Characterization of Aδ- and C-Fibers Innervating the Plantar Rat Hindpaw One Day After an Incision

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Pogatzki, Esther M., G. F. Gebhart, and Timothy J. Brennan. Characterization of Aδ- and C-fibers innervating the plantar hindpaw one day after an incision. J Neurophysiol 87: 721–731, 2002; 10.1152/jn.00208.2001. Primary hyperalgesia after tissue injury is suggested to result from sensitization of primary afferent fibers, but sensitization to mechanical stimuli has been difficult to demonstrate. In the companion study, sensitization of mechano-responsive Aδ- and C-fibers did not explain pain behaviors 45 min after an incision in the rat hindpaw. In the present study, we examined mechanical response properties of Aδ- and C-fibers innervating the glabrous skin of the plantar hindpaw in rats 1 day after an incision or sham procedure. In behavioral experiments, median withdrawal thresholds to von Frey filaments were reduced from 522 mN before to 61 mN 2 h after incision; median withdrawal thresholds after sham procedure were stable (522 mN). Responses to a nonpunctate mechanical stimulus were increased after incision. In neurophysiological experiments in these same rats, 67 single afferent fibers were characterized from the left tibial nerve 1 day after sham procedure (n = 39) or incision (n = 28); electrical stimulation was used as the search stimulus to identify a representative population of Aδ- and C-fibers. In the incision group, 11 fibers (39%) had spontaneous activity with frequencies ranging from 0.03 to 39.3 imp/s; none were present in the sham group. The median response threshold of Aδ-fibers was less in the incision (56 mN, n = 13) compared with sham (251 mN, n = 26) group, mainly because the proportion of mechanically insensitive afferents (MIAs) was less (8 vs. 54% after sham procedure). Median C-fiber response thresholds were similar in incised (28 mN, n = 15) and sham rats (56 mN, n = 13). Responsiveness to monofilaments was significantly enhanced in Aδ-fibers 1 day after incision; stimulus response functions of C-fibers after incision and sham procedure did not differ significantly. Only Aδ-fibers but not C-fibers sensitized to the nonpunctate mechanical stimulus. The size of receptive fields was increased in Aδ- and C-fibers 1 day after incision. The results indicate that sensitization of Aδ- and C-fibers is apparent 1 day after incision. Because sensitization of afferent fibers to mechanical stimuli has been difficult to demonstrate, others have suggested that central sensitization may play a role in primary mechanical hyperalgesia. In the majority of electrophysiological studies of mechanosensitive nociceptors, no changes in response threshold to mechanical stimuli have been noted (Baudonn et al. 1991; Campbell et al. 1979; Schmelz et al. 1996; Thalhammer and LaMotte 1982, 1983; Meyer and Campbell 1981). Because sensitization of afferent fibers to mechanical stimuli has been difficult to demonstrate, others have suggested that central sensitization may play a role in primary mechanical hyperalgesia. In the majority of electrophysiological studies of mechanosensitive nociceptors, no changes in response threshold to mechanical stimuli have been noted (Baudonn et al. 1991; Campbell et al. 1979; Schmelz et al. 1996; Thalhammer and LaMotte 1982, 1983; Meyer and Campbell 1981). Likewise, the majority of mechanosensitive afferent fibers investigated in the preceding study (Hämäläinen et al. 2002) did not sensitize 45 min after an incision was made in their receptive field (RF).

It was noted, however, that some afferent fibers with very high response thresholds before the experimental incision reduced their response threshold and expanded their RFs 1 h after the incision (Hämäläinen et al. 2002). It was suggested that these fibers, termed mechano-insensitive afferents (MIAs) (Handwerker et al. 1991; Meyer et al. 1991), likely contribute to mechanical hyperalgesia. This corresponds with recent studies demonstrating that MIAs have the capability to become responsive to mechanical stimuli under conditions of inflammation (Davis et al. 1993; Handwerker et al. 1991; Kress et al. 1992; Neugebauer et al. 1989). Further investigations support the suggestion that MIAs may play a role in inflammation-induced mechanical hyperalgesia (Schmelz et al. 1996; Schmidt et al. 2000).

The contribution of MIAs to mechanical hyperalgesia caused by an incision is still not clear. In the preceding study, only some MIAs sensitized after incision and the reduction in response threshold of individual MIAs was not great (Hämäläinen et al. 2002). The experimental injury, a surgical incision in the plantar aspect of the rat hindpaw, leads to a
remarkable reduction in paw withdrawal threshold to mechanical stimuli for several days, representing mechanical hyperalgesia (Brennan et al. 1996). Pain behaviors in these rats and the time course of mechanical hyperalgesia have similarities to patients’ pain reports in the postoperative period (Tverskoy et al. 1994).

In the present study, we further examined mechanisms that contribute to primary mechanical hyperalgesia in the postoperative period. Pain behaviors and response properties of single afferent fibers to mechanical stimuli were studied in rats 1 day after an incision or sham procedure. An electrical search stimulus was used to identify a representative population of afferent fibers innervating the incision site at the plantar aspect of the hindpaw. This strategy reduces bias (relative to a “natural” search stimulus such as tapping) with regard to response threshold and thus relative contributions of mechanosensitive afferents and MIAs to mechanical hyperalgesia 1 day after an incision. Portions of these data have been reported in abstract form (Pogatzki et al. 2000).

METHODS

General

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

Forty-one adult male Sprague-Dawley rats (250–350 g, Harlan, Indianapolis, IN) were used. Rats were housed individually; food and water were available ad libitum. Neurophysiological experiments were performed in the same rats after behavioral testing. At the end of the protocol, all rats were killed with an overdose of pentobarbital sodium.

Plantar incision

An incision was made under 1.5–2% halothane anesthesia delivered via a nose cone similar to that described previously (Brennan et al. 1996). Briefly, the plantar aspect of the left hindpaw was prepared, and a 1-cm longitudinal incision through skin, fascia, and muscle was made. In the present study, incision was started 12 mm distal from the edge of the heel (see Fig. 1). The skin was closed with two 5-0 nylon sutures, and the wound was covered with antibiotic ointment. Control rats underwent a sham procedure that included halothane anesthesia, sterile preparation of the plantar area, and topical antibiotics, but they received no incision.

Pain behaviors

Behavioral tests were undertaken to establish the magnitude of mechanical hyperalgesia 2 h and 1 day after plantar incision. To assess baseline pain behavior, 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. All rats were pretested for response to a nonpunctate mechanical stimulus (plastic disk) and withdrawal threshold to von Frey filaments as described previously (Brennan et al. 1996). Briefly, withdrawal to punctate stimulation was tested by applying calibrated nylon von Frey monofilaments (Stoelting, Wood Dale, IL) to an area adjacent to the intended incision (Fig. 1). Each von Frey filament (15, 30, 42, 65, 73, 98, 149, and 265 mN) was applied once beginning with 15 mN until a withdrawal response occurred. The lowest force from three tests producing a response was considered the withdrawal threshold; if there was no paw withdrawal, 522 mN was recorded.

The nonpunctate mechanical stimulus, a 5-mm clear plastic disk attached to a von Frey filament (bending force 400 mN), was applied directly on the intended incision site (Fig. 1). A positive response was defined as a withdrawal (flinch) or a passive lifting of the foot without bending the filament; response frequency was calculated from three repeated tests. After assessing baseline pain behaviors, an incision or sham procedure was made; pain behavior was tested 2 and 20 h later.

Electrophysiological studies

The same rats assessed for pain behavior after an incision or sham procedure were studied in electrophysiological experiments. Twenty-four hours after incision or sham procedure, anesthesia was induced by an intraperitoneal injection of pentobarbital (Nembutal; 50 mg/kg). The surgical preparation was made as described in the companion article (Hämäläinen et al. 2002). Briefly, the internal jugular vein, the common carotid artery, and the trachea were cannulated; rats were artificially ventilated, and experiments were terminated if mean arterial blood pressure fell below 90 mmHg. Body temperature was recorded with a probe placed on the right foot of the rat; normothermia was maintained by an underbody heating pad and overhead lamp with feedback control.

For single-fiber recordings from the left tibial nerve, the sciatic nerve was exposed and a pool for warm mineral oil was made. The tibial nerve was detached from the sural and peroneal nerves, and all were cut proximal. Afferent fibers were identified in fine filaments teased from the distal tibial nerve that was in continuity with the hindpaw. Two needle electrodes were inserted subcutaneously 9 mm proximal and distal to the incision site; electrical stimulation (10- to 100-V and 0.5- to 1-ms pulses) was used to search for afferent fibers with a RF adjacent to the plantar incision. We limited the study to afferent fibers with a RF at least 4–5 mm from a stimulation electrode. Because the RFs of primary afferent fibers in the rat are reported to not increase more than 5 mm after mechanical injury or inflammation (Andrew and Greenspan 1999; Reeh et al. 1987), it is unlikely that afferent fibers recorded in the present study were sensitized by insertion of needle electrodes. The placement of needle electrodes after the sham procedure was the same.

The nerve filament was subdivided until a single action potential was discriminated. Conduction velocity (CV) of an individual fiber was calculated from the response latency determined using a stimulus strength just above threshold. At the end of the experiment, the distance from the cathode to the recording electrode was measured and used to calculate CV.

Nerve activity was amplified, filtered, and displayed on a digital oscilloscope. Spike shape was continuously monitored by analog delay; single-unit action potentials were discriminated, and peristimulus time histograms (PSTHs, 1-s binwidth) were created via a data acquisition system (spike2/CED1401 program). All data were also recorded and stored on video tape.

Characterization of afferent fibers

Response properties of single afferent fibers with RFs on the plantar aspect of the left midfoot were recorded in rats 2 h after incision or sham procedure. When more than one afferent fiber was studied in a rat, the RFs of fibers did not overlap. In general, tapping of the foot and mechanical testing was kept to a minimum to avoid tissue damage and potential sensitization of afferent fibers.

Afferent fibers were classified as Aδ- or C-fibers if their CV was between 2.5 and 30 m/s or less than 2.5 m/s, respectively; faster conducting fibers (Aβ-fibers) were not studied. Ongoing spontaneous activity was recorded for each fiber over a 5-min period and averaged. An afferent fiber with a mean activity >0.1 Hz (a minimum of 30 imp
during the 5-min period recorded to assess spontaneous activity) was considered spontaneously active.

To characterize mechanical response properties, a brush (number 4 camel’s hair artist’s brush) and the plastic disk was applied once to the RF. The the plastic disks were applied for 2–3 s. The brush stimulus was applied for 2–3 s by stroking vertically at the hindpaw. Subsequently, calibrated von Frey filaments (2, 6, 10, 16, 29, 56, 78, 92, 110, 147, and 262 mN) were applied in ascending order to the low-threshold area of the RF; each filament was applied once for 3 s. The interstimulus interval was 10–20 s. Using 1-s binwidth, the peak activity of the fiber was the greatest rate during application of the stimulus.

The mechanical response threshold of each fiber was defined as the lowest force that caused either activation of the fiber if no spontaneous activity was present or an increase in fiber activity by at least 2 SDs above mean spontaneous activity. The next strength filament must also have excited the fiber. If a fiber did not respond to the 262-mN filament, a filament with a force of 608 mN was applied. Some fibers did not respond to any von Frey filament, in which case a pinch stimulus (a small curved forceps) was applied, and 608 mN was designated the response threshold.

To characterize mechanical response properties, a brush (number 4 camel’s hair artist’s brush) and the plastic disk was applied once to the camel’s considered spontaneously active.

Behavioral data were compared using nonparametric tests; Friedman’s test for within-group and the Kruskal-Wallis test and Mann-Whitney rank-sum test for between-group comparisons were used (Siegel and Castellan 1988).

To compare mechanical response properties of afferent fibers assessed in rats 1 day after incision and rats 1 day after sham procedure, a two-way ANOVA for repeated measurements and an unpaired t-test was used. Nonparametric analyses (χ² test, Mann-Whitney test, and Wilcoxon matched pair test) were used where appropriate. All results are expressed as median or means ± SE when appropriate. P < 0.05 was considered statistically significant.

RESULTS

Behavioral experiments

In 27 rats, the median withdrawal threshold to von Frey filaments did not change after sham procedure (Fig. 1A). Mechanical hyperalgesia to von Frey filaments was apparent only after a plantar incision (n = 14). The median withdrawal threshold decreased from 522 mN before to 61 mN 2 h and 61

Statistical analysis

Behavioral data were compared using nonparametric tests; Friedman’s test for within-group and the Kruskal-Wallis test and Mann-Whitney rank-sum test for between-group comparisons were used (Siegel and Castellan 1988).
Spontaneous activity. None of 39 fibers in the sham group had spontaneous activity, whereas 11 of 28 fibers (39%) in the incision group \((P < 0.05)\) were spontaneously active (Table 1). Spontaneous activity was stable over the entire recording period, which usually lasted 50–60 min. No afferent fibers developed spontaneous activity during testing. Both \(\alpha\)- and \(\beta\)-fibers in incised rats exhibited spontaneous activity. An example of a spontaneously active \(\alpha\)-fiber with a high rate of activity is shown in Fig. 2A; an example of an \(\alpha\)-fiber with a low rate of activity (studied in another rat) is shown in Fig. 2B. Five of the 11 fibers that were spontaneously active 1 day after incision exhibited modest increases in activity (<3 imp/s), whereas 6 fibers had mean rates >15 imp/s (Fig. 2C).

To confirm that the origin of spontaneous activity arose from the RF that was adjacent to the incision, 0.1 ml of 2% lidocaine was infiltrated into the RF of four spontaneously active fibers. Spontaneous activity stopped immediately after lidocaine infiltration into the RFs of three fibers (see example in Fig. 2D). A second infiltration of 0.05 ml lidocaine was required to cease spontaneous activity of one fiber.

Mechanical response properties. Qualitative response properties of nociceptors studied in sham and incision groups did not differ; that is, none of the \(\alpha\)- or \(\beta\)-fibers responded to the brush stimulus or to punctate von Frey filaments <6 mN.

Response threshold. The distribution and summary of mechanical response thresholds of \(\alpha\)- and \(\beta\)-fibers studied 1 day after the sham procedure or incision is shown in Figs. 3 and 4. There was a significant reduction in the median response threshold of \(\alpha\)-fibers in the incision group \((P < 0.05, \text{Fig. 3B})\), and this is clear from inspection of the distribution illustrated in Fig. 3A. In the sham group, 14 of the 26 \(\alpha\)-fibers (54%) responded only to punctate stimuli \(\geq 262\) mN (including 5 fibers that responded only to pinch and assigned a response threshold of 608 mN). In the incision group, in contrast, only 1 of 13 \(\alpha\)-fibers (8%) responded to punctate stimuli \(\geq 262\) mN (Fig. 3A).

It is interesting to note that there were many fewer \(\beta\)-fibers (3) than \(\alpha\)-fibers (14) in their respective sham groups with response thresholds \(\geq 262\) mN. This is reflected in the median response thresholds of these fibers (251 mN for \(\alpha\)-fibers, 56 mN for \(\beta\)-fibers). Thus, although inspection of Fig. 4A suggests a modest shift in the distribution of individual response thresholds of \(\beta\)-fibers in the incision group, reduction of the median response threshold of \(\beta\)-fibers to 28 mN after an incision was not significant (Fig. 4B).

Response magnitude. Increases in peak responses of both \(\alpha\)- and \(\beta\)-fibers in sham and incision groups were apparent as stimulus intensity was increased. Examples of stimulus-response functions of \(\alpha\)-fibers following the sham procedure or an incision are shown in Fig. 5: summary data are given in Fig. 6. Mean stimulus-response functions of \(\alpha\)- but not \(\beta\)-fibers in the incision groups differed significantly from their corresponding sham groups; peak responses after incision were significantly greater in \(\alpha\)-fibers (Fig. 6, \(F = 4.47, P < 0.05\)) but not \(\beta\)-fibers (\(F = 3.65, P = 0.07\)).

RF size. Mechanical RF areas in incised rats were significantly greater than RF areas in sham rats. Typical examples of RFs of \(\alpha\)- and \(\beta\)-fibers are shown in Fig. 7A and B, respectively. The mean estimated RF size of \(\alpha\)-fibers (Fig. 7C) was greater \((P < 0.05)\) in incised rats \((120.8 \pm 34.5\) mm\(^2\)) relative to the complementary sham group \((13.5 \pm 34.0\) mm\(^2\)). Similarly, the mean estimated RF of \(\beta\)-fibers was greater \((P < 0.05)\) in incised rats \((167.8 \pm 17.9\) mm\(^2\)) vs. \(33.5 \pm 8.6\) mm\(^2\), respectively; Fig. 7D).

Blunt probe. In the sham group, 6 of 26 \(\alpha\)-fibers were activated by the plastic disk, whereas a greater proportion \((P < 0.05)\) of \(\alpha\)-fibers in incised rats \((9 \pm 13\) fibers, 69%) responded to this stimulus (Table 1). The proportion of \(\beta\)-fibers responding to the blunt probe in incised rats \((12 \pm 15\) fibers, 88%) was not different from in sham rats \((7 \pm 13\) fibers, 54%). For \(\alpha\)-fibers, the peak response to the blunt mechanical stimulus was greater in the incision compared with the sham group \((P < 0.05)\); responsiveness to this nonpunctate stimulus was not significantly different for \(\beta\)-fibers after an incision (Fig. 8).

TABLE 1. Properties of \(\alpha\)- and \(\beta\)-fibers in sham and incised rats

<table>
<thead>
<tr>
<th></th>
<th>(\alpha)-Fibers</th>
<th>(\beta)-Fibers</th>
</tr>
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<tbody>
<tr>
<td>Number of afferent fibers</td>
<td>26</td>
<td>13</td>
</tr>
<tr>
<td>Conduction velocity, m/s</td>
<td>(6.3 \pm 1.1)</td>
<td>(7.5 \pm 1.7)</td>
</tr>
<tr>
<td>Spontaneous activity fibers</td>
<td>0/26 (0)</td>
<td>5/13 (38)*</td>
</tr>
<tr>
<td>Mechanical response properties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median response threshold to punctate mechanical stimuli, mN</td>
<td>261</td>
<td>28*</td>
</tr>
<tr>
<td>Mean estimated receptive field area, mm(^2)</td>
<td>(13.5 \pm 4.0)</td>
<td>(120.8 \pm 34.5*)</td>
</tr>
<tr>
<td>Number of fibers responding to the nonpunctate stimulus</td>
<td>6/26 (23)</td>
<td>9/13 (69)*</td>
</tr>
<tr>
<td>Mechano-insensitive afferents</td>
<td>14/26 (54)</td>
<td>1/13 (8)*</td>
</tr>
</tbody>
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Values are means \(\pm\) SE. Numbers in parentheses for conduction velocity are ranges; other numbers in parentheses are percentages. * \(P < 0.05\) vs. sham.
FIG. 2. Spontaneous activity in afferent fibers 1 day after plantar incision. A: peristimulus time histogram of spontaneous activity recorded from a C-fiber 1 day after incision (binwidth 1 s). The mean firing frequency in this example was 33.2 imp/s. Inset: digitized oscilloscope trace from the original recording for the indicated area. The mechanical receptive field (RF) of this fiber (black area) was adjacent to and includes the incision. B: spontaneous activity recorded from an Aδ-fiber in another rat 1 day after incision (binwidth 1 s). Similar to the fiber shown in A, the RF was adjacent to the incision site. C: mean activity (imp/s averaged over 5 min) of all 11 spontaneously active fibers in the incision group. Two of 5 spontaneously active Aδ-fibers, and 4 of 6 spontaneously active C-fibers had firing rates greater than 15 imp/s. D: lidocaine (LA) was infiltrated into the RF of a spontaneously active fiber. Spontaneous activity (same fiber as in A) stopped immediately after injection of lidocaine.

FIG. 3. Mechanical response thresholds of Aδ-fibers. A: distribution of response thresholds for Aδ-fibers; the bars represent the number of Aδ-fibers with response thresholds of given forces (mN) after sham procedure (□) and after incision (■). B: the median response threshold of Aδ-fibers in the sham and the incision group is shown; the median response threshold of Aδ-fibers in the incision group was less. The results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines). †P < 0.05 vs. sham.
**Discussion**

Aδ- and C-fibers were activated and sensitized to mechanical stimuli 1 day after incision; spontaneous activity and RF size were increased. In incised rats, the mechanical response threshold was reduced in Aδ-fibers, and responsiveness to punctate mechanical stimuli was greater relative to control rats. More Aδ-fibers were responsive to the blunt mechanical stimulus, and responsiveness was increased after incision.

**Methodology**

The population of afferent fibers studied in the sham group in the current study differs from the population of control afferents in the companion paper (Hämäläinen et al. 2002). In the latter study, the control afferents were recorded from rats that had pentobarbital anesthesia and no incision made. In the current paper, afferents from the sham group had halothane anesthesia, antibiotics, and preparation of the hindpaw the day before recording and pentobarbital anesthesia the next day. It is unlikely that there is any effect of halothane anesthesia, sterile preparation and antibiotics on the afferents 24 h later. Behaviors do not change before and after (Fig. 1) a sham procedure (Zahn and Brennan 1999). Pentobarbital is routinely used for recording of primary afferents. Therefore the difference in the populations of the afferents between the control groups in these two studies is likely related to the search criteria (see following text).

**Spontaneous activity in Aδ- and C-fibers 1 day after incision**

In agreement with others, spontaneous activity was not present in any cutaneous Aδ- or C-fibers in control rats (Ahlgren et al. 1992; Andrew and Greenspan 1999; Hylden et al. 1989). Persistent, sustained spontaneous activity occurred in 38% of Aδ- and 40% of C-fibers 1 day after incision, indicating that individual fibers have different capacities to be spontaneously activated following the incision. A similar proportion of spontaneously active fibers has been reported after induction of inflammation (Ahlgren et al. 1992; Kocher et al. 1987).

Although spontaneous activity in afferent fibers has been reported after a variety of tissue injuries and under inflammatory conditions, it is not a consistent finding. In general, two types of preparations are used to study activation and sensitization of afferent fibers after injury. Population studies compare properties of afferent fibers in a group of animals after tissue injury or inflammation with the properties of fibers in uninjured control animals. In the majority of these studies, a greater proportion of spontaneously active fibers has been reported relative to controls (Ali et al. 1999; Andrew and Greenspan 1999; Baik-Han et al. 1990; Han et al. 2000; Hylden et al. 1989; Kajander and Bennett 1992; Kocher et al. 1987; Michaelis et al. 1995). In acute preparations, the nerve is first transected, activity and response properties characterized, and then the RF injury occurs. Activity and responses of the afferents after injury are compared with preinjury responses. Under these conditions, spontaneous activity in afferent fibers was not evident after incision (Hämäläinen et al. 2002) or did not

**Figure 4.** Mechanical response thresholds of C-fibers. A: distribution of response thresholds for C-fibers; the bars represent the number of fibers with response thresholds of given forces (mN) after sham procedure (■) and after incision (○). B: the median response threshold of C-fibers was similar in the sham and the incision groups. The results are expressed as median (horizontal line) with 1st and 3rd quartiles (boxes), and 10th and 90th percentiles (vertical lines).

**Figure 5.** Examples of responses to von Frey filaments in sham (A) an incised rats (B and C) are shown (binwidth, 1 s). The site of application of the von Frey filament to the lowest threshold area of the RF of the fiber (black circle) is shown.
persist longer than 1–2 h after mechanical injury (Reeh et al. 1987) or induction of cutaneous inflammation (Randich et al. 1997).

The inconsistency between acute preparations and population studies may be related to the time recordings were made after injury. Sustained spontaneous activity in afferent fibers after incision may require several hours to develop. In the companion paper, recording time after incision was limited to 1–2 h; this could explain why spontaneous activity was not observed. Joint afferents developed spontaneous activity 2–3 h after injection of koalin-carageenan (Schaible and Schmidt

FIG. 6. Summary (mean ± SE) of stimulus-response functions. A: mean maximum responses of Aδ-fibers in the incision group (●, n = 13) were significantly greater (F = 4.47, P < 0.05) than responses in the sham group (○, n = 26). The broken horizontal line represents the mean peak response of Aδ-fibers that is produced by the strongest filament (262 mN) in rats after sham procedure; a filament with this force usually did not produce withdrawal in the behavioral experiments. The gray area outlines the range of forces that produced hindpaw withdrawal in 95% of the incised rats in the behavioral experiments; responses of Aδ-fibers at these forces are not greater than peak responses to forces that did not produce hindpaw withdrawal in rats after sham procedure. B: mean maximum responses of C-fibers in the incision group (●, n = 15; P > 0.05) compared with the sham group (○, n = 13). Responses of C-fibers at forces producing withdrawal in behavioral experiments (gray area) were less than the peak responses to the strongest filament that did not produce hindpaw withdrawal in rats after sham procedure (broken horizontal line).

FIG. 7. Typical examples of RFs from Aδ-fibers (A) and C-fibers (B); the black area depicts the RF determined using a von Frey filament with a force twice the response threshold of the individual fiber. Summary data are shown in C and D. One day after incision, the mean RF area of Aδ-fibers (C) and C-fibers (D) was greater in the incision group. Data are expressed as means ± SE. *P < 0.05 vs. sham.

FIG. 8. Summary of the peak response to the blunt mechanical stimulus in Aδ-fibers (A) and C-fibers (B). A: the mean peak response of Aδ-fibers to the blunt stimulus in the incision group (●, n = 13) was significantly greater (P < 0.05 vs. sham) than responses in the sham group (○, n = 26). B: mean peak responses of C-fibers to the blunt stimulus were not different between the sham group (○, n = 13) and incision group (●, n = 16). The brackets are the SE.
Contributions of spontaneous activity to pain behavior after plantar incision

Spontaneous pain behaviors have been defined in particular animal models of pain and correlated with ongoing activity of primary afferent fibers (Han et al. 2000; McCall et al. 1996; Puig and Sorkin 1996). After plantar incision, no tonic pain behavior occurs after incision like the licking, biting, and flinching behaviors caused by formalin injection. We have characterized nonevoked pain behavior based on weight bearing on the incised hindpaw (Brennan et al. 1996; Zahn et al. 1998); rats had increased pain scores for several days after plantar incision. The spontaneous discharge in \( \alpha \) and \( \delta \) fibers may contribute to nonevoked pain behaviors described for the same incision. In the present study, the majority of spontaneously active \( \alpha \) and \( \delta \) fibers had activity greater than 15 Hz after incision. Perhaps licking, biting, and flinching do not occur because the proportion of \( \delta \) and \( \alpha \) fibers activated by formalin is greater (McCall et al. 1996; Puig and Sorkin 1996).

Sensitization to mechanical stimuli

In the present study, \( \alpha \)-fibers had lower response thresholds in incised rats. As suggested by others, threshold reduction of mechanosensitive nociceptors may not be a sufficient predictor for sensitization of afferent fibers (Andrew and Green 1999; Campbell et al. 1979, 1988; Cooper et al. 1991; Thalhammer and LaMotte 1982). Stimulus-response functions evaluate a range of stimuli and demonstrate, in some studies, mechanical sensitization of nociceptors (Ahlgren et al. 1997; Andrew and Green 1999; Cooper et al. 1991; Tanner et al. 1998). The peak responses of \( \alpha \)-fibers to a range of von Frey filaments 1 day after incision was significantly enhanced in the present study. Increased spontaneous activity was not subtracted to compare peak afferent firing rates because total discharge in fibers may be critical for transmission of afferent information to spinal neurons. Peak responses of \( \alpha \)-fibers after incision tended to be greater compared with controls but were not significantly different.

In the companion paper, a reduction in response threshold of afferent fibers 45 min after incision was limited, and, except for RF expansion, responsiveness to punctate stimuli did not change greatly (Hämäläinen et al. 2002). The same mechanisms (e.g., loss of DRRs, loss of axonal transport, and shorter time after incision) that were suggested to limit the development of spontaneous activity in the acute preparation may also impair the development of sensitization to mechanical stimuli. Furthermore, only a distinct group of afferent fibers may sensitize to mechanical stimuli after incision, and this group may not have been studied in great detail in the acute preparation. For example, tapping and applying pressure to the plantar region were used to identify afferent fibers (Hämäläinen et al. 2002). This may have biased selection to mechanosensitive fibers with lower response thresholds. From work by others, mechanosensitive fibers may be less likely to reduce response threshold than \( \alpha \)-high-threshold mechanoreceptors (Reeh et al. 1987). C-fibers did not reduce their response thresholds regardless of their mechanosensitivity (Reeh et al. 1987).

Increased responsiveness to the blunt mechanical stimulus occurred in \( \alpha \)-fibers from incised rats, and more \( \alpha \)-fibers were responsive. The same mechanism(s) responsible for a sensitization of \( \alpha \)-fibers, e.g., a reduction in response threshold and increase in responsiveness, may increase responses to the blunt mechanical stimulus.

RF expansion

One day after incision, the estimated RF areas increased approximately ninefold and fivefold in \( \alpha \) and \( \delta \) fibers, respectively. Because the RF area was assessed by applying a filament with a force approximately twice the response threshold and response thresholds in \( \alpha \)-fibers were less in incised rats, the RF expansion may be relatively underestimated in the incised group. One underlying mechanism for primary afferent RF expansion may be sensitization of insensitive branches of mechanosensitive fibers (Schmelz et al. 1994, 1996). RFs of fibers studied in incised rats may have expanded into the incision that was made in less responsive afferent branches.

The percentage RF expansion was greater 1 day after incision compared with what was found 45 min after incision (Hämäläinen et al. 2002). Again, the mechanisms discussed above may explain these differences. Also, this disparity may be a result of the placement of the incision as near as possible to the low-threshold site of the primary afferent RF (Hämäläinen et al. 2002). The RF expansion may be less when the injury occurs at the most sensitive area rather than outside to the most sensitive area because silent branches of the afferents may not be injured when the incision is placed adjacent to the most sensitive area (Thalhammer and LaMotte 1982; Treede et al. 1992).

MIAs

In the present study, electrical stimulation was used to search for fibers. As a result, a significantly greater proportion of fibers with very high mechanical response thresholds was identified in sham rats (see Fig. 9A, example). Criteria by...
incised rats, only 8% of Aδ-fibers were MIAs. Because the same search stimulus was used in both groups, some mechanosensitive fibers after incision could have been Aδ-MIAs that were sensitized after incision. This suggests that Aδ-MIAs are important for a reduction in median response threshold to mechanical stimuli after incision. This is further supported by a greater number of Aδ-MIAs that reduced their response threshold 45 min after incision compared with mechanosensitive Aδ-fibers (Hämäläinen et al. 2002) and is consistent with results by others (Reeh et al. 1987). The proportion of C-MIAs did not differ significantly in the incision and sham groups (Fig. 9C); this is reflected in a similar median response threshold of C-fibers after incision compared with sham procedure. As suggested by others (Reeh et al. 1987), C-fibers may reduce their response threshold less after injury than Aδ-fibers.

Sensitization of Aδ-MIAs supplying the glabrous skin of the rat may not be uniform after incision. Only a proportion of MIAs reduced their response threshold 1 h after incision (Hämäläinen et al. 2002); one Aδ-fiber studied 1 day after incision met the criteria of a mechano-insensitive fiber, suggesting that no sensitization occurred. Only some MIAs supplying the knee joint of the cat (Schaible and Schmidt 1988), hairy skin of the rat (Kress et al. 1992), or hairy skin of the monkey (Davis et al. 1993) sensitized after application of chemicals or inflammatory mediators to their RF.

**FIG. 9.** Mechano-insensitive afferent fibers (MIAs). A: an example of an Aδ-MIA from the sham group; this fiber responded only to a filament with a force of 608 mN. B: relative proportion of mechanosensitive Aδ-fibers and Aδ-MIAs after sham procedure and after incision. A smaller proportion of Aδ-MIAs was recorded in rats 1 day after incision. The proportion of Aδ-fibers with response thresholds (RT) \( \leq 92 \) mN was greater after incision than after sham procedure \((P < 0.05)\); in behavioral experiments, 95% of the rats withdrew to this or lower forces 20 h after incision. C: proportion of mechanosensitive C-fibers and C-MIAs after sham procedure and after incision.

**Contribution of mechanical sensitization to pain behavior.**

Activation and sensitization of primary afferents may explain the reduced withdrawal thresholds observed behaviorally. First, the magnitude of the decrease in response threshold of Aδ-fibers correlated to the reduced withdrawal threshold in rats 1 day after incision compared with controls. A greater proportion of Aδ-fibers responded to forces \( \leq 92 \) mN in incised rats (Fig. 9B); these filaments produced hindpaw withdrawal in 95% of the incised rats. Therefore a greater number of Aδ-fibers will be activated and may contribute in part to the withdrawal.

Second, enhanced responsiveness of Aδ-fibers to punctate mechanical stimuli occurred in incised rats. However, in general, the peak responses produced by filaments causing withdrawal in incised rats (gray area in Fig. 5, A and B) were not much greater than the peak responses produced by the strongest filament in sham rats, which usually did not produce withdrawal (262 mN, horizontal broken line in Fig. 5). Therefore enhanced responsiveness of afferent fibers by itself does not explain the withdrawal in incised rats. Under inflammatory conditions, an enhanced responsiveness of afferent fibers to a range of mechanical stimuli was remarkable without a great reduction in response threshold (Andrew and Greenspan 1999; Cooper et al. 1991). However, these studies did not assess pain behaviors to make a direct comparison of neurophysiological and behavioral results; enhanced afferent fiber discharge limited to mechanical stimuli with high intensities may not contribute to reduced withdrawal thresholds in behavioral experiments (Andrew and Greenspan 1999; Cooper et al. 1991).

Third, expansion of RFs of Aδ- and C-fibers occurred that may be important for mechanical hyperalgesia after incision. An increase in RF size will cause more fibers to be activated by the punctate or nonpunctate stimulus and produce greater input to dorsal horn neurons.
Sensitization of afferent fibers to mechanical stimuli may only contribute in part to mechanical hyperalgesia after incision; central sensitization may also be important. Perhaps spontaneous activity in afferent fibers, particularly the high-frequency discharge of C-fibers, may produce sustained depolarization of dorsal horn neurons, and this may amplify responses to mechanical stimuli (Sandkühler 2000). Increased background activity and mechanical sensitization of dorsal horn neurons occurs as early as 1 h after incision (Vandermeulen and Brennan 2000). Noiceptive specific and wide dynamic range neurons develop background activity, expanded RFs, and enhanced responses to mechanical stimuli (Vandermeulen and Brennan 2000). However, only wide dynamic range neurons and not noiceptive specific neurons were activated by the same forces that produced withdrawal in behavioral experiments after plantar incision. Therefore sensitization of wide dynamic range neurons may transmit the behavioral responses (Vandermeulen and Brennan 2000); the spontaneous activity in Aδ- and C-fibers could amplify responses to mechanical stimuli, including low-threshold mechanoreceptors at the same wide dynamic range neuron. The spontaneous activity in Aδ- and C-fibers could also amplify responses to mechanical stimuli in nociceptive specific neurons; however, the nociceptive specific neurons may only sensitize to strong mechanical stimuli greater than 100 mN. These forces are greater than the withdrawal threshold after incision.

Finally, the nonevoked pain behavior we have observed after incision may not only be pain at rest. Because the scoring is based on weight bearing, there may be a mechanical component to the behavior. Mechanical sensitization of afferent fibers after incision could contribute to decreased weight bearing in an awake and ambulating rat.

Conclusion

The present study demonstrates that peripheral sensitization to mechanical stimuli contributes to pain behaviors after plantar incision. Sensitization of Aδ- and C-fibers occurs to the same mechanical stimuli that produce pain behaviors. Both groups of fibers increased their RF size, permitting more fibers to be activated by a stimulus. Spontaneous activity in afferents occurs; this may contribute to nonevoked pain behavior and mechanical hyperalgesia.

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