Acute Effect of an Incision on Mechanosensitive Afferents in the Plantar Rat Hindpaw

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Hämäläinen, Minna M., G. F. Gebhart, and Timothy J. Brennan. Acute effect of an incision on mechanosensitive afferents in the plantar rat hindpaw. J Neurophysiol 87: 712–720, 2002; 10.1152/jn.00207.2001. The purpose of this study was to examine which primary afferent fibers are sensitized to mechanical stimuli after an experimental surgical incision to the glabrous skin of the rat hindpaw. Afferent fibers teased from the L4 dorsal root or the tibial nerve were recorded in anesthetized rats. The mechanical response properties of each fiber were characterized before and 45 min after an incision (or sham procedure) within the mechanical receptive field. Sensitization is characterized by an expansion of the mechanical receptive field, an increase in spontaneous activity, an increase in response magnitude, or a decrease in response threshold. After incision, the background activity and response properties of Aβ-fibers (n = 9) to mechanical stimuli were unchanged. Four of 13 mechanosensitive Aδ-fibers exhibited sensitization after the incision; response threshold decreased, response magnitude increased, or receptive field size increased. Background activity of Aδ-fibers was not increased by the incision. Sensitization was observed in 4 of 18 mechanosensitive C-fibers 45 min after the incision. Background activity of C-fibers was not increased by the incision. In a group of mechanically insensitive afferent fibers (MIAs), 3 of 7 Aδ-fibers and 4 of 10 C-fibers sensitized 45 min after incision. Response threshold was decreased in only 2 of 17 MIAs; receptive field size increased in 7 of 17 MIAs. Aδ-fibers did not sensitize after the incision, and only 8 of 31 (26%) mechanosensitive Aδ- and C-fibers gave evidence of sensitization. In a group of MIA Aδ- and C-fibers, a greater percentage of 17 fibers studied (41%) were sensitized after incision. In this model, the principal effect of an incision, when examined 45 min after the insult, is an increase in receptive field size of the afferents, particularly those characterized as MIAs. To the extent that the mechanical hyperalgesia characterized in the same model is initiated in the periphery, it would appear that spatial summation of modestly increased response magnitude is important to the development of hyperalgesia.

INTRODUCTION

Postoperative pain is a common form of persistent, acute pain. Pain after surgery is present at rest and is exacerbated by activities like coughing and ambulating (Kehlet 1994) as well as by mechanical probing (Inagaki et al. 1993; Johansson et al. 1994; Richmond et al. 1993). Increased pain to mechanical probing, mechanical hyperalgesia, is an important feature of postoperative pain. Mechanical hyperalgesia can be contributed to by either or both peripheral and central mechanisms.

Primary hyperalgesia, an increased response to stimulation at the site of injury, and secondary hyperalgesia, an increased response to stimulation in uninjured tissue, are both present after surgery (Richmond et al. 1993; Stubhaug et al. 1997). This reveals that both peripheral and central mechanisms contribute to postoperative pain (Treede et al. 1992). The present study focused on peripheral mechanisms of mechanical hyperalgesia.

The characteristic features of experimental peripheral fiber sensitization are a lowering of response threshold, an increase in response magnitude to suprathreshold stimuli, an increase in spontaneous activity, or an increase in receptive field (RF) size (Handwerker and Reeh 1991; Meyer 1995; Treede 1995; Treede et al. 1992). Experimentally, nociceptors have been shown to sensitize to thermal stimulation (Treede et al. 1992). However, correlation of fiber sensitization with primary mechanical hyperalgesia has been reported only rarely despite testing in a variety of peripheral injury models (Ahlgren et al. 1992; Andrew and Greenspan 1999; Cooper et al. 1991; Handwerker and Reeh 1991; Meyer et al. 1991; Neugebauer et al. 1989; Reeh et al. 1986; Schäible and Schmidt 1988; Steen et al. 1992; Treede 1995; Treede et al. 1992). This could be due to differences in experimental methods, peripheral injury models, experimental animals, and/or tissue studied (e.g., glabrous vs. hairy skin).

The purpose of this study was to clarify which afferent fibers are activated and sensitized to punctate and blunt mechanical stimuli after an incision to the glabrous skin of the rat hindpaw (Brennan et al. 1996; Zahn and Brennan 1999b). We have addressed this objective by recording mechanosensitive afferent fibers innervating the plantar aspect of the rat hindpaw using standard teased-fiber techniques before and 45 min after incision. In contrast with the original rat model of incisional pain (Brennan et al. 1996), we used a modified version of the incision (described in METHODS), which was performed within the RF of the recorded fiber as near as possible to the low-threshold, mechanosensitive site. Complementary behavioral experiments showed that this modified incision induces similar pain behaviors, i.e., the reduced withdrawal threshold and increased responses to a blunt mechanical stimulus are behaviors suggestive of pain, indicating primary mechanical hyperalgesia as in the original model.

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METHODS

General

The experimental protocols were reviewed and approved by The University of Iowa animal care and use committee. The animals were treated in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals issued by the International Association for the Study of Pain (Zimmermann 1983).

Behavioral studies

PLANTAR INCISION. Twelve adult male Sprague-Dawley rats (300–350 g; Harlan, Indianapolis, IN) were used for behavioral studies. Rats were anesthetized with 2% halothane delivered via a nose cone, and each received an intramuscular injection of penicillin (Flocillin) 30,000 IU in the triceps muscle. The original rat model of incisional pain employed a 1-cm-long incision, with or without muscle involvement (Brennan et al. 1996). For the present experiment, a 5-mm-long incision of skin and fascia was made in the plantar aspect (heel, midfoot, or distal pad area, see Fig. 1) of the right hindpaw. No underlying muscle was incised. After hemostasis with gentle pressure, the incision was closed with one 6-0 nylon ophthalmic suture on an FS-2 needle. The wound site was covered with a mixture of polymixin B, neomycin, and bacitracin ointment. After surgery, rats were allowed to recover in their cages until behavioral testing. The suture was removed under brief halothane anesthesia after testing on the second postoperative day.

PAIN BEHAVIORS. On the day of an experiment, rats were placed individually on an elevated plastic mesh floor covered with a clear plastic cage top (21 × 27 × 15 cm) and allowed to acclimate. The behavioral experiments were not blinded. Baseline pain behaviors (pre) were measured as follows.

Withdrawal responses to punctate mechanical stimulation were determined using calibrated von Frey filaments applied from underneath the cage through openings (12 × 12 mm) in the plastic mesh floor to an area adjacent to the intended incision (Zahn and Brennan 1999b). Location of the testing site is shown in Fig. 1. Each von Frey filament (11, 37, 50, 63, 106, 162, 228 mN) was applied once starting with 11 mN and continuing until a withdrawal response occurred or 228 mN was reached. The median force producing a response, determined from three tests given over a 10-min period, was considered the withdrawal threshold. For this study, 522 mN was recorded as the withdrawal threshold if there was no withdrawal response to the next lowest filament (228 mN).

To measure responses to a blunt, nonpunctate mechanical stimulus, a 5-mm diam, circular plastic disk attached to a von Frey filament (400 mN) was applied from underneath the cage through openings in the plastic mesh floor directly on the intended incision site. A response was considered positive if the rat withdrew or the blunt stimulus raised the hindpaw, indicating inability to bear weight on the disk. This test was repeated three times within approximately 1 min, from which a mean response frequency was calculated.

Rats were tested for responses to punctate and nonpunctate stimuli before incision (pre) as described above and again 40–50 min after incision. Responses to the same stimuli were determined daily for the next 3 days (1 day, 2 days, 3 days). After the final test (3 days) rats were killed by an overdose of pentobarbital sodium. In previous studies, a sham procedure (anesthesia, antibiotics, and sterile preparation) did not affect pain behaviors (Zahn and Brennan 1999b). No sham group was examined in the current study.

Electrophysiological studies

SURGICAL PROCEDURES. A total of 75 adult male Sprague-Dawley rats (400–450 g) was used. Rats were initially anesthetized by an intraperitoneal injection of pentobarbital (Nembutal; 50 mg/kg). A tracheal cannula was inserted for artificial ventilation. A carotid artery was cannulated to monitor blood pressure, and mean arterial pressure was maintained ±90 mmHg. A jugular vein was cannulated for constant infusion of pentobarbital (5–10 mg·kg⁻¹·h⁻¹) to maintain anesthesia. At the end of preparative surgery, rats were paralyzed with pancuronium bromide (2 mg/kg), and supplementary doses were given at approximately 1-h intervals. Rectal temperature was maintained at approximately 37°C by a servo-controlled electric heating lamp. For determination of conduction velocity, the right sciatic nerve was cannulated to monitor blood pressure, and mean arterial pressure was maintained ±90 mmHg. A jugular vein was cannulated for constant infusion of pentobarbital (5–10 mg·kg⁻¹·h⁻¹) to maintain anesthesia. At the end of preparative surgery, rats were paralyzed with pancuronium bromide (2 mg/kg), and supplementary doses were given at approximately 1-h intervals. Rectal temperature was maintained at approximately 37°C by a servo-controlled electric heating lamp. For determination of conduction velocity, the right sciatic nerve

FIG. 1. Behavioral data. A: punctate hyperalgesia: withdrawal threshold before and after incision. The results are pooled and expressed as median (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines). B: nonpunctate hyperalgesia: response frequency before and after incision. The data are expressed as means (solid squares) ± SE (vertical lines). C: diagram of the plantar aspect of the rat foot showing site of application of von Frey filament (solid circle) and site of application of plastic disk (large dashed circle). Twelve rats underwent skin and fascia incision at one of the locations shown (n = 4 per group). *P < 0.05 vs. pre by Friedman and Dunnett’s test.
was exposed and silver hook electrodes were placed around the nerve, insulated with Reprosil® from the surrounding tissue, and the wound was closed. For single fiber recording, a laminectomy was performed to expose the L₅ dorsal root, which was cut close to its entry into the spinal cord and from which fibers were teased. Spinal clamps supported the vertebral caudal and rostral to the exposed spinal cord. A pool for warm mineral oil was made over the exposed cord, and the right hindpaw was fixed on clay. In some experiments, the sciatic nerve was exposed, and the tibial nerve was isolated from the nerve trunk and cut proximally for single fiber recording. One fiber innervating glabrous skin was recorded from the sural nerve. In these experiments, the conduction velocity was determined electrically by inserting needle electrodes into the hindpaw just outside the mechanical RF of the fiber.

RECORDING AND STIMULATION PROCEDURES. Standard teased-fiber techniques (Campbell et al. 1979) were used to record from the central processes of primary afferent fibers. Only fibers innervating the plantar aspect of the hindpaw were studied. Recordings were made from the nerve filament that was placed on the platinum bipolar electrode; a fine strand of connective tissue was placed across the other pole of the electrode. At the end of each experiment, the distance between the recording and stimulation electrodes was measured with a piece of thread to calculate conduction velocity. Stimulus parameters for electrical excitation of fibers ranged from 5 V and 0.5 ms to 20 V and 2 ms at 1 Hz, depending on fiber type (with needle electrodes from 5 V and 0.5 ms to 100 V and 2 ms). Nerve activity was amplified and filtered using standard techniques. Amplified signals were led to a digital oscilloscope and an audiometer and also taped. The action potentials of single units were isolated using a window discriminator whose output was used to create peristimulus time histograms (PSTHs) via a data acquisition system (spike2/CED1401 program).

Characterization of afferent fibers

Fibers were classified as C-fibers if their conduction velocity was <2.5 m/s, as Aδ-fibers if their conduction velocity was between 2.5 and 30 m/s, and as Aβ-fibers if their conduction velocity was >30 m/s. The mechanical response threshold was determined using calibrated von Frey filaments (1, 2, 4, 8, 10, 24, 40, 59, 86, 95, 131, 235, 539 mN) applied to the low-threshold, mechanosensitive site of the RF. The mechanical response threshold was defined as the minimum force necessary to evoke one action potential; none of the fibers studied were spontaneously active. The original threshold response was tested a second time for agreement. If necessary a third application was made. Testing was limited to the minimum required. The mapping of mechanical RFs was done with a von Frey filament at a bending force approximately twice the response threshold. The RF was examined once before the incision, and the border was again mapped while depicted on a diagram of the plantar hindpaw.

After incision, the RF was redrawn on the diagram. The area of each RF (pre- and postincision) was estimated by measuring the length and width of the RF on the drawing. The change in RF was calculated as the percentage change in size. Responses of fibers to brush (camel’s hair brush) and to a blunt nonpunctate mechanical stimulus were also determined. The duration of stimulation (von Frey, brush or the blunt probe) was approximately 2–3 s. The time interval between stimuli was approximately 10 s.

Characterization of an afferent fiber was followed by recording of background activity for 5 min, after which a 5-mm-long incision of skin and fascia was made within the RF as near as possible to the low-threshold, mechanosensitive site. The incision was closed with one 6-0 nylon suture. Forty-five minutes (and in some cases 90 min) after the incision, mechanical responses of the fiber were tested again. Some fibers were tested as described above following a sham operation. In these sham experiments, preparative surgery and testing were done as described above except no incision was made in the hind paw. More than one fiber was studied in 26 rats. In these experiments, single fiber responses were characterized, a sham operation was performed, and the postsham operation responses recorded. Later, a second fiber with a RF distant from the previous sham test site, was isolated and characterized, and an incision was made. No rat received more than one incision. In the current study, sensitization was characterized as a decrease in mechanical response threshold, increase in background activity, increased response magnitude to mechanical stimuli at threshold, or an increase in the size of the RF.

Statistical analysis

BEHAVIORAL DATA. Data are presented as the median withdrawal threshold for the punctate stimulus and mean ± SE for the withdrawal frequency. Data were compared using nonparametric analyses. Friedman’s and a one-tailed Dunnett’s tests for within-group comparisons and the Kruskal-Wallis and Dunn’s test for between-group differences were used. P < 0.05 was considered statistically significant.

ELECTROPHYSIOLOGICAL DATA. Data are presented as median or means ± SE where appropriate. Responses of fibers were quantified by measuring the peak discharge rate for each stimulus intensity applied to the low-threshold site of the RF. Comparisons of thresholds before and after incision (or sham) were made using Wilcoxon’s signed-rank test. Comparisons between the responses of fibers before and 45 min after incision were made using a two-way ANOVA or paired r-test. P < 0.05 was considered statistically significant.

RESULTS

Behavioral studies

The effect of a small incision on withdrawal threshold to von Frey filaments was studied in 12 rats. The incision was performed in the heel area, in the midfoot, or in a distal area (n = 4 per group, Fig. 1, A–C). The median withdrawal threshold decreased from 522 mN (pre) to 50 mN 45 min after incision (Fig. 1A). The mean response frequency to nonpunctate stimuli increased from 0 ± 0% before surgery (pre) to 70 ± 8% 45 min after surgery (Fig. 1B). Figure 1C shows the location of testing sites and the incisions. The response frequency was less at the midfoot at 45 min (F2,12 = 10.0; P < 0.05); all other comparisons at other time points were not different. These data are pooled. Small skin and fascia incisions that we used to activate and sensitize the RF of primary afferents produce behaviors as we have observed for a similar larger incision (Brennan et al. 1996; Zahn and Brennan 1999b; Zahn et al. 1997).

Electrophysiological studies

FIBER SAMPLE. A total of 101 afferent fibers was identified and characterized from the tibial/sural nerves (n = 49) or the L₅ dorsal root (n = 52). Because no differences were observed in results, the data from tibial/sural nerves and the L₅ dorsal root were pooled. Based on conduction velocity (CV), 31 were classified as Aβ-, 30 as Aδ-, and 40 as C-fibers (Fig. 2). The mean CV for Aβ-fibers was 39.8 ± 1.7 (SE) m/s, 15.2 ± 1.4 m/s for Aδ-fibers, and 1.2 ± 0.1 m/s for C-fibers. The distribution of mechanical thresholds for different fiber types is presented in Fig. 3. All Aβ-fibers (n = 31) responded to light brush and the nonpunctate stimulus. None of the Aδ- or C-fibers responded to light touch, and most gave increasing responses to graded punctate mechanical stimuli. Some fibers were activated only by pinch or monofilaments ≥235 mN (10

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Aβ- and 12 C-fibers). These fibers were considered to be mechanically insensitive afferents (MIA) and are analyzed separately from other fibers in this study (Meyer et al. 1991). We were able to complete the protocol in 20/22 MIAs (see following text).

**EFFECT OF INCISION ON MECHANICAL RESPONSES OF Aβ-FIBERS.** Nine Aβ-fibers were studied both before and after incision. Making the incision in the RF evoked a discharge in all Aβ-fibers. The median mechanical response threshold was 10 mN before and 10 mN 45 min after incision (Fig. 4A). The median mechanical response threshold was also unchanged in rats undergoing the sham procedure (i.e., received no incision; Fig. 4B). When response magnitude of Aβ-fibers to different von Frey filaments was examined, the stimulus-response function was significantly attenuated after incision ($F = 4.21, P < 0.05$; Fig. 4C). As a group, Aβ-fibers thus appeared to desensitize somewhat after the sham procedure ($F = 10.77, P < 0.05$, Fig. 4D) or the incision, suggesting that the passage of time per se was responsible. We also evaluated responses of fibers at the mechanical response threshold; significant changes were not observed after incision (Fig. 4E) or the sham procedure (Fig. 4F). Generally, response magnitude in both groups was reduced. Background activity was not affected by an incision in any Aβ-fibers. RF size did not change after the sham procedure or the incision.

**EFFECT OF INCISION ON RESPONSES OF MECHANOSENSITIVE Aδ- AND C-FIBERS.** An incision in the RF evoked a discharge in all mechanosensitive Aδ- and C-fibers studied. The median mechanical response threshold of Aδ-fibers was 59 mN before and 40 mN 45 min after the incision ($n = 13$, Fig. 5A). The median mechanical response threshold was unchanged in sham-treated rats ($n = 5$, Fig. 5B). When response magnitude of Aδ-fibers to different von Frey filaments was measured, the stimulus-response function was attenuated significantly after incision ($F = 10.2, P < 0.05$, Fig. 5C). This attenuation was not observed in Aδ-fibers recorded in rats following the sham procedure ($n = 5$, $F = 1.48$, Fig. 5D). The maximum response of Aδ-fibers at response threshold was not changed after incision (Fig. 5E) or the sham procedure (Fig. 5F). Background activity was not increased by an incision in any Aδ-fibers. RF expansion occurred in 4 of 13 incised mechanosensitive Aδ-fibers, but did not occur in any fibers from 5 sham-treated rats. Altogether, four mechanosensitive Aδ-fibers were sensitized after incision. In one fiber, response threshold decreased, peak response to mechanical stimulation at threshold increased, and RF size increased (Fig. 6). All three remaining fibers exhibited expansion of their RFs; one also decreased threshold. The mean
increase in RF size in the four fibers was 330% of preincision RF size.

The median response threshold of mechanosensitive C-fibers was 40 mN before and 40 mN 45 min after the incision (Fig. 7A). In sham procedure animals, the median mechanical response threshold was 50 mN before and 59 mN after sham procedure (Fig. 7B). The stimulus-response function of C-fibers was not affected by incision (Fig. 7C). As a group, no changes in mean response magnitude at threshold were observed after incision or sham procedure (Fig. 7, E and F). Background was not increased by an incision in any C-fibers. Two of 18 mechanosensitive C-fibers increased RF size after incision (to 150 and 343% of the preincision RF size); the sham procedure did not increase RFs (0 of 6). Four mechanosensitive C-fibers were sensitized after incision: one decreased threshold only, one increased response magnitude at threshold, and one had only an expanded RF. The fourth sensitized C-fiber had an expanded RF and increased response magnitude at threshold after 45 min (Fig. 8).

FIG. 5. A: the median mechanical response threshold of Aδ-fibers was 59 mN before and 40 mN after incision (n = 13). B: the median mechanical response threshold 40 mN was unchanged in rats (n = 5) that received no incision. C when maximum responses were measured for Aδ-fibers, there was a significant attenuation of the stimulus-response function after the incision (F = 10.2, P < 0.05). D: the stimulus-response function was not attenuated in rats that received no incision (F = 1.48, P > 0.05). E and F: maximum responses of Aδ-fibers at threshold were not changed after incision (E) or in rats that received no incision (F).

FIG. 6. Example of sensitized Aδ-fibers. Before incision threshold was 59 mN; after incision threshold was 24 mN. The receptive field (RF) expanded to include the area accompanied by the dotted semi-circle.

FIG. 7. A: the median mechanical response threshold of C-fibers was 40 mN before and after incision (n = 18). B: the median mechanical response threshold was 50 mN before and 59 mN after sham procedure (n = 6). C and D: maximum responses of C-fibers were not changed after incision (C) or in rats that received no incision (D). E and F: maximum responses of C-fibers at threshold were not changed after incision (E) or in rats that received no incision (F).

FIG. 8. Example of a sensitized C-fiber. Forty-five minutes after incision, the fiber exhibited enhanced responsiveness at threshold and an expanded RF (small dotted circle). Ninety minutes later, the response at threshold at returned to baseline, but the RF expansion was greater (large dotted circle).
EFFECT OF INCISION ON MIAs. Seventeen MIAs were studied both before and after incision; three additional MIAs were studied following the sham procedure. An incision in the RF evoked a discharge in all MIAs. The median mechanical response threshold was 235 mN before and 235 mN 45 min after incision (Fig. 9A). In sham animals, the median mechanical response threshold was also 235 mN before and 45 min later (n = 6, Fig. 9B). When comparing the mean maximum responses of all MIA Aδ- or C-fibers at threshold, they were not significantly changed after incision (Fig. 9C). Background activity was not increased by an incision in any MIA Aδ- or C-fiber. Similarly, maximum responses of MIAs at threshold were unchanged in rats that received no incision (Fig. 9C). Overall, 7 of 17 MIA fibers had expanded RFs after incision to a mean 304 ± 48% of preincision RF size; this did not occur after the sham procedure (0 of 3).

Three of seven MIA Aδ-fibers were sensitized; either response threshold decreased or response magnitude at threshold increased. All three fibers exhibited expanded RFs. Four of 10 MIA C-fibers were sensitized; all had only expanded RFs. An example of an MIA C-fiber with RF expansion only at 45 min is given in Fig. 10.

EFFECT OF INCISION ON RESPONSES TO A NONPUNCTATE STIMULUS. Responses of fibers to a blunt probe were determined before and 45 min after incision. No changes in responses were observed in the three groups of fibers after incision (Fig. 11). An increase in response to the blunt probe was defined as an increase in the peak rate of ≥10 imp/s after incision. Three Aδ- and one C-fiber increased peak response by ≥10 imp/s. No responses of MIAs were increased. No fiber subjected to a sham procedure was sensitized to the blunt mechanical stimulus.

DISCUSSION

In the present experiments, we have shown behaviorally that a 5-mm-long incision of the plantar aspect of the rat hindpaw produces increased responsiveness suggesting hyperalgesia to punctate and nonpunctate mechanical stimuli. This hyperalgesia is obvious within 45 min after incision and lasts several days. In an attempt to examine a peripheral neuronal correlate for this hyperalgesia, the responses of single fibers innervating the plantar aspect of the hindpaw to mechanical punctate and nonpunctate stimuli were measured before and 45 min after an incision in the RF. The percentage of fibers that were sensitized after the surgical incision was greatest among those with high response thresholds, particularly those designated as MIA.

It is surprising that no increase in ongoing or background activity was present after incision. We expected to see spontaneous activity because increased background activity occurs in dorsal horn neurons after incision at the same time interval that we have been unable to measure any increase in afferent fiber activity (Vandermeulen and Brennan 2000). Others have noted persistent increased background activity while recording single afferents before and after pressure injury (Reeh et al. 1987), after knee joint inflammation (Schaible and Schmidt 1988), and after carrageenin-induced myositis (Berberich et al. 1988).

Even though a remarkable decrease in punctate withdraw threshold occurs as early as 45 min after incision, the mechanical sensitivity of Aδ- and C-fibers as a group did not increase. MIAs as a group did not develop increased responsiveness to the mechanical stimuli. Also, we could not identify any particular subpopulation of these fibers that likely by itself medi-
maximum responses of MIA A-fibers were not affected by incision or sham procedure. There were neither changes in maximum responses to nonpunctate stimuli after incision.

...bers did not have changes in maximum responses to nonpunctate stimulus were not affected by incision.

...sensitization seemed to increase (e.g., Fig. 10).

Altogether, 15 of 48 fibers developed sensitization to punctate stimuli; usually RF expansion occurred. RF expansion of many Aδ- and C-fibers could permit a far greater number of fibers to be activated by application of a single monofilament after incision. Thus RF expansion could be sufficient to produce spatial summation of inputs at the dorsal horn and cause a withdrawal response.

In support of this, we have examined the response properties of dorsal horn neurons before and after an incision. Evidence of sensitization occurs in both wide dynamic range (WDR) and high-threshold (HT) neurons because RF expansion and increased background activity occur in both types (Vandermeulen and Brennan 2000; Zahn and Brennan 1999a). After incision, WDR neurons respond to the same forces that produce withdrawal in behavioral studies. HT neurons do not respond to the filaments that produce withdrawal. In uninjured tissue, WDR neurons are activated by low-threshold mechanoceptors and nociceptors; HT neurons only respond to strong nociceptive stimuli. This suggests that a facilitatory interaction between mechanoreceptors and nociceptors in the WDR neuron transmits the withdrawal responses observed behaviorally.

The importance of central contributions to the development and maintenance of mechanical hyperalgesia has been documented (Cervero and Laird 1996; Neugebauer and Schaible 1990; Ren et al. 1994; Simone et al. 1991; Woolf and King 1990; Woolf et al. 1994). Cervero and Laird (1996) posited an interaction between low-threshold mechanoceptors and nociceptors in the form of axon reflexes. They suggest that there could be a presynaptic link between low-threshold mechanoceptors and nociceptors at the spinal cord level. The activity in the low-threshold mechanoceptors evokes primary afferent depolarization (PAD) of the nociceptors. It is suggested that in hyperalgesic states, PAD is strong enough to evoke orthodromic action potentials in nociceptors. In this way, low-threshold mechanoceptors could activate nociceptors and cause withdrawal responses via central mechanisms.

Even when heat sensitization of afferent fibers is present after inflammation or injury, many other studies have had difficulty finding evidence of sensitization to punctate mechanical stimuli. However, some experiments attempting to acutely induce sensitization in afferent fibers have been successful (Ahlgren et al. 1992; Berberich et al. 1988; Cooper et al. 1991; Davis et al. 1993; Junger and Sorkin 2000; Reeh et al. 1987; Schaible and Schmidt 1988; Schmidt et al. 1995). Others have found evidence of enhanced mechanical responsiveness by characterizing afferent fibers from a group of animals treated with an inflammmogen and compared these responses to fibers from an untreated control group (Andrew and Greenspan 1999; Cooper et al. 1991; Schaible and Schmidt 1985). We were not able to detect increased responses to the non-punctate/blunt mechanical stimulus in any group of fibers. In behavioral studies, application of the plastic disk causes either withdrawal or, because of limited weight bearing, lifting of the foot. The behavioral response may depend on ongoing nociceptor activation that limits weight bearing and contributes to lifting of the foot by pressure from the disk. In addition, the blunt mechanical stimulus may activate more afferent fibers after incision because of their expanded RFs documented in the present study. Alternatively, it is possible that peripheral mechanisms contribute an insignificant role in the behavior observed. That is, spatial summation significant enough to enhance responses of dorsal horn neurons to mechanical stimulation after incision (Zahn and Brennan 1999a) may contribute most to behavioral hyperalgesia early after incision.

In the companion paper, evidence of increased background activity and mechanical sensitization (decreased threshold and increased responsiveness) was present in afferent fibers 1 day after incision (Pogatzki et al. 2002); these results were not found in the present study (45 min to 1 h after incision). Several methodological aspects of the present study and companion paper merit comment. First, in the present study, the incision was made as near as possible to the low-threshold, mechanosensitive site of the RF. Thus, the incision was made as near as possible to the most sensitive area without cutting it. It is possible that greater changes in mechanical responsiveness could have resulted if the test site was a few millimeters away from the low-threshold, mechanosensitive site and the incision was made through the low-threshold site. Also, the percentage RF expansion was greater 1 day after incision compared with which was found 45 min after incision (Pogatzki et al. 2002). The RF expansion may be less when the injury occurs adjacent to the most sensitive area rather than outside this zone (Thalhammer and LaMotte 1982; Treede et al. 1992).

Second, the disparity between the acute preparation in the current study that characterized afferents and then attempted to sensitize them and population studies that compare fibers from a sham group to an incised group (Pogatzki et al. 2002) may be
caused by the time recordings that were made after the injury. Sustained spontaneous activity in afferents may require several hours to develop. However, this delay is not consistent with spontaneous activity in dorsal horn neurons immediately after incision and sustained for the 1–2 h.

The time for testing of mechanical responses of single fibers in the current study was selected based on behavioral studies. Within 45 min after the incision, there were significant pain behaviors evident. Because making the incision in the RF evoked a strong response in all fibers, could it be possible that central sensitization by primary afferent fibers is the result of a short-lasting, high-intensity burst of activity in response to the incision? This is unlikely because previous studies have shown that the injury discharge does not contribute to later pain behaviors in this incisional model (Pogatzki and Brennan 1999).

Third, the search strategy to find the RF was tapping, and in some cases, pinching the glabrous skin of the hindpaw. It is possible that this strategy favored only certain types of afferents. Because electrical stimulation was used to search for fibers in the companion study (Pogatzki et al. 2002), a significantly greater proportion (54%) of Aβ-fibers with very high mechanical response thresholds was identified in sham rats, and these may have a greater propensity to develop spontaneous activity and to decrease threshold after incision.

Incisions are a common cause of pain and hyperalgesia; yet, few have studied how they produce pain. Campbell et al. (1988) have examined the effect of a tissue cut on primary afferent fiber sensitization. C mechano-heat fibers from the hairy skin of the primate sensitized to heat stimuli immediately after incision and sustained for the time recordings that were made after the injury. The changes in response properties that occurred do not totally account for the changes observed in complementary behavioral studies.

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