the discharge rate was significantly higher in the LB neurons than in cells without LB input (Fig. 4A and B). In a previous study of our group, only 5% of the dorsal horn neurons having input from the GS muscle exhibited background activity [10]. The majority of the non-myelinated afferents (75%) of the paraspinal tissues in the rat likewise showed background activity [5]. These results suggest that neurons supplying structures in the low back are much more active at rest than those from the hindlimb muscles. Another possible explanation for the high proportion of active neurons in the present study and that of [5] is that the surgical exposure of the low back muscles and fascia has sensitized the receptors in those tissues. However, this explanation is not satisfactory, because in the study by Hoheisel and colleagues [10] the GS muscle was likewise surgically exposed.

4.5. Acute sensitizing effect of NGF on the spinal dorsal horn neurons

In the control animals, only 5.3% of the electrically identified dorsal horn neurons in segment L3 had the RFs in low back structures. This percentage increased significantly to 23.7% one day after intramuscular injection of NGF into the MF at the vertebral level L4 and L5. A trend in the same direction was found also in the spinal segment L5 after NGF injection. In human subjects, intramuscular injection of NGF at a concentration of 0.8 μM into the masseter muscle evoked long-lasting mechanical allodynia and hyperalgesia starting one day after the injection [24]. Intramuscular injection of NGF causes both peripheral and central sensitization to mechanical stimulation. Peripheral sensitization of primary afferent fibers has been confirmed in the rat masseter muscle [18]. Intracellular recording of the dorsal horn neurons in vivo having input from the gastrocnemius-soleus (GS) muscle showed that intramuscular injection of 0.8 μM NGF into that muscle one day prior to the experiment sensitized the dorsal horn neurons to electrical stimulation of the muscle nerve [14], i.e. the increased responsiveness was due to central sensitization. Whether the NGF effects of the present study are due to central or peripheral sensitization cannot be answered at present. The background activity of the LB neurons was not different in the control and the NGF-injected animals. Assuming that the background activity in dorsal horn neurons is responsible for spontaneous pain in humans, this result fits with the observation by Svensson and colleagues [24] that their subjects did not feel pain at rest 1 day after injection of NGF.

4.6. Long-term sensitizing effect of CFA on spinal dorsal horn neurons

In behavioral experiments, intramuscular injection of CFA into the MF resulted in a decrease in the mechanical withdrawal threshold 6 and 12 days later while that of the GS was unchanged. These results suggest that chronic sensitization of the MF was

induced by the CFA treatment, but systemic sensitizing effects did not occur. At least the mechanical pain threshold of the GS muscle was unchanged after CFA into the MF.

When the data from the segments L2 and L3 were pooled, the proportion of LB neurons was significantly higher in the CFA group (6 days) than in the control group. CFA-induced sensitization of the LB neurons likely is the reason for the mechanical allodynia seen in the behavioral experiments. A similar long-term sensitization of LB neurons due to overuse and/or poor coordination of low back muscles and other structures might be one of the mechanisms responsible for chronic low back pain in patients.

In conclusion: (1) The spinal segment L2 had much more input from low back structures than the segments L3 and L5, (2) the approximate center of the RF of the LB neurons was always shifted 2–3 segments caudad relative to the spinal segment recorded from, (3) LB neurons can easily be sensitized to mechanical stimulation. Acute sensitization can be induced by prior intramuscular injection of NGF, and chronic sensitization by CFA 6 or 12 days prior to the experiment. Sensitization of the dorsal horn neurons having input from the low back may be a mechanism contributing to chronic low back pain in patients.

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