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Mechano- and thermosensitivity of injured muscle afferents

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Kirillova I, Rausch VH, Tode J, Baron R, Jänig W. Mechano- and thermosensitivity of injured muscle afferents. *J Neurophysiol* 105: 2058–2073, 2011. First published February 9, 2011; doi:10.1152/jn.00938.2010.— Injury of limb nerves leading to neuropathic pain mostly affects deep somatic nerves including muscle nerves. Here, we investigated the functional properties of injured afferent fibers innervating the lateral gastrocnemius-soleus muscle 4–13 h [time period (TP) I] and 4–7 days (TP II) after nerve crush in anesthetized rats using neurophysiological recordings from either the sciatic nerve (165 A-, 137 C-fibers) or the dorsal root L_5 (43 A-, 28 C-fibers). Ongoing activity and responses to mechanical or thermal stimulation of the injury site of the nerve were studied quantitatively. Of the electrically identified A- and C-fibers, 5 and 38% exhibited ectopic activity, respectively, in TP I and 51 and 61%, respectively, in TP II. Thus all afferent fibers in an injured muscle nerve developed ectopic activity since ~50% of the fibers in a muscle nerve are somatomotor or sympathetic postganglionic. Ongoing activity was present in 50% of the afferent A-fibers (TP II) and in 53–56% of the afferent C-fibers (TP I and II). In TP II, mechanical, cold, and heat sensitivity were present in 91, 63, and 52% of the afferent A-fibers and in 50, 40, and 66% of the afferent C-fibers. The cold and heat activation thresholds were 5–27 and 35–48°C, respectively, covering the noxious and innocuous range. Most afferent fibers showed combinations of these sensitivities. Mechano- and cold sensitivity had a significantly higher representation in A- than in C-fibers, but heat sensitivity had a significantly higher representation in C- than in A-fibers. These functional differences between A- and C-fibers applied to large- as well as small-diameter A-fibers. Comparing the functional properties of injured muscle A- and C-afferents with those of injured cutaneous A- and C-afferents shows that both populations of injured afferent neurons behave differently in several aspects.

nerve injury; ectopic impulse activity; mechanosensitivity; neuropathic pain

AFFERENT FIBERS INNERVATING skeletal muscle are large-diameter myelinated [Aα- (*group I*) or Aβ- (*group II*) fibers], small-diameter myelinated (Aδ- or *group III* fibers), or unmyelinated (C- or *group IV* fibers). The large-diameter myelinated fibers innervate muscle spindles or Golgi-tendon organs. They are mechanosensitive and involved in regulation of length and tension of skeletal musculature. Afferent muscle Aδ-fibers are nociceptive, low pressure-sensitive, or contraction-sensitive. Unmyelinated muscle afferents are nociceptive, low pressure-sensitive, contraction-sensitive, stretch-sensitive, thermosensitive, or show combinations of these sensitivities (Adreani et al. 1997; Adreani and Kaufman 1998; Hayes et al. 2005; Kaufman et al. 1983; Mense and Meyer 1985, 1988; Mense and Stahnke 1983; Taguchi et al. 2005; for review, see Kumazawa and Mizumura 1977, Mense 1993). In vitro investigation of muscle afferent neurons using calcium imaging of dorsal root ganglion (DRG) cells show that small-diameter muscle afferent neurons may be divided, according to their chemosensitivity, into low and high metabolite-responsive afferent neurons (Light et al. 2008). The small-diameter muscle afferent neurons are involved in activation of cardiovascular and respiratory systems during muscular exercise (Kaufman and Forster 1996; Mitchell et al. 1983), in sensations during exercise, as well as muscle fatigue, deep pressure sensation (Graven-Nielsen et al. 2004; Rivers and Head 1908), and pain sensations (Marchettini et al. 1996; Simone et al. 1994; Torebjörk et al. 1984).

Which functional ectopic discharge properties are shown by muscle afferents after injury to the muscle nerve? These ectopic discharge properties may be important in the generation of neuropathic pain following peripheral nerve injury. In a chronically injured skin nerve, most injured ectopically active A-fibers are mechanosensitive, but very few are thermosensitive, whereas injured ectopically active afferent C-fibers show mechano-, cold, or heat sensitivity or combinations of these sensitivities (Gorodetskaya et al. 2003, 2009; Grossmann et al. 2009a,b; Jänig et al. 2009).

Here, we investigated the ectopic discharge properties of injured myelinated and unmyelinated muscle afferents following crush lesion of the lateral gastrocnemius-soleus nerve in the rat. Ongoing activity and responses to mechanical, cold, or heat stimuli applied to the injury site of the nerve were quantitatively studied 4–13 h [time period (TP) I] and 4–7 days (TP II) after nerve crush. The results show that practically all injured afferent A-fibers and C-fibers of a muscle nerve can be activated by mechanical and/or thermal stimuli of the lesion site, about half of them also exhibiting ongoing activity.

METHODS

Seventy-seven male Wistar rats (399 ± 6.3 g body wt, range 300–570 g) were used for this study. In 22 rats, the lateral gastrocnemius-soleus nerve was crushed 7.2 ± 0.3 h (range 4–13 h) before the recording from afferent fibers started (TP I). In 55 rats, the lateral gastrocnemius-soleus nerve was crushed under general anesthesia [pentobarbital sodium (Narcoren; Merial, Hallbergmoos, Germany), 60 mg/kg ip] and aseptic conditions 4–7 days before the neurophysiological experiments (TP II). The nerve was exposed in the left hindlimb. Under visual control through a stereomicroscope, a length of ~0.5 mm of the nerve was crushed about 5–10 mm proximal to its entry into the skeletal muscle, using fine watchmaker forceps. The crush was repeated 3 times each for a few seconds, resulting in a translucent nerve. The wound was closed, and healing was uneventful. In the time period of 4–7 days after nerve injury, nerve fibers do not regenerate to the skeletal muscle, assuming a regeneration speed of 1–3 mm/day (Sunderland 1978, 1991).

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Anesthesia and Animal Maintenance

For the neurophysiological experiments, anesthesia and maintenance of the rats were the same as described previously (Gorodetskaya et al. 2003, 2009; Grossmann et al. 2009a,b). The rats were anesthetized by pentobarbital (60 mg/kg ip initially; 10 $mg \cdot kg^{-1} \cdot h^{-1}$ during surgery and the experiments). During the neurophysiological recordings, the rats were ventilated with air enriched by 20% O_2 at a rate of 70 strokes per minute. The mean arterial blood pressure measured via a catheter in the tail artery was always ≥80 mmHg. The acid-base status regularly determined was in the range of pH 7.4, P_{CO_2} = 35–40 mmHg, $P_{O_2} \geq 100$ mmHg. At the end of the experiments, the animals were killed under deep anesthesia by an intravenous injection of a saturated potassium chloride solution. All experiments were approved by the local animal care committee of the state administration and were conducted in accordance with German federal law.

Recording and Electrical Stimulation

In 55 rats, the left lateral gastrocnemius-soleus nerve and the sciatic nerve up to the sciatic notch were exposed. In these experiments, the sciatic nerve was put on a rigidly fixed, small, black Perspex platform ~10 mm proximal to the separation of the gastrocnemius-soleus nerves from it. A pool was formed from the skin flaps and filled with warm (30°C) paraffin oil.

In 20 rats, the lateral gastrocnemius-soleus nerve and the lumbar dorsal root L_4 or L_5 were exposed. The afferent neurons innervating the lateral gastrocnemius-soleus muscle project through the spinal nerve L_4 (45%) or the spinal nerve L_5 (55%) (Swett et al. 1991). In these rats, a laminectomy was performed by removing the dorsal processes and attached dorsal arches of the vertebra L_2 to L_6. Dorsal roots and spinal cord were covered by paraffin oil in a pool made of the surrounding skin. The dorsal root L_4 or L_5 was put on a rigidly fixed, small, black Perspex platform. Before isolating filaments from the sciatic nerve or from the dorsal root L_4 or L_5 the common peroneal nerve (after carefully separating it from the sciatic nerve), the sural nerve and the tibial nerve were transected.

On the black Perspex platform, fine strands were teased out from the dorsal site of the sciatic nerve or from the dorsal root L_4 or L_5 and put on a platinum electrode for recording, the indifferent electrode being connected to the nearby tissue. The signals recorded from the

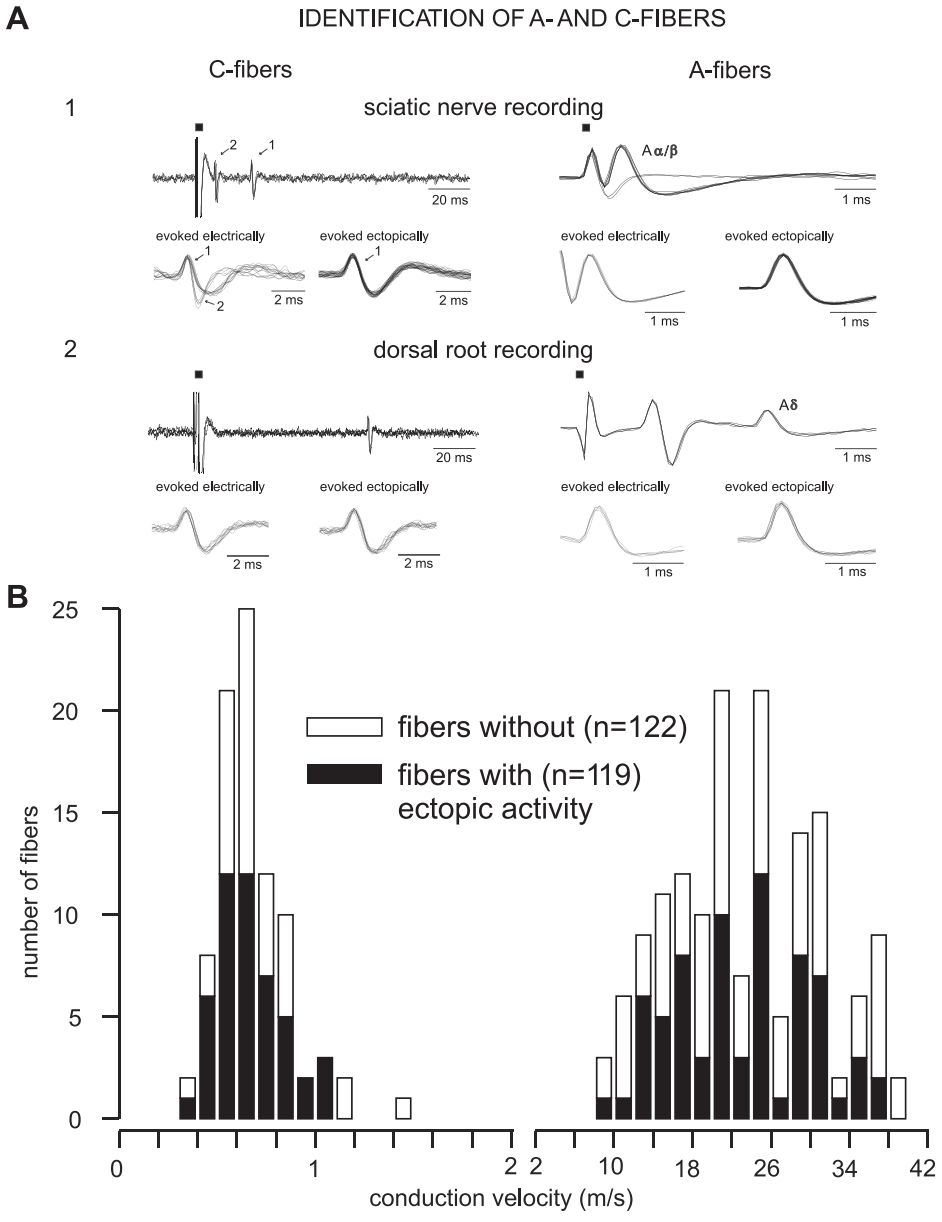

Fig. 1. Identification and conduction velocity of A- and C-fibers in the lateral gastrocnemius-soleus nerve. *A*: the conduction velocity was determined from the latency of the responses in filaments isolated from the sciatic nerve (*A1*) or the dorsal root L_4 or L_5 (*A2*) to single-pulse stimulation of the gastrocnemius-soleus nerve (*top* records, several times superimposed; stimulus indicated by the square). These electrically evoked responses were compared with the action potentials occurring spontaneously or being evoked by mechanical or thermal stimulation of the nerve injury site (*bottom* records, several times superimposed). The A-fiber in *A1* was an Aα/β-fiber and in *A2* probably an Aδ-fiber (conduction velocity 12 m/s). *B*: distribution of conduction velocities in TP II (4–7 days after nerve crush). Data are from 23 experiments in which all electrically identified fibers were tested. Black parts of the columns: fibers showing ongoing activity and/or responses to mechanical and/or thermal stimuli applied to the nerve injury site. Open parts of the columns: fibers that did not show ongoing activity, mechanosensitivity, or thermosensitivity.

afferent nerve fibers in the nerve strands were differentially amplified, filtered (120 Hz to 1–1.2 kHz for unmyelinated fibers, 120 Hz to 12 kHz for identifying myelinated fibers), and fed through window discriminators. The lateral gastrocnemius-soleus nerve was isolated from the surrounding tissue by a semiflexible plastic sheet on a length of 5–6 mm between the site where it was connected to the skeletal muscle and the site where it joined the sciatic nerve the injury site being in the middle. The nerve was positioned on a pair of platinum electrodes for electrical stimulation with square-wave pulses of 0.1-ms (A-fibers) or 0.5-ms (C-fibers) duration at strengths of up to 30 V (A-fibers 0.3–2 V; C-fibers 3–30 V). The distance between the proximal stimulation electrode and the recording electrode at the sciatic nerve or the recording electrode at the dorsal root was 13.7 ± 0.3 mm (range 10–20 mm) and 74.6 ± 1.1 mm, respectively. The stimulation electrodes were removed for mechanical or thermal stimulation of the injury site. In TP I 88% of the A-fibers and 62% of the C-fibers and in TP II almost all A-and C-fibers were identified electrically on the base of their conduction velocities (by measuring the latency of the responses to single-pulse stimulation of the gastrocnemius-soleus nerve; see *top* records in Fig. 1, *A1* and *A2*). The distinction between afferent A- and C-fibers in the rat based on the conduction velocity of their axons was made according to Lawson and coworkers (Lawson 2005; Lawson and Waddell 1991; Waddell et al. 1989). Fibers conducting at >2 m/s were considered to be myelinated, and fibers conducting at ≤2 m/s as unmyelinated. Lawson and Waddell (1991) show that afferent neurons with axons conducting at 1.3–2 m/s have either C- or Aδ-fibers. These fibers were rare in our sample (Fig. 1*B*).

In the remaining A- or C-fibers (most of them in TP I), the exact latency of response to electrical stimulation of the muscle nerve was not measured because of the overlap of the electrically evoked action potentials. However, in these cases, it was always possible to classify the fibers as A- or C-fibers on the basis of the size and shape of the action potential in relation to the electrically or ectopically evoked action potentials (Blenk et al. 1996). Under our filter conditions (see above), action potentials recorded from A-fibers were usually two-

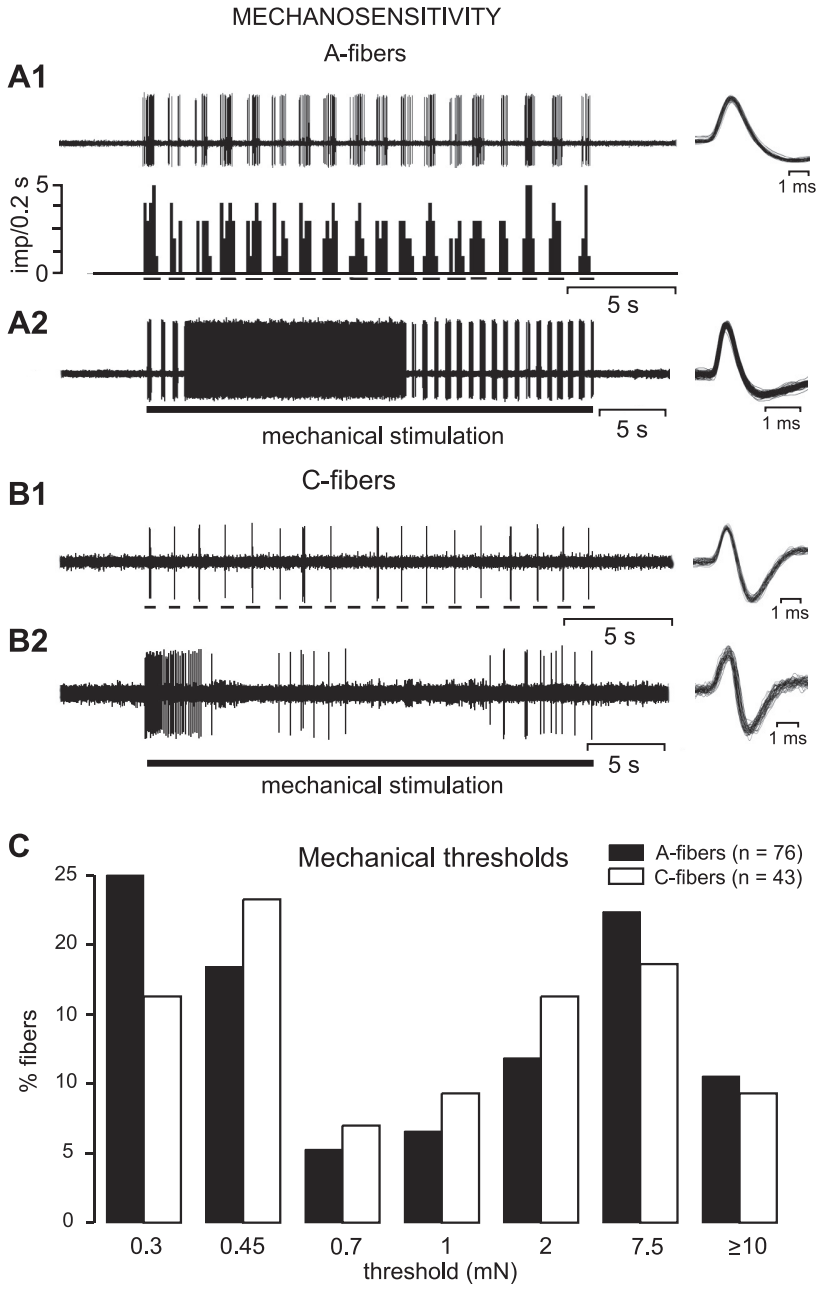

Fig. 2. Mechanosensitivity of A- and C-fibers. *A* and *B*: responses of injured A- (*A*) and C-fibers (*B*) to mechanical stimulation of the nerve. Mechanical stimuli were applied with glass fiber von Frey filaments at suprathreshold strengths and at a rate of 70 stimulations/min under visual control through a stereomicroscope. In *A1* and *B1*, the single stimuli are indicated to show the phasic responses of the afferent fibers. The A-fiber in *A2* switched from phasic responses to a tonic response during repetitive stimulation. The C-fiber in *B2* did not respond phasically to most repetitive stimuli. *Insets* on the *right*: action potentials several times superimposed. *C*: distribution of activation thresholds of injured A- and C-fibers to mechanical stimulation of the nerve. The strength of the von Frey filament applied to the receptive field in the nerve generating ≥3 action potentials in the 1st 10 s of stimulation in fibers being silent or ≥3 additional action potentials in fibers with ongoing activity ≤0.5 impulse (imp)/s was defined as activation threshold. In fibers with ongoing activity >0.5 imp/s, the threshold was an increase of activity by 50%. Thresholds of A-fibers were measured in time period (TP) II. Distributions of thresholds of C-fibers were not significantly different between TP I and II ($P > 0.05$, Wilcoxon-Mann-Whitney *U* test). The data from both time periods were therefore pooled together. Activation thresholds of A-fibers and C-fibers were not significantly different in TP II ($P > 0.05$, Wilcoxon-Mann-Whitney *U* test).

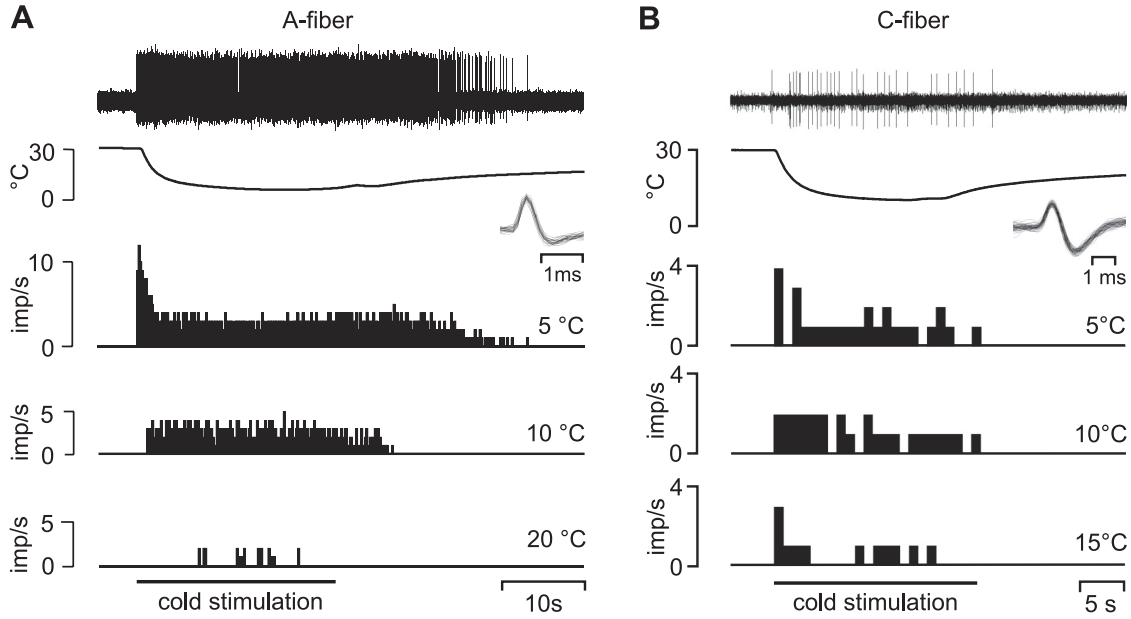

Fig. 3. Cold sensitivity of A- and C-fibers. *A*: graded responses of an A-fiber to cold stimulation of the nerve. The fiber was silent at 30°C. *Inset*: action potential several times superimposed. Data are from TP II. *B*: graded responses of a C-fiber to cold stimulation of the nerve. The fiber was silent at 30°C. *Inset*: action potentials several times superimposed. Data are from TP II.

phasic and larger than action potential recorded from C-fibers (see superimposed action potentials in Fig. 1*A*, *right*, and Figs. 2*A*, 3*A*, 6*A*, and 7). Action potentials recorded from C-fibers were usually triphasic (see superimposed action potentials in Figs. 1*A*, *left*, and Figs. 2*B*, 3*B*, and 6*B*).

Mechanical and Thermal Stimulation of the Muscle Nerve

All physiological stimuli were applied from 3-mm proximal to 3-mm distal to the injury site of the nerve.

Mechanical stimulation. Mechanosensitivity of injured muscle afferents was tested first qualitatively with a fine-tipped blunt glass rod (tip diameter 0.5 mm). Activation thresholds were determined with calibrated von Frey fiberglass filaments with circular plain tips of 0.5-mm diameter and forces of 0.3–10 mN (Marstock^{nervtest}; Fruhstorfer, Marburg, Germany). The mechanical stimuli were applied repetitively at 70 stimulations/min to the nerve, which was attached to and held in position by the semiflexible plastic sheet that isolated the nerve from the surrounding tissue. This way of mechanical stimulation was reproducible for the weak von Frey filaments but less so for von Frey filaments >10 mN. Therefore, we could reliably determine the mechanical thresholds of the injured afferent fibers but not the stimulus-response functions for mechanical stimuli using von Frey filaments >10 mN. An afferent fiber was considered to be mechanosensitive if it generated ≥3 action potentials in 10 s during the repetitive mechanical stimulation of the nerve in silent fibers or ≥3 additional action potentials in fibers with ongoing activity ≤0.5 impulse (imp)/s. In fibers with ongoing activity of >0.5 imp/s, mechanosensitivity was defined as an increase of activity by ~50% during the repetitive mechanical stimulation.

Thermal stimulation. Thermal sensitivity of the injured nerve fibers was tested qualitatively using a fine-tipped metal rod (tip diameter 1.5 mm) at temperatures of 5 or ~50°C. Thermal sensitivity was tested quantitatively using a water-perfused thermode that was positioned on the nerve at the injury site. The length of the contact site with the nerve was 3 mm. The temperature was measured at this contact site (see Gorodetskaya et al. 2003). The nerve fibers were stimulated with a series of either cold stimuli of 5–28°C or heat stimuli of 35–50°C in descending or ascending order, respectively. All thermal stimuli lasted for approximately 20–30 s starting from a baseline temperature of 30°C. A fiber was considered to be cold- or heat-activated either if at least 3–5 action potentials were evoked during a stimulus in a silent fiber or 3–5 additional action potentials in fibers with ongoing activity ≤0.5 imp/s or if the rate of ongoing activity increased by 50% in fibers with ongoing activity >0.5 imp/s.

Data Analysis

Neural activity, temperature of the thermode for stimulation of the injured nerve, arterial blood pressure, and electrocardiogram and endotracheal pressure were simultaneously fed into a computer using the Spike II System (Cambridge Electronic Design, Cambridge, United Kingdom). Data analysis was performed offline using the general purpose capture and analysis package Spike II. In this analysis, size and shape of the action potentials recorded from the nerve fibers were used to discriminate the signals recorded from different afferent fibers (see *bottom* records in Fig. 1, *A1* and *A2*). These parameters were continuously monitored to guarantee that we were recording from the same fiber since the action potentials sometimes changed in size and shape with time. Quan-

Table 1. *Percentages of electrically identified fibers with ongoing and/or evoked ectopic activity*

	4–13 Hours Rec Sciatic N.	χ^2-Test	4–7 Days Rec Sciatic N.	χ^2-Test	4–7 Days Rec Dorsal R.
A-fibers	12:244 (5.4%)	$P < 0.000$	79:155 (51%)	$P < 0.05$	41:61 (67.2%)
C-fibers	39:103 (37.9%)	$P < 0.02$	52:86 (60.5%)	$P < 0.05$	20:24 (83%)

Values are percentages of electrically identified fibers with ongoing activity (OA) and/or activity evoked by mechanical and/or thermal stimulation of the nerve injury site. The fibers recorded from the sciatic nerve (Rec Sciatic N.) were obtained in 19 experiments (4–13 h) and 23 experiments (4–7 days). The fibers recorded from the dorsal root (Rec Dorsal R.) L_4 or L_5 were obtained in 19 experiments.

Table 2. *Numbers and discharge characteristics of A-fibers 4–13 h (TP I) and 4–7 days (TP II) after crush injury of the lateral gastrocnemius-soleus nerve*

	4–13 Hours			4–7 Days		
	Rec Sciatic Nerve	Rec Dorsal Root	Total	Rec Sciatic Nerve	Rec Dorsal Root	Total
Number of fibers with ectopic activity	12	2	14	153	41	194
Individual Discharge Characteristics						
OA	4	1	5 (35.7%)	79	18	97 (50%)
Mechanosensitivity	8	1	9 (64.3%)	140	37	177 (91.2%)
Cold sensitivity	0	0	0%	103	19	122 (62.9%)
Heat sensitivity	0	0	0%	87	14	101 (52.2%)
Combinations of Discharge Characteristics						
Mech only/OA	8:0	1:0	9:0 (64.3%)	33:11	13:4	46:15 (23.7%)
Cold only/OA	0	0	0%	6:1	0	6:1 (3.1%)
Heat only/OA	0	0	0%	4:2	0	4:2 (2.1%)
Mech and cold/OA	0	0	0%	25:5	10:2	35:7 (18%)
Mech and heat/OA	0	0	0%	11:5	5:2	16:7 (8.2%)
Cold and heat/OA	0	0	0%	1:1	0	1:1 (0.5%)
Mech, heat, and cold/OA	0	0	0%	71:52	9:6	80:58 (41.2%)
OA only	4	1	5 (35.7%)	2	4	6 (3.1%)

Numbers and discharge characteristics of injured afferent A-fibers 4–13 h [time period 1 (TP I)] and 4–7 days (TP II) after crush injury of the lateral gastrocnemius-soleus nerve. The numbers behind the colon indicate the numbers of fibers with OA (included in the number before the colon). The percentages of individual discharge characteristics are expressed in respect to the total number of fibers showing ectopic discharges. Mech, mechanosensitivity.

titative measurements are either expressed as means ± SE or medians ± interquartile range. For statistical analysis, the χ^2-test or the Wilcoxon-Mann-Whitney *U* test was used.

RESULTS

Muscle afferent fibers are usually referred to as *groups I–IV* afferent fibers, *groups I* and *II* afferents being large-diameter myelinated (Aα/β-) fibers, *group III* afferents small-diameter myelinated (Aδ-) fibers, and *group IV* afferents unmyelinated (C-) fibers. In our study, we had no criteria to discriminate between *group I*, *II*, or *III* fibers (Fig. 1*B*). Therefore, we will use throughout the text the terms A-fibers (or myelinated fibers) and C-fibers (or unmyelinated fibers).

Proportions of Injured Muscle A- and C-Fibers with Ectopic Activity

The term "ectopic activity" of an afferent fiber denotes activity originating spontaneously at the injury site or DRG cells of afferent neurons and activity evoked by mechanical or thermal stimulation of afferent neurons at the injury site along their axons or at the DRG.

Table 3. *Numbers and discharge characteristics of C-fibers 4–13 h (TP I) and 4–7 days (TP II) after crush injury of the lateral gastrocnemius-soleus nerve*

	4–13 Hours			4–7 Days		
	Rec Sciatic Nerve	Rec Dorsal Root	Total	Rec Sciatic Nerve	Rec Dorsal Root	Total
Number of fibers with ectopic activity	39	8	47	20	98	118
Individual Discharge Characteristics						
OA	23	2	25 (53.2%)	12	54	66 (55.9%)
Mechanosensitivity	29	6	35 (74.5%)	11	48	59 (50%)
Cold sensitivity	5	6	11 (23.4%)	14	33	47 (39.8%)
Heat sensitivity	22	3	25 (53.2%)	12	66	78 (66.1%)
Combinations of Discharge Characteristics						
Mech only/OA	13:8	1:0	14:8 (29.8%)	1:0	15:7	16:7 (13.6%)
Cold only/OA	0	2:0	2:0 (4.3%)	2:1	6:1	8:2 (6.8%)
Heat only/OA	9:5	0	9:5 (19.1%)	2:2	30:18	32:20 (27.1%)
Mech and cold/OA	3:1	2:1	5:2 (10.6%)	4:2	8:2	12:4 (10.2%)
Mech and heat/OA	11:7	1:1	12:8 (25.5%)	2:2	17:7	19:9 (16.1%)
Cold and heat/OA	0	0	0	4:2	11:9	15:11 (12.7%)
Mech, heat, and cold/OA	2:1	2:0	4:1 (8.5%)	4:2	8:7	12:9 (10.2%)
OA only	1	0	1 (2.1%)	1	3	4 (3.4%)

Numbers and discharge characteristics of injured afferent C-fibers 4–13 h (TP I) and 4–7 days (TP II) after crush injury of the lateral gastrocnemius-soleus nerve. The numbers behind the colon indicate the numbers of fibers with OA. The percentages of individual discharge characteristics are expressed in respect to the total number of fibers showing ectopic discharges.

This study is based on the recording from 208 injured afferent A-fibers and 165 injured afferent C-fibers that could be activated by electrical stimulation of the lateral gastrocnemius-soleus nerve and exhibited at least 1 form of discharge property (ongoing activity, mechanosensitivity, cold sensitivity, and/or heat sensitivity). In TP I (4–13 h after nerve injury), 5.4% of the electrically identified A-fibers and 37.9% of the electrically identified C-fibers isolated from the sciatic nerve exhibited ectopic activity (Table 1).

In 23 experiments of TP II (4–7 days after nerve injury), we studied systematically all nerve fibers in the filaments, which were isolated from the sciatic nerve and activated by electrical stimulation of the lateral gastrocnemius-soleus nerve for their ectopic ongoing and evoked activity and for their conduction velocity. In these experiments, 51% of the electrically identified A-fibers and 60.5% of the electrically identified C-fibers were ectopically active (Table 1). Thus the proportion of ectopically active afferent A- and C-fibers significantly increased in TP II compared with TP I ($P < 0.0001$ for A-fibers and $P < 0.02$ for C-fibers, χ^2-test). The distribution of the conduction velocity of these fibers shows a clear separation between C- and A-fibers, only 1 fiber conducting in the range of 1.3–2 m/s. The distribution of the conduction velocities of A-fibers did not show a separation between Aδ- (*group III*) and Aα/β- (*group I/II*) fibers (Fig. 2*B*).

About 50% of the myelinated axons in the gastrocnemius-soleus nerve are somatomotor axons (Mitchell and Schmidt 1983; Peyronnard et al. 1986; Sittiracha and McLachlan 1986), and 45% of the unmyelinated axons are sympathetic postganglionic axons (Baron et al. 1988). Assuming that injured somatomotor and postganglionic axons do not exhibit spontaneous or evoked ectopic activity (Schmelz et al. 1998; Schmidt et al. 1995), we conclude that all or most myelinated and unmyelinated fibers showing no ectopic activity are efferent fibers (Fig. 1*B*) and that therefore all or most injured muscle afferents show ectopic activity in TP II. This conclusion is supported by the finding that the proportions of electrically identified A- and C-fibers that were isolated from the dorsal root L_4 or L_5 and exhibited ectopic activity were significantly higher than the proportions of electrically identified A- and C-fibers with ectopic activity that were isolated from the sciatic nerve (Table 1).

Seventy-nine percent of the A-fibers and 83% of the C-fibers with ectopic activity were recorded from filaments isolated from the sciatic nerve, and the remaining fibers were isolated from the dorsal root L_5 (see *top* of Tables 1 and 2). There were no statistically significant differences in the functional characteristics (incidence of ongoing activity, mechano- or thermosensitivity, or their combinations) between the afferent fibers isolated from the sciatic nerve and the afferent fibers isolated from the dorsal root. Therefore,

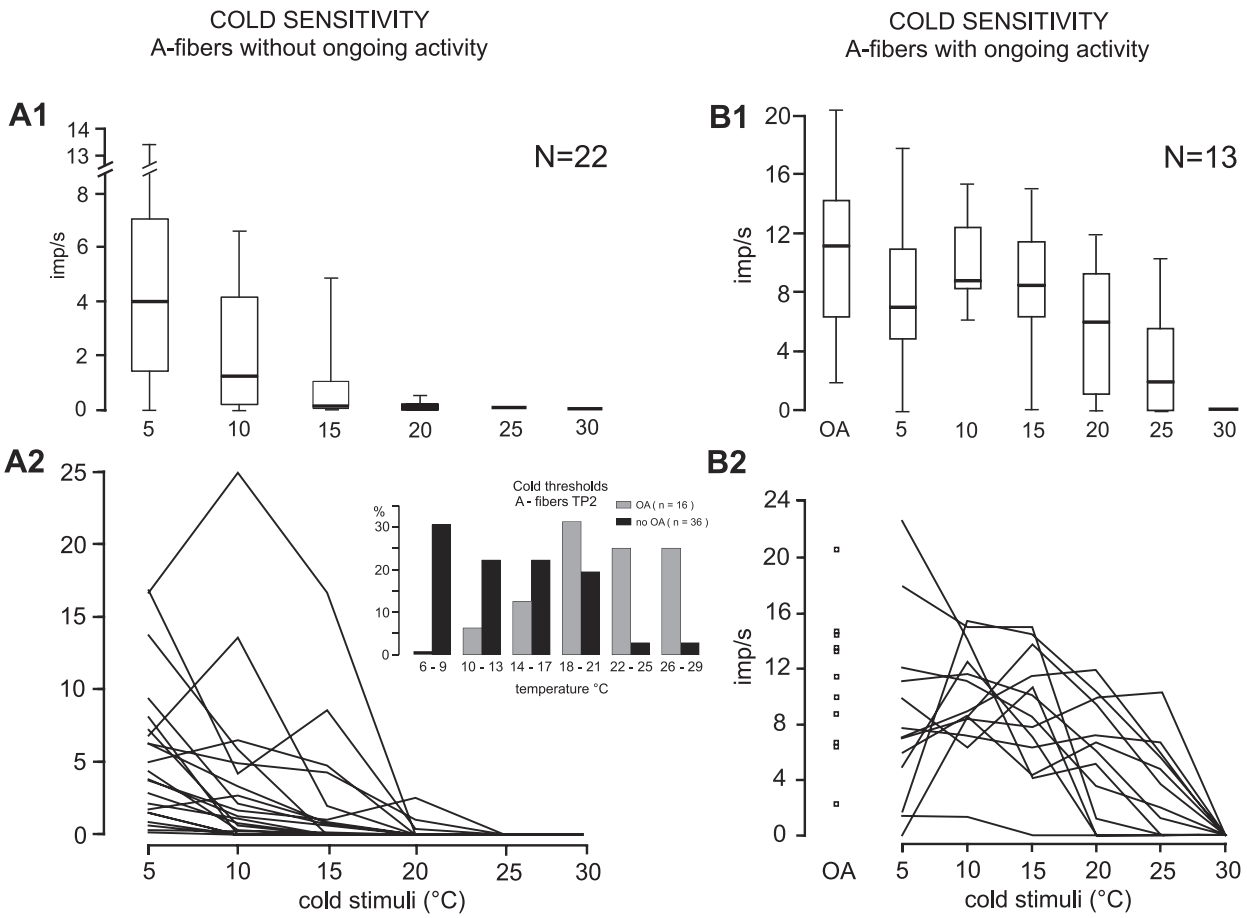

Fig. 4. Stimulus-response functions of A-fibers to cold stimuli in TP II. The afferent fibers were separated into silent fibers [no ongoing activity (OA); *A1* and *A2*] and fibers with OA (*B1* and *B2*). *A2* and *B2* show the responses of single-afferent fibers, and *A1* and *B1* the population responses (median plus interquartile range). The rate of OA was subtracted from the response to cold stimuli. The ordinate scales are the activity in impulses per second during stimuli of 20-s duration (see Fig. 3*A*). *Inset* in *A2*: distribution of cold thresholds of A-fibers.

both populations of afferent fibers will be described together.

We will first describe the discharge properties of the injured A- and C-fibers evoked by physiological stimulation. Then we will describe the ongoing activity and the pattern of discharge of the injured afferent fibers. The numbers of afferent neurons exhibiting individual and combinations of discharge characteristics to the physiological stimuli are shown in Tables 2 and 3. All percentages will be expressed with respect to the total numbers of afferent A- or C-fibers that exhibited at least one type of ectopic activity.

Mechanosensitivity

A-fibers. In TP I, only very few A-fibers showed mechanosensitivity (Table 2). In TP II, 91% of the injured A-fibers with ectopic activity were mechanosensitive. Most A-fibers showed brisk responses with high instantaneous frequencies to repetitive mechanical stimulation at ~1 Hz (Fig. 2*A1*). Some of them switched to tonic discharge during this repetitive mechanical stimulation (Fig. 2*A2*). The mechanical thresholds ranged from 0.3 to >10 mN (median 0.7 mN, $n = 76$) and showed a bimodal distribution with modal values of about 0.3–0.45 and 7.7 mN (Fig. 2*C*). Although not systematically investigated, it was obvious that the mechanosensitive A-fibers were also very stretch sensitive. During the positioning of the thermode on the lateral gastrocnemius-soleus nerve (for thermal stimulation), the nerve was always slightly stretched leading sometimes to strong activation of the highly mechanosensitive A-fibers.

C-fibers. The frequency of C-fibers with mechanosensitivity was 74.5% in TP I and 50% in TP II (Table 2). In TP II, it was significantly lower than in A-fibers (59:118 C-fiber vs. 177:194 A-fibers; $P < 0.000$, χ^2-test). The magnitude of the responses to mechanical stimulation of the nerve injury site was lower than in A-fibers. Mechanosensitive C-fibers responded only with one impulse to a brief stimulus applied with a suprathreshold von Frey filament or a glass rod (Fig. 2*B1*), or they responded rather irregularly to repetitive mechanical stimulation of the nerve injury site (Fig. 2*B2*). The thresholds ranged from 0.3 to >10 mN (median 0.7 mN, $n = 43$) and showed a bimodal distribution similar to that of A-fibers with modal values of 0.45 and 7.5 mN (Fig. 2*C*). They were statistically not significantly different from those in A-fibers ($P > 0.05$, Wilcoxon-Mann-Whitney U test).

Cold Sensitivity

A-fibers. In TP I, cold sensitivity was absent in injured A-fibers. In TP II, 63% of the A-fibers with ectopic activity were cold-sensitive, showing graded responses to cold stimuli (Fig. 3*A*). Cold-sensitive A-fibers with ongoing activity showed larger responses to cold stimuli than silent cold-sensitive A-fibers (Fig. 4, *A* and *B*). The maximal responses in spontaneously active A-fibers occurred mostly at 10–15°C (Fig. 4*B2*) and in silent A-fibers at <10°C (Fig. 4*A2*). The thresholds to cold stimuli ranged from 6 to 27°C. They were significantly lower in spontaneously active A-fibers than in silent A-fibers (*inset* in Fig. 4*A2*; $P < 0.001$, Wilcoxon-Mann-Whitney U test).

C-fibers. The frequency of C-fibers with cold sensitivity was higher in TP II (40%) than in TP I (23.4%; $P < 0.05$, χ^2-test). It was significantly lower than that of injured cold-sensitive A-fibers in TP II (47:118 vs. 122:194 fibers; $P < 0.000$, χ^2-test; Tables 2 and 3). The responses to cold stimuli were mostly tonic (Fig. 3*B*) and varied considerably between C-fibers (Fig. 5*B*). In most C-fibers, these responses were maximal at temperatures of ≤10°C (Fig. 5*B*). The thresholds ranged from 5 to 20°C (median 15°C; *inset* in Fig. 5). The discharge rate at 5°C was significantly lower in C-fibers than in cold-sensitive A-fibers ($P < 0.001$, Wilcoxon-Mann-Whitney U test; Figs. 4 and 5).

Heat Sensitivity

A-fibers. Heat sensitivity in injured A-fibers was absent in TP I and present in 52% of the fibers in TP II. The responses to heat stimuli of 20- to 30-s duration applied to the nerve injury site were graded (Fig. 6*A*). In most heat-sensitive A-fibers, the activation outlasted the stimulus by 1–4 min. The latency of the responses varied, starting immediately, during the stimulus, or even after the stimulus (Fig. 7). The discharge pattern during heat stimulation was regular in 21.9%, bursting in 37.5%, or bursting followed by regular discharges in 40.6% of the heat-sensitive A-fibers. These patterns correlated with the pattern of ongoing activity. Thus A-fibers with a regular ongoing discharge mostly showed a regular discharge pattern during and after heat stimulation (Fig. 7), and A-fibers with a

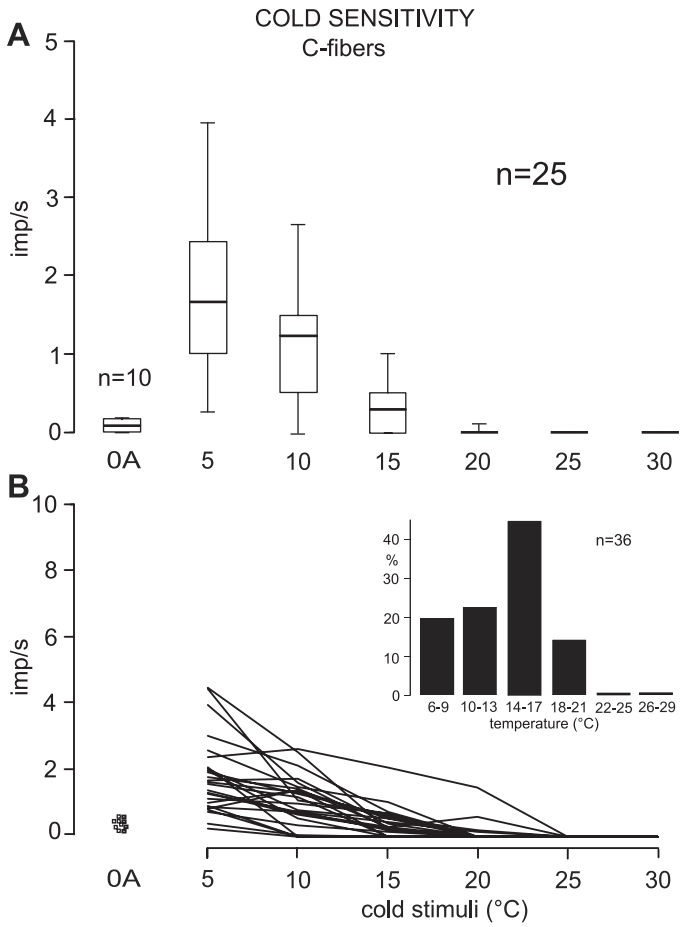

Fig. 5. Stimulus-response functions of C-fibers to cold stimuli in TP II. *A*: population responses (median plus interquartile range). *B*: responses of single afferent fibers. The rate of OA in 10 fibers was subtracted from the response to cold stimuli. The ordinate scales are the activity in impulses per second during stimuli of 20-s duration (see Fig. 3*B*). *Inset*: distribution of cold thresholds of C-fibers in TP I and II.

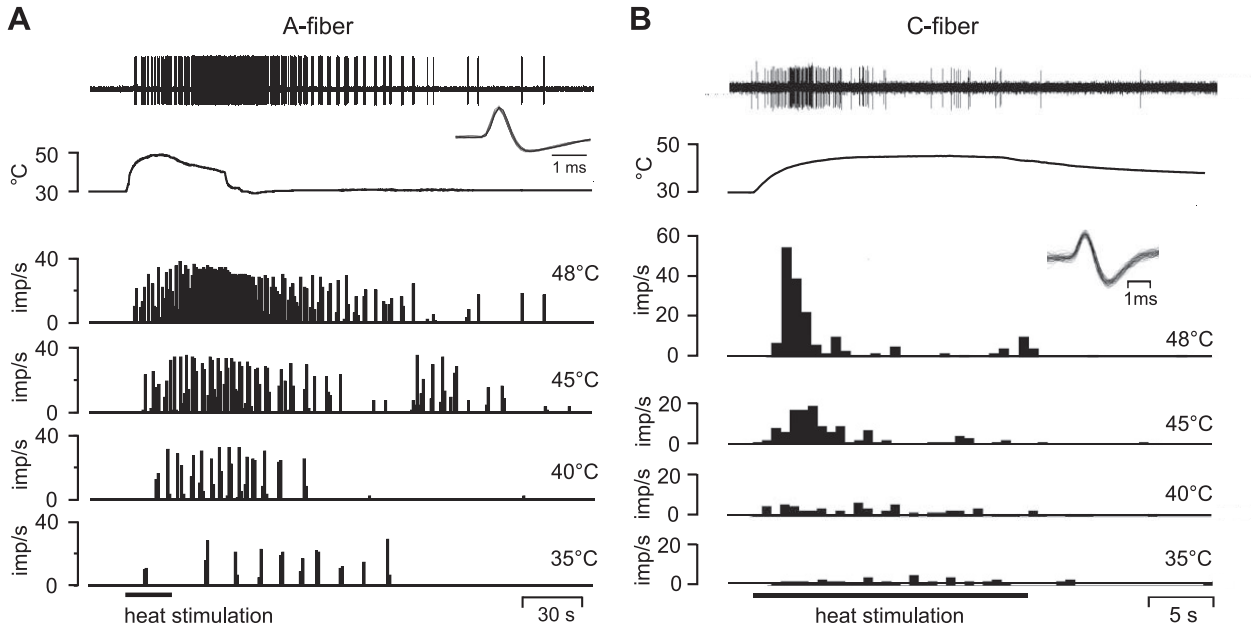

Fig. 6. Responses of an A- (*A*) and a C-fiber (*B*) to graded heat stimuli. Note the long-lasting response of the A-fiber and the bursting activity. *Insets*: action potentials several times superimposed. Data are from TP II.

bursting ongoing discharge a bursting discharge during heat stimulation (Fig. 6*A*). A-fibers with ongoing activity showed significantly stronger and longer responses to heat stimulation than silent heat-sensitive A-fibers at stimuli of 40 and 45°C (Fig. 8; 40°C, $P < 0.0002$; 45°C, $P < 0.01$, Wilcoxon-Mann-Whitney *U* test). The heat threshold was significantly lower in spontaneously active A-fibers than in silent A-fibers (*inset* in Fig. 8*A*; $P < 0.01$, Wilcoxon-Mann-Whitney *U* test).

C-fibers. Heat sensitivity was present in 53.2% of the injured C-fibers in TP I and in 66.1% in TP II. In TP II, its frequency was significantly higher than in A-fibers (78:118 vs. 101:194 fibers; $P < 0.02$, χ^2-test). As in the heat-sensitive A-fibers, the thresholds ranged from 35 to 50°C (median 40°C; *inset* in Fig. 9*C*). The responses to heat stimuli were phasic, tonic, or bursting. They did not outlast the stimulus and were not delayed with respect to the stimulus (Fig. 6*B*). The stimulus-response functions varied between individual C-fibers (Fig. 9*B*). The discharge rate during heat stimuli of 20- to 30-s duration was significantly lower in C- than in A-fibers (Figs. 8 and 9*A*; $P < 0.002$ for 48°C, Wilcoxon-Mann-Whitney *U* test).

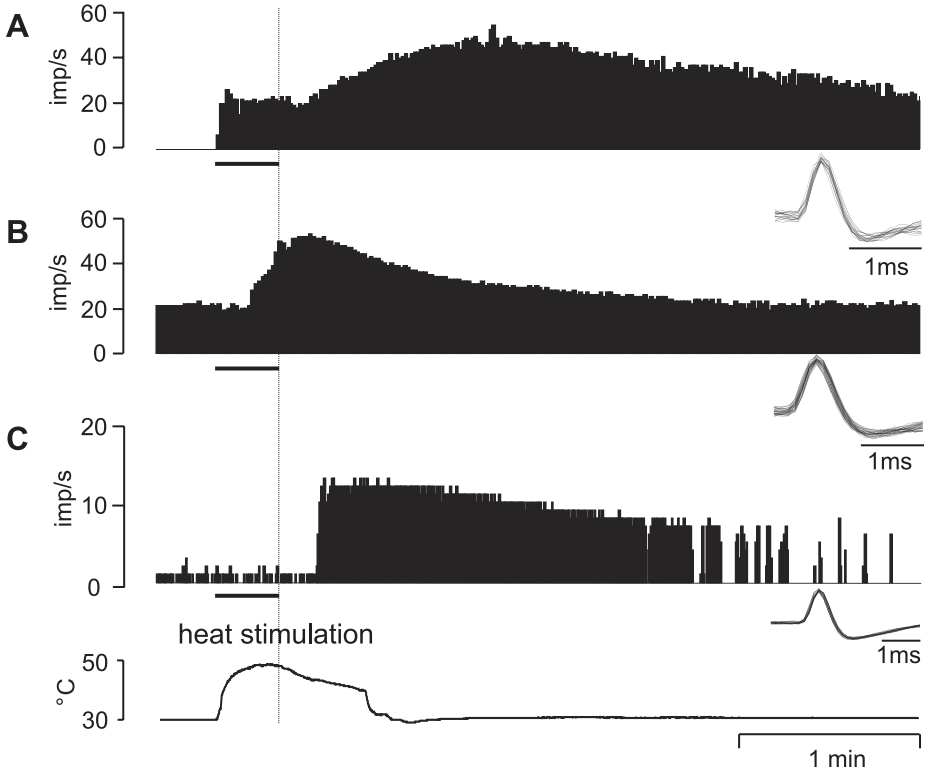

Fig. 7. Different latencies of responses of 3 A-fibers to heating stimuli applied to the nerve injury site. The dotted line indicates the end of the stimulus. The activation outlasted the stimulus in these fibers. The fiber in *A* was immediately activated and increased its activity after the stimulus for ~1 min. The fiber in *B* started to be activated in the middle of the heat stimulus. The fiber in *C* started to be activated >10 s after the stimulus when the temperature was still ~43°C.

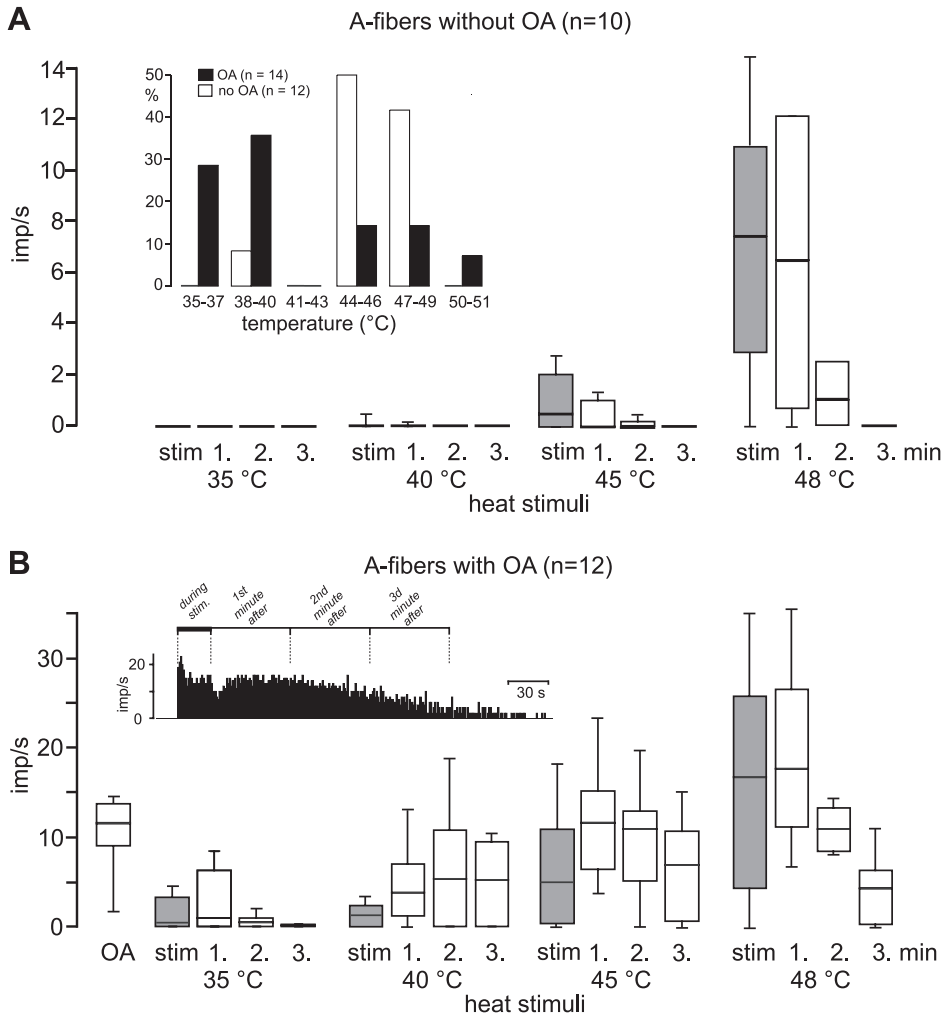

Fig. 8. Stimulus-response functions of A-fibers to heat stimuli (stim). The fibers were separated into silent fibers [no ongoing activity (OA); *A*] and fibers with OA (*B*). The responses were separated into those during stimulation (gray columns) and those in the 1, 2, and 3 min after stimulation (open columns; see *inset* in *B*). The OA was subtracted from the responses to heat stimuli. Medians and interquartile range are shown. *Inset* in *A*: distribution of activation thresholds to heat stimuli. Data are from TP II.

Ongoing Activity

A-fibers. In TP I, ongoing activity in A-fibers was almost absent. In TP II, 50% of the A-fibers exhibited ongoing activity. The rate of ongoing activity in these fibers measured before the fibers were activated by mechanical or thermal stimuli ranged from 0.01 to 38.8 imp/s (means ± SE, 10.9 ± 0.9 imp/s, median 9.5 imp/s, $n = 96$; Fig. 10). The pattern of ongoing activity measured in 94 A-fibers was irregular (20.2%), regular (23.4%), or bursting (56.4%), showing in the interval histograms of the activity one peak or two to three peaks. Some 9% of the A-fibers discharged in doublets and/or triplets; these fibers usually had a regular pattern of ongoing activity. The mean discharge rate was significantly higher in afferent fibers with regular activity than in afferent fibers with bursting or irregular activity [22.8 ± 1.6 imp/s (regular, $n = 22$) vs. 10.4 ± 0.8 imp/s (bursting, $n = 53$), $P < 0.0002$; 10.4 ± 0.8 imp/s (bursting, $n = 53$) vs. 0.95 ± 0.23 (irregular, $n = 19$), $P < 0.0001$, Wilcoxon-Mann-Whitney *U* test]. Frequency and rate of ongoing activity of the fibers were not correlated with their responsiveness to one of the physiological stimuli.

The rate of ongoing activity was not higher in A-fibers conducting at ≥20 m/s (11.7 ± 1.4 imp/s, $n = 53$) than in A-fibers conducting at <20 m/s (10.9 ± 1.5 imp/s, $n = 26$), most of the latter being possibly Aδ- (*group III*-) fibers. The rates of ongoing activity in fibers isolated from the sciatic nerve and fibers isolated from the dorsal root were not significantly different [11.7 ± 1.0 imp/s ($n = 79$) vs. 9.8 ± 2.4 imp/s ($n = 17$)].

C-fibers. Fifty-three percent of the C-fibers had ongoing activity in TP I and 56% in TP II. The rate of ongoing activity ranged from 0.02 to 6.95 imp/s (median 0.32 imp/s, $n = 85$; Fig. 10). In TP I, the rate of ongoing activity was significantly higher than in TP II [0.13–7.0, median 0.5 imp/s ($n = 25$) vs. 0.01–6.4, median 0.22 imp/s ($n = 60$); $P < 0.02$, Wilcoxon-Mann-Whitney *U* test]. The rate of ongoing activity was significantly lower in C-fibers than in injured A-fibers whether conducting at ≥20 or <20 m/s ($P < 0.0001$, Wilcoxon-Mann-Whitney *U* test). Frequency and rate of ongoing activity in C-fibers of the fibers were not correlated with their responsiveness to mechanical or thermal stimuli.

The pattern of ongoing activity in C-fibers measured in 94 A–fibers was irregular (53%; 0.01–1.95, median 0.2 imp/s, $n = 45$), irregular bursting (34.1%; 0.06–2.1, median 0.33 imp/s, $n = 29$), or regular (12.9%; 0.18–6.95, median 2.4 imp/s, $n = 11$). The rate of ongoing activity was significantly higher in regularly discharging C-fibers than in C-fibers discharging irregularly or irregularly in bursts ($P < 0.000$). The rates of ongoing activity in fibers isolated from the sciatic nerve and fibers isolated from the dorsal root in TP II were

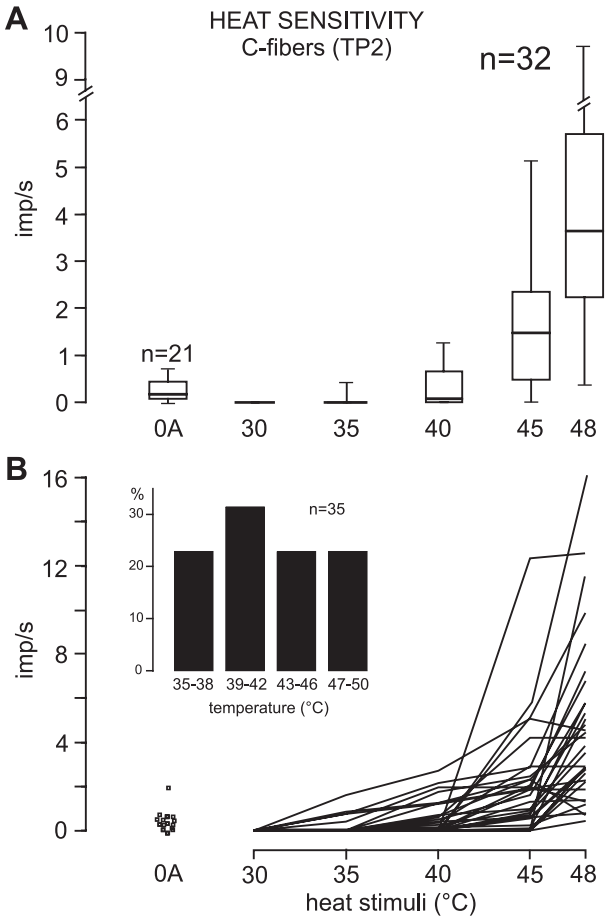

Fig. 9. Stimulus-response functions of C-fibers to heat stimuli. The OA in 21 fibers was subtracted from the responses to heat stimuli. Medians and interquartile range are shown. *Inset*: distribution of activation thresholds to heat stimuli. Data are from TP II.

not significantly different [0.02–6.4, median 0.24 imp/s ($n = 48$) vs. 0.01–0.94, median 0.21 imp/s ($n = 12$)].

Patterns of Discharge

The *bottoms* of Tables 2 and 3 show the discharge patterns in injured muscle afferent fibers in TP I and II. These patterns of activation are illustrated graphically in Fig. 11 for TP II. The following differences between A- and C-fibers in TP II need to be emphasized.

1) Cold sensitivity and mechanosensitivity have a significantly higher representation in A- than in C-fibers ($P < 0.000$, χ^2-test).

2) Heat sensitivity has a significantly higher representation in C- than in A-fibers ($P < 0.02$, χ^2-test).

3) Cold or heat sensitivity is associated with mechanosensitivity in >90% of the cold- or heat-sensitive A-fibers but in only 40–50% of the cold- or heat-sensitive C-fibers. This corresponds to the observation that 48% of the C-fibers are only thermo- (cold- and/or heat-) sensitive but not mechanosensitive compared with 5.9% of the A-fibers ($P < 0.000$, χ^2-test).

4) The patterns of discharge produced by mechanical or thermal stimuli are not significantly different in A-fibers conducting at <20 m/s [most of them probably being Aδ- (or *group III*) afferents] and in A-fibers conducting at ≥20 m/s [all of them being Aα/β- (or *group II* and *I*) afferents]. However, the patterns of both are significantly different from those in C-fibers as shown for the total population of A-fibers in Fig. 11.

DISCUSSION

Here, we have described for the first time the responsiveness of myelinated and unmyelinated muscle afferents to mechanical and thermal stimuli applied to the injury site of the nerve 4–13 h (TP I) and 4–7 days (TP II) after crush lesion of the muscle nerve. The overall results follow. *1*) Almost all afferent A- and C-fibers develop ongoing activity and/or mechano- and/or thermosensitivity. *2*) A high proportion of C-fibers but very few A-fibers show mechanosensitivity and/or thermosensitivity already hours after nerve injury. *3*) Mechanosensitivity is particularly prominent in A-fibers. *4*) A high proportion of both A- and C-fibers exhibits cold and/or heat sensitivity, which is mostly combined with mechanosensitivity. *5*) Afferent fibers being thermosensitive only are rare in A-fibers but common in C-fibers. *6*) Ongoing activity is present in close to 50% of the A-fibers (in TP II) and in >50% of the C-fibers. *7*) The functional characteristics of A-fibers conducting at <20 m/s are significantly different from those of C-fibers.

Proportions of Injured Fibers with Ectopic Activity

About 50% of the myelinated fibers in the gastrocnemius-soleus nerve are somatomotor fibers (Mitchell and Schmidt 1983; Peyronnard et al. 1986; Sittiracha and McLachlan 1986), and 45% of the unmyelinated fibers are postganglionic (Baron et al. 1988). These motor fibers do not develop ectopic ongoing activity or responsiveness to physiological stimuli when injured (Schmelz et al. 1998; Schmidt et al. 1995; A. Teliban, F. Bartsch, M. Struck, and W. Jänig, unpublished observations). Therefore, practically all injured muscle afferent A- and C-fibers show at least one type of activity elicited by mechanical or thermal stimulation or generated spontaneously (Table 4).

Functional Response Characteristics of Injured and Intact Muscle Afferents

Not unexpectedly, most injured A-fibers and 50–80% of the injured C-fibers were mechanosensitive compared with the response properties of intact muscle afferents (Mense 1993; Taguchi et al. 2005). The high degree of mechanosensitivity of injured myelinated muscle afferents as reflected in their low threshold to mechanical stimulation with von Frey filaments and in their high stretch sensitivity (Fig. 2*C*) has already been demonstrated by Johnson and Munson (1992) and Proske et al. (1995). The high mechanosensitivity of afferent C-fibers in an injured muscle nerve is described here for the first time. The thresholds clearly show a bimodal distribution both in A- and C-fibers with modal values of 0.3–0.45 and 7.5 mN (Fig. 2*C*). Whether this is related to low-threshold (nonnociceptive) and high-threshold (nociceptive) mechanosensitive intact muscle afferents we do not know and needs to be studied.

The high frequency of cold and heat sensitivity of injured muscle afferents is remarkable. These thermal sensitivities are mostly combined with mechanosensitivity, although some 50% of the C-fibers are only thermosensitive. Heat sensitivity is already present in TP I (i.e., hours after nerve crush) in ~50% of the C-fibers with ectopic activity, and this frequency re-

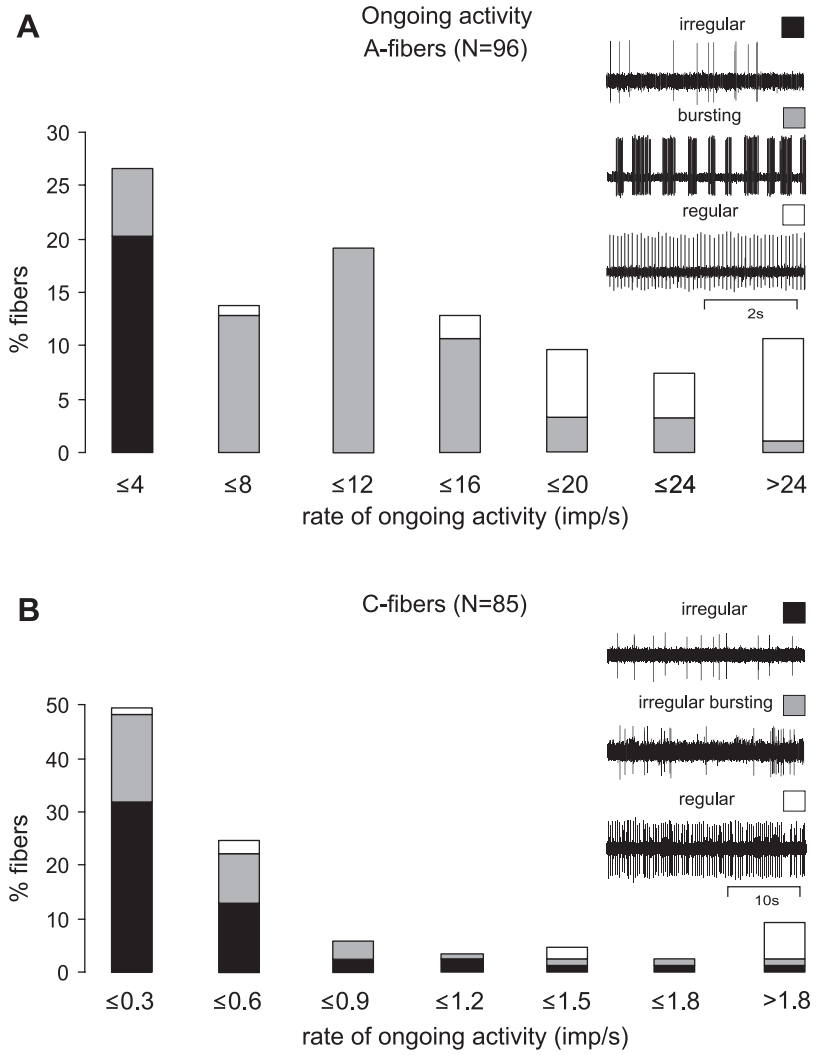

Fig. 10. Distribution of rate of ongoing activity (OA) in A- (*A*) and C-fibers (*B*). OA in A-fibers is from TP II. OA in C-fibers is from TP I ($n = 25$) and TP II ($n = 60$). The rate of OA in C-fibers was significantly higher in TP I than in TP II (see text). *Insets*: examples of irregular, bursting, and regular OA in A-fibers (*A*) and of irregular, irregular bursting, and regular OA in C-fibers (*B*).

mains as high in TP II, the thresholds ranging from 35 to 50°C (Fig. 8*B*). Heat sensitivity is absent in A-fibers in TP I but expressed in some 50% of these afferent fibers in TP II. Heat sensitivity has been reported in about 40–60% of intact muscle mechanosensitive C-fibers (Kumazawa and Mizumura 1977; Taguchi et al. 2005). Furthermore, ~50% of intact muscle mechanosensitive C-fibers are capsaicin-sensitive (Hoheisel et al. 2004). Activation of intact muscle A-fibers by heating the muscle has not been reported in the literature. Thus heat sensitivity is a novel functional characteristic of injured muscle A-fibers but not of injured muscle C-fibers. Whether heat sensitivity of injured muscle afferents contributes to neuropathic pain requires further study.

In TP I, cold sensitivity is absent in A-fibers and present in some 20% of C-fibers with ectopic activity. Its frequency increases to some 40–60% in both groups of fibers in TP II. Cold sensitivity has not been reported in intact muscle A-fibers and only in a few intact muscle C-fibers (Kumazawa and Mizumura 1977; Mense 1986, 1993; Taguchi et al. 2005). Thus cold sensitivity is a novel functional property of injured muscle afferents. The cold thresholds to excite the A- or C-fibers are largely in the noxious range, although some cold-sensitive A-fibers with ongoing activity can be excited by stimuli of 25–30°C. Whether the cold sensitivity of injured muscle afferents contributes to neuropathic pain or deep somatic dys- and paresthesias may appear unlikely but needs to be studied.

About 50% of the injured muscle A- and C-afferents show ongoing activity. Whether noninjured muscle afferents have ongoing activity is very much debated. However, it has been shown by some groups that intact muscle Aδ-afferents (Kumazawa and Mizumura 1977) and up to 90% of the intact muscle C-fibers exhibit a low rate of ongoing activity (Berberich et al. 1988; Diehl et al. 1993; Kumazawa and Mizumura 1977; Taguchi et al. 2005). The rates and frequencies of ongoing activity are the same in the populations of injured muscle A- and C-fibers isolated from the sciatic nerve and in the populations of injured A- and C-fibers isolated from the dorsal root L_4 or L_5. This supports the notion that ongoing activity in injured muscle afferent neurons may primarily originate in the periphery at the injured site and not in the DRG (Devor 2006; Wall and Devor 1983). This agrees with the finding that peripheral nerve injury occurring remotely from the DRG is followed by ongoing activity originating in the DRG in some 20% of muscle afferent neurons that have small-diameter myelinated axons but not in the DRG cells of muscle afferent neurons with unmyelinated axons (and not in the DRG cells of cutaneous afferent neurons). The rate of ongoing activity originating in the DRG in muscle afferent

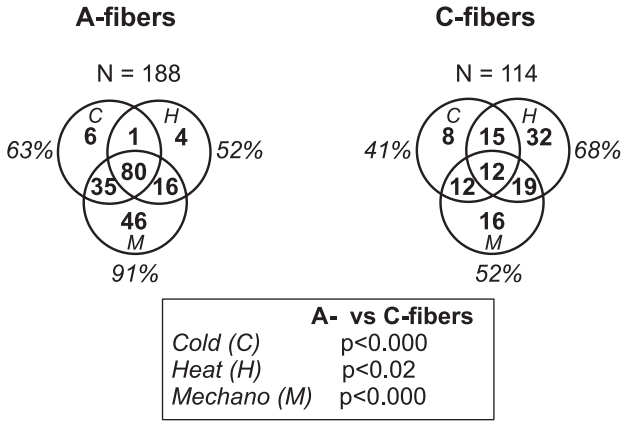

Fig. 11. Patterns of cold, heat, and mechanosensitivity in A- and C-fibers projecting in the injured muscle nerve in TP II. The total numbers do not include afferents that have only ongoing activity (6 A-fibers and 4 C-fibers). The total numbers are therefore lower than in Tables 2 and 3. Numbers in italic show the proportions of cold, heat, or mechanosensitive afferents. χ^2-Test was used for comparison of the frequencies of cold, heat, or mechanosensitivity between A- and C-fibers.

neurons is 0.1–15 imp/s (mean ~2 imp/s) in neurons with irregular discharges and 2.2–7.1 imp/s (mean 5.4 imp/s) in neurons with bursting discharges (Michaelis et al. 2000). These rates of ongoing activity originating in the DRG in injured muscle A-fiber neurons are lower than those originating in peripheral injured A-fibers as reported here.

Comparison of Injured Muscle A- and C-Fibers

Muscle afferents consist functionally of three large groups: large-diameter myelinated (Aα/β-) fibers, small-diameter myelinated (Aδ-) fibers, and C-fibers. The muscle Aδ- and C-fibers are involved in functions related to activation of the cardiovascular and respiratory system during exercise ("ergoreceptor" function), deep pressure sensation, muscle fatigue, nociception, and pain (Graven-Nielsen et al. 2004; Kaufman and Forster 1996; Mense 1993; Mitchell et al. 1983; Rivers and Head 1908; Simone et al. 1994; Torebjörk et al. 1984). Thus these small-diameter muscle afferents have common functions that are different from those of the large-diameter muscle afferents. Therefore, we expected that slowly conducting injured myelinated afferents exhibit discharge patterns similar to injured C-fibers. To our surprise, injured myelinated afferents conducting at $<$20 m/s and those conducting at $\geq$20 m/s were functionally practically identical. However, both populations of myelinated fibers were significantly different in their frequency of cold, heat, and mechanosensitivity as well as rate of ongoing activity from the population of injured C-fibers (Tables 2 and 3, Fig. 11).

We have divided the nerve fibers according to Lawson and Waddell (1991) and Waddell et al. (1989) into A-fibers (conduction velocity $>$2 m/s) and C-fibers (conduction velocity $\leq$2 m/s). Our distribution of conduction velocity is very similar to that shown by Waddell et al. (1989) for afferent fibers that have their cell bodies in the DRG L_4 or L_5, which also contain the cell bodies of the afferent neurons projecting in the lateral gastrocnemius-soleus nerve (Swett et al. 1991). Waddell et al. (1989) and Lawson and Waddell (1991) describe that Aδ-fibers conduct at 2–12 m/s and Aα/β-fibers at $>$12 m/s, however, in rats with a weight ranging from 120 to 190 g. The weight of our rats was more than twice as high. The division between Aδ-fibers and Aα/β-fibers may therefore be closer to 20 m/s in our group of rats. For this reason, we have compared the functional properties of A-fibers conducting at $<$20 m/s with those of C-fibers.

Comparison of Functional Characteristics of Injured Muscle Afferents and Injured Cutaneous Afferents

Table 4 compares the ectopic activities (ongoing activity, mechanosensitivity, thermosensitivity) developed by myelinated and unmyelinated muscle afferents and cutaneous afferents following nerve crush (see Grossmann et al. 2009a, Jänig et al. 2009 for data obtained on injured cutaneous afferents).

1) Four to seven days after a nerve injury, almost all muscle A-afferents and C-afferents exhibit ectopic activity, but only ~50% of the cutaneous afferents.

2) In the first 13 h after nerve injury, the frequency of muscle C-fibers with ectopic activity (ongoing activity and mechano- and thermosensitivity) is significantly higher in a muscle nerve than in a skin nerve, whereas the frequency of A-fibers with mechanical ectopic activity is higher in an injured skin nerve than an injured muscle nerve (Blenk et al. 1996; Michaelis et al. 1995, 1999).

3) The mechanical thresholds are significantly lower in injured muscle A- and C-afferents than in injured cutaneous A- and C-afferents (Gorodetskaya et al. 2003).

4) The cold sensitivity of cutaneous C-fibers was divided into type 1 cold sensitivity and type 2 cold sensitivity (see Grossmann et al. 2009a,b; Jänig et al. 2009). Type 1 cold-sensitive C-fibers behave like (nonnociceptive) cool receptors. Most of them have a high rate of ongoing activity at temperatures of 30°C on the surface of the nerve and are activated by small cooling steps and inhibited by heating stimuli. Some are additionally activated at strong heat stimuli. They are with a few exceptions mechanoinsensitive (Grossmann et al. 2009a). This type of cold-sensitive afferent neuron does not appear to project to skeletal muscle.

5) The functional characteristics of cold-sensitive muscle C-afferents resemble those of type 2 cold-sensitive cutaneous C-afferents: most of these cold-sensitive cutaneous and muscle afferents are mechanosensitive or mechano- and heat-sensitive (Fig. 11). The threshold for activation by cold stimuli is high in both (Fig. 4*A*).

6) The frequency of A-fibers with cold sensitivity is significantly higher in an injured muscle nerve than in an injured cutaneous nerve.

7) The frequency of A-fibers with ongoing activity is higher in an injured muscle nerve than in an injured skin nerve but not the frequency of C-fibers with ongoing activity. The rates of ongoing activity are not significantly different between injured muscle and cutaneous C-fibers.

8) Injured cutaneous afferents show transduction characteristics for physiological stimuli that are qualitatively and quantitatively similar to those of intact cutaneous afferents. Thus the frequencies of the functionally different populations of cutaneous afferent fibers are the same for intact and injured afferent neurons (Gorodetskaya et al. 2009; Jänig et al. 2009). The same may apply to heat and mechanosensitivity of injured muscle afferents but not for cold sensitivity.

Table 4. *Comparison of injured muscle afferents (4–7 days after nerve crush) and injured cutaneous afferents (4–14 days after nerve crush)*

	A-Fibers			C-Fibers		
	Muscle N. Gastrocnem.-Soleus Lat. $n = 194$	Skin N. Suralis $n = 43$	U Test or χ^2-Test	Muscle N. Gastrocnem.-Soleus Lat. $n = 118$	Skin N. Suralis $n = 135$	U Test or χ^2-Test
% Efferent axons	50% Somatomotor	A few somatomotor	†	45% Postganglionic	15% Postganglionic	†
% Electrically activated fibers with ectopic activity	46%	42%	†	56%	49%	†
OA						
Rate	0.01–38.8 i/s (9.5 i/s), $n = 96$	0.4, 2.8, 6.4 i/s		0.02–7.0 i/s (0.32 i/s), $n = 65$	Non-C1: 0.1–1.9 i/s (0.3 i/s), $n = 50$; C1: 0.12–10 i/s (2.2 i/s), $n = 28$	ns
Frequency	50%	7%	$P < 0.001$	55.9%	57.70%	ns
Mechanosensitivity						
Threshold	0.3 to >10 mN (1 mN), $n = 76$	0.7–70 mN (7.5 mN), $n = 38$	$P < 0.001$	0.3 to >10 mN (0.7 mN), $n = 43$	0.45 to >70 mN (10 mN), $n = 73$	$P < 0.001$
Frequency	91.2%	98%	ns	50%	54%	ns
Cold sensitivity						
Threshold	OA: 10–27°C (22.5°C), $n = 16$; silent: 5–26°C (12°C), $n = 36$	3–14°C (7°C), $n = 5$		5–20°C (15°C), $n = 36$	Type C1: 17–22°C (18.5°C), $n = 29$; Type C2: 3–21°C (5°C), $n = 29$	
Frequency	62.9%	11.60%	$P < 0.001$	39.8%	42.80%	ns
Heat sensitivity						
Threshold	OA: 35–50°C (40°C), $n = 14$; silent: 35–50°C (45°C), $n = 12$	36–48°C (40°C), $n = 7$		35–50°C (41°C), $n = 35$	35–49°C (41.5°C), $n = 74$	ns
Frequency	52.2%	16.30%	$P < 0.001$	66.1%	62.20%	ns

Values are ranges (medians). Comparison of the functional characteristics of injured muscle afferents (4–7 days after nerve crush, TP II) and injured cutaneous afferents (4–14 days after nerve crush). Data on injured cutaneous afferents are from Grossmann et al. (2009b) and Jänig et al. (2009). N. Gastrocnem.-Soleus Lat., lateral gastrocnemius-soleus nerve; ns, not significant; OA, ongoing activity or afferents with OA; silent, silent afferents; i/s, impulses per second; type C1/C2, nonnoxious/noxious cold sensitivity (see Grossmann et al. 2009b, Jänig et al. 2009). Data on cutaneous afferents are from Jänig et al. (2009). All percentages (frequencies of afferent fibers with a particular functional property) are expressed with respect to the total number of fibers. †The number of afferent fibers with ectopic activity is significantly higher in the injured muscle nerve than in the injured sural nerve (N. Suralis) since 50 and 45% of the axons in the muscle nerve are somatomotor or postganglionic, respectively, yet very few and 15% in the sural nerve.

9) Cutaneous afferent neurons with C-fibers shrink and may die after axotomy; injured cutaneous afferent A-neurons only shrink (Degn et al. 1999; Hu and McLachlan 2003; Jänig and McLachlan 1984; Lekan et al. 1997; Tandrup et al. 2000). These cellular changes do not occur in axotomized primary afferent neurons innervating skeletal muscle (Hu and McLachlan 2003). The differential pathobiological changes in cutaneous and muscle afferent neurons following axotomy may imply that activity in injured muscle afferent neurons is becoming more important in the generation of pain, dysesthesias, and paresthesias than activity in injured cutaneous afferent neurons in chronic conditions.

These differences and similarities between injured muscle and cutaneous afferent fibers are remarkable. They indicate that neuropathic pain, paresthesias, and dysesthesias in patients with nerve injury may also be related to the activity in injured muscle afferents and possibly other injured deep somatic afferents.

Axonal Mechano- and Thermosensitivity

Cold and heat sensitivity of cutaneous afferent neurons with C-fibers may extend over the axon membrane in the nerve but not mechanosensitivity (Hoffmann et al. 2008; Teliban et al. 2011). Almost all nonnociceptive cold-sensitive cutaneous C-fibers (type 1 cold-sensitive C-fibers) exhibit axonal cold sensitivity. About 30–40% of the nociceptive cold- or heat-sensitive C-fibers show axonal cold or heat sensitivity (Teliban et al. 2011). Whether this axonal thermosensitivity is also present in muscle afferent neurons with C-fibers or A-fibers we do not know. In TP I (4–13 h after nerve crush), heat and cold sensitivity of muscle A-fibers was absent, and only very few A-fibers were mechanosensitive (Table 2). This shows indirectly that axonal mechano- and thermosensitivity are probably absent in myelinated muscle afferents since we tested the axons up to ~3 mm rostral to the injury site. However, axonal mechano- and thermosensitivity of muscle A- and C-afferents need to be investigated systematically.

Recently, it has been shown that perineural inflammation generated by complete Freund's adjuvant applied to the sciatic nerve of rats is followed by axonal mechanosensitivity of C- and Aδ-afferents innervating deep somatic tissues but not of those innervating skin (Bove et al. 2003; Dilley and Bove 2008; Dilley et al. 2005). This ectopic axonal mechanosensitivity during neuritis may be related to the high mechanosensitivity of injured muscle afferents as shown by us.

Functional Types of Injured Muscle Afferents and Transduction Mechanisms

The molecular transduction mechanisms underlying the mechanosensitivity and thermosensitivity of injured (and intact) muscle afferents have to date not been investigated. The transduction mechanisms underlying the mechanosensitivity are poorly understood (Hu et al. 2006; Julius and McCleskey 2006; Lewin and Moshourab 2004). However, in view of the wide range of mechanical thresholds of injured afferents showing distinct groups of high- and low-threshold afferents, it is not far-fetched to assume that several mechanisms contribute to the mechanosensitivity.

Heat sensitivity of muscle afferents may be dependent on the transient receptor potential V1 (TRPV1) channel (Caterina 2007). It has to be shown that injured heat-sensitive muscle A- and C-afferents can be excited and/or desensitized by capsaicin or similar agonists of the TRPV1 receptor. Indeed, ~50% of the intact muscle C-fibers is capsaicin-sensitive (Hoheisel et al. 2004). Cold sensitivity of injured muscle afferents probably is barely mediated by the menthol-sensitive TRPM8 channel (Bandell et al. 2004; Bautista et al. 2007; Belmonte et al. 2009; Madrid et al. 2006; McKemy et al. 2002; Story et al. 2003) but by other channels, one being possibly the TRPA1 channel (Bandell et al. 2004; Jordt et al. 2004; Karashima et al. 2009; Story et al. 2003). However, it will have to be shown whether cold-sensitive muscle A- and C-afferents can be excited by the agonist menthol, as is the case in type 1 cold-sensitive cutaneous afferents (A. Teliban, F. Bartsch, M. Struck, and W. Jänig, unpublished observations), and that probably other channels (e.g., the TRPA1 channel) are involved in the novel cold sensitivity of injured muscle A- and C-afferents.

Conclusions

Three to seven days after lesion of a muscle nerve, almost all injured afferent A- and C-fibers develop ongoing and/or evoked activity originating at the injury site. Four to thirteen hours after nerve injury, this ectopic activity is almost absent in A-fibers but already present in many C-fibers. Mechanosensitivity is prominent in the majority of A- and C-fibers. Heat and cold sensitivity in injured A-fibers and cold sensitivity in injured C-fibers appear to be novel functional properties. Whether and to what degree the discharge properties of injured muscle afferents contribute to deep neuropathic pain, dysesthesias, and paresthesias as well as other functional changes in patients with nerve injury has to be explored.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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