Myelinated Mechanically Insensitive Afferents From Monkey Hairy Skin: Heat-Response Properties

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INTRODUCTION

Mechanically and heat-sensitive A-fiber nociceptors (AMHSs) from monkey skin exhibit two distinct heat transduction mechanisms (Treede et al. 1995). Type I AMHS have a delayed onset of response to heat stimuli, and their discharge rate typically increases during a prolonged heat stimulus. Their median heat threshold is high (>53°C), and they are found in glabrous and hairy skin. These fibers encode the sustained pain during a prolonged, intense heat stimulus and the primary hyperalgesia to heat that develops after a burn to the glabrous skin (Meyer and Campbell 1981b). In contrast, type II AMHS have a short-latency adapting response to heat stimuli. Their median heat threshold is low (46°C), and they are found only in hairy but not glabrous skin. The response properties and conduction velocities of type II AMHSs are such that this fiber type is an ideal candidate to subserve the sensation of “first pain” to stepped heat stimuli. Although first pain to heat is a prominent sensation in psychophysical studies, relatively few type II AMHS are evident with conventional neurophysiological search techniques. This paradox prompted us to consider whether other search techniques might demonstrate a higher incidence of type II AMHS.

Most investigations of A-fiber nociceptors depended on a technique that uses responses to mechanical stimuli to locate cutaneous receptive fields (e.g., Adriaensen et al. 1983; Burgess and Perl 1967; Fitzgerald and Lynn 1977; Georgopoulos 1976; Treede et al. 1995). This common search technique may introduce a selection bias, especially because recent studies showed that sensitivity of nociceptors to heat and chemical stimuli may be unrelated to their mechanical sensitivity (Davis et al. 1993; Schmidt et al. 1995). Indeed more than one-half of the A-fiber nociceptor population exhibits high mechanical thresholds (>6 bar (≈100 mN) and thus are unlikely to be found with a mechanical search technique (Meyer et al. 1991). The prime motivation for this study was to determine the heat-response characteristics of mechanically insensitive afferents (MIAs).

An electrical search technique (Meyer et al. 1991) was used to determine the receptive field locations of all A-fiber afferents in monkey cutaneous nerves. We demonstrate that A-fiber MIAs, like mechanically sensitive afferents, exhibit two distinct heat transduction mechanisms. However, a large proportion of the MIAs exhibit a type II response, whereas a large proportion of mechanically sensitive afferents exhibit a type I response.

METHODS

A standard teased fiber technique (e.g., Campbell and Meyer 1983) was used to record from single primary afferents in cuta-
neous, peripheral nerves in monkeys (Macaca fascicularis, 5–7 kg). The monkeys were initially sedated by intramuscular injection of ketamine (10 mg/kg), and anesthesia was maintained with a mixture of pentobarbital sodium (3 mg kg\(^{-1}\) h\(^{-1}\)) and morphine sulfate (0.5 mg kg\(^{-1}\) h\(^{-1}\)). At the beginning of each experiment, penicillin G (450,000 U) was administered for prophylaxis against infection. To minimize muscle artifacts as well as to facilitate respiratory control, the animals were paralyzed with pancuronium bromide (0.1 mg/kg) and artificially ventilated (end-tidal pCO\(_2\) maintained at 32±40 Torr). The electrocardiogram was monitored aurally and visually. Changes in heart rate were used to monitor the level of anesthesia. Core temperature was measured via a rectal probe and maintained ~38°C with use of circulating water heating pads. Dextrose (5%) in normal saline was infused intravenously throughout the experiment to maintain hydration.

An incision was made in the skin over the cutaneous nerve of interest, and the edges of the incision were tied to a ring to form a well into which paraffin oil was placed. At the proximal end of this well, small bundles of axons were cut away from the nerve trunk and dissected to fine strands that were placed on the recording electrode. The strands, from which recordings were made, were cut proximally so that only centripetally conducted action potentials were recorded. A bipolar, nerve stimulation electrode (cathode proximal) was placed on the nerve 37.3±6.3 mm (mean±SD) distal to the recording electrode but within the well. The number of fibers at the recording electrode activated after stimulation at the nerve stimulation electrode was determined. Small strands were dissected for recording to enable each myelinated fiber to be identified on the basis of its electrical threshold, latency, and action-potential shape. A search was then made to locate the receptive field for each of these fibers. For every receptive field that was located, mechanical-electrical interaction consisting of latency shifts in the electrically evoked action potential by intervening responses to natural stimuli (Hallin and Torebjörk 1974; Meyer et al. 1985; Schmelz et al. 1995) or collision techniques (Meyer et al. 1985) were used to verify that a receptive field was associated with the action-potential shape identified from stimulation at the nerve stimulation electrode.

A fiber was included in the study if 1) its receptive field was located in the hairy skin, 2) its conduction velocity was clearly in the myelinated fiber range (≥2.5 m/s), 3) no cutaneous injury occurred within 2 cm of the receptive field before study, and 4) the fiber innervated the skin and not subcutaneous tissue (as demonstrated by gently pulling the skin aside relative to the underlying tissue and reapplying the stimulus). Mechanically sensitive fibers were classified as nociceptors if they responded to pinching of the skin but did not respond to blunt pressure (200-g brass rod, 1-cm diam, 0.26 bar) or light stroking. Low-threshold mechanoreceptors were divided into those with rapidly and slowly adapting responses.

To locate the receptive fields of receptors with low or moderate mechanical thresholds, the skin was pinched firmly in a way so as not to damage the skin. Low-threshold thermoreceptors were identified by their spontaneous activity that could be modified by mild warming of the skin with a heat lamp (only cold fibers were observed in this study, which were silenced by mild heating). For most mechanically sensitive afferents, the receptive field area was mapped under a microscope by marking the skin with dye at spots where the fiber responded to a 0.5-mm-diam nylon monofilament, which exerted a force of 200 mN (10 bar). For fibers with a high mechanical threshold, an 18-bar probe (0.7-mm diam, 720 mN) was used for mapping. After waiting several minutes, the threshold to mechanical stimulation was determined with the use of calibrated nylon monofilaments (von Frey type, Stoelting aesthesiometer set) with pressures ranging from 0.1 to 18 bar. As in a previous study (Meyer et al. 1991), we considered any fiber with a mechanical threshold >6 bar to be an MIA. Based on the calibrated von Frey set used in our laboratory, this corresponds to fibers that did not respond to a 5.1-bar (0.36-mm-diam, 51-mN) probe but did respond to the next higher 7.3-bar (0.51-mm-diam, 98-mN) probe.

An electrical search technique (Meyer et al. 1991) was used to locate an electrical receptive field (eRF) for afferents that were not responsive to the pinch stimulus or the low-intensity thermal stimuli. A saline-soaked cotton swab was used as a monopolar, cathodic search electrode. To locate the eRF, the fiber of interest was first excited transcaneously at a position just distal to the nerve stimulation electrode, and the time for the action potential to reach the recording electrode was recorded. As the search electrode was moved distally along the course of the nerve, the action-potential latency increased gradually. A region was usually found in which the suprathermal latency no longer increased as the probe was moved distally. In this region, the latency at threshold usually increased abruptly and the electrical threshold decreased (Meyer et al. 1991). This region of longest conduction latency and minimum threshold was defined as the eRF of the fiber. The reduced threshold and longer latency is thought to be associated with the superficial, branching structure of the cutaneous nociceptor terminals.

The conduction velocity of the fiber under study was estimated at the end of each experiment from electrical stimulation at the eRF. For this purpose, two needles were inserted next to the eRF borders, and stimuli of three times the threshold were used. The conduction distance was determined by the length of a piece of suture placed along the presumed path of the nerve between the receptive field and the recording electrode. Conduction velocities were also obtained from latencies measured after stimulation at the nerve stimulation electrode.

The responsiveness of nociceptors to heat stimuli was tested with a laser thermal stimulator system that delivered stepped increases in skin temperature with rise times of ~100 ms (Meyer et al. 1976). The threshold for heat stimuli was tested with stimuli of 1-s duration presented every 30 s and with stimuli of 30-s duration presented every 60 s. They were given in runs of ascending stimulus intensities in 1°C steps from 39 to 53°C. The base temperature (38 or 35°C) was maintained for 1 min before the first stimulus and between stimuli. The sequence was stopped after the first evoked response occurred. A 2-min, stimulus-free interval between repeated threshold runs was previously found to yield reproducible results in C-fiber nociceptors (Treede et al. 1990) and was also used in this study. Fatigue was examined with use of three 49°C stimuli presented with a repetition period of 30 s.

The response of each nociceptor to an intense (53°C) heat stimulus of 30-s duration was used to classify its heat sensitivity in a manner similar to that done previously in mechanically sensitive afferents (Treede et al. 1995). The response latency was defined as the time between stimulus onset and the initiation of the first action potential. The propagation time along the nerve from the receptive field to the recording site (estimated with electrical stimuli at the receptive field) was subtracted to represent the times of action-potential initiation at the receptive field. We also determined the latency-to-peak firing. For this purpose, peristimulus frequency histograms were constructed in the following manner: the instantaneous action-potential frequency was calculated for each pair of stimuli at the receptive field (Treede et al. 1995) and slowly adapting responses.

For statistical analysis of incidences, we used \( \chi^2 \)-tests. For group comparisons, nonparametric tests were used (Mann-Whitney U test, Wilcoxon matched-pairs test) because several variables were either not normally distributed or had only rank scale character. Standard deviations are used as indicators of variability despite

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nonnormal distributions. P-values of 0.05 and below were considered to be significant.

RESULTS

A total of 118 strands were investigated in 32 experiments that used the superficial radial (n = 26), medial antebrachial cutaneous (n = 3), and superficial peroneal (n = 3) nerves. Each strand contained 2.3 ± 1.4 (SD) A-fibers that could be activated from the nerve stimulation electrode. The conduction velocity distribution (Fig. 1) revealed two peaks, one at 15 m/s and the other at 43 m/s. The boundary between these two fiber groups was placed at the minimum between these two peaks, which was 30 m/s. Of the 263 fibers identified, 114 were classified as Aβ-fibers and 149 as Aδ-fibers.

The receptor type was identified for 77 Aβ- and 79 Aδ-fibers (Table 1). For the majority of Aβ-fibers, a low-threshold mechanoreceptive terminal was identified. Nine of the 50 nociceptors were Aβ-fibers, the fastest nociceptor having a conduction velocity of 70 m/s. Eight of these nine fibers exhibited a type 1 heat response.

Most cutaneous A-fiber nociceptors (43/50) responded to the range of heat stimuli used in this study. Of the seven heat-insensitive nociceptors, two responded to high-intensity von Frey probes and one to squeezing with small forceps (true high-threshold mechanoreceptors). Three of the seven fibers did not respond to any of the natural stimuli used in this study, and one responded vigorously to an ice stimulus but not to mechanical stimuli.

Four heat-insensitive fibers did not have a cutaneous receptive field and were excluded from further analysis. Their eRF did not move when the skin was moved. They either had a mechanical threshold of 18 bar (n = 2) or did not respond to this highest stimulus intensity used (n = 2). Adequate mechanical stimuli for deep afferents were joint or tendon compression near the metacarpophalangeal joint of the index finger, forced thumb movements, or compression of the web between thumb and index. The latter fiber exhibited a slowly increasing discharge to constant blunt pressure.

Classification of heat responses

Response latency and peak discharge latency to an intense heat stimulus (53°C, 30 s), which causes a burn injury, were used to classify the heat responses in a manner similar to that done by us previously to classify mechanically sensitive

TABLE 1. Properties of A-fibers identified with electrical nerve stimulation

<table>
<thead>
<tr>
<th></th>
<th>Aβ Fibers</th>
<th>Aδ Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of fibers</td>
<td>114</td>
<td>149</td>
</tr>
<tr>
<td>Conduction velocity*</td>
<td>47.1 ± 10.9 m/s</td>
<td>15.4 ± 6.0 m/s</td>
</tr>
<tr>
<td>Electrical threshold²</td>
<td>1.0 ± 1.0 V</td>
<td>8.3 ± 12.8 V</td>
</tr>
<tr>
<td>Number of receptors identified</td>
<td>77</td>
<td>79</td>
</tr>
<tr>
<td>Receptor types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-threshold mechanoreceptors</td>
<td>67</td>
<td>24</td>
</tr>
<tr>
<td>RA/PC</td>
<td>39</td>
<td>17</td>
</tr>
<tr>
<td>SA</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Low-threshold cold receptors</td>
<td>1†</td>
<td>10</td>
</tr>
<tr>
<td>Nociceptors</td>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>Heat sensitive</td>
<td>8</td>
<td>35</td>
</tr>
<tr>
<td>MSA</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>MIA</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Heat insensitive</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>MSA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MIA</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cold</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Subcutaneous receptors</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SD. RA/PC, rapidly adapting or Pacinian-like low-threshold mechanoreceptor; SA, slowly adapting low-threshold mechanoreceptor; MSA, mechanically sensitive afferent, mechanical threshold <6 bar (≤50 mN); MIA, mechanically insensitive afferent, mechanical threshold >6 bar (≥100 mN); and Cold, nociceptor that responded only to intense cold stimulus. * Conduction velocity from nerve stimulation electrode. † Threshold at nerve stimulation electrode for 0.1-ms duration pulse. ‡ Low-threshold cold receptor with 24-m/s conduction velocity.
afferents (Treede et al. 1995). Examples of the two types of heat response that were observed in this study are shown in Fig. 2. In some fibers the latency to the first action potential was long (e.g., Fig. 2A; >6 s), the heat-evoked discharge increased with time in spite of constant skin temperature, and the peak discharge occurred near the end of the stimulus. This type I heat response was found in a few MIAs but was more typical for mechanically sensitive fibers. For other heat-sensitive afferents, the latency to the first action potential was short (e.g., Fig. 2B, 0.136 s), the heat-evoked discharge adapted during a constant heat stimulus, and the peak latency was <1 s. This type II heat response was found predominantly among the MIAs.

A scatter plot of peak latency as a function of response latency is shown in Fig. 3A. Because the peak discharge cannot occur before the first action potential, all data points lie above the y = x diagonal. A type II heat response (O; ●) was exhibited by 13 of the 35 A-fiber nociceptors, for which a response to the 53°C, 30-s stimulus was recorded. The distribution of the type I heat responses (□; ■) suggests that there may be two subgroups with median response latencies of 0.9 s (Fig. 3A, top left; n = 12) and 9.7 s (Fig. 3A, top right; n = 10). The significant difference in response latency (P < 0.002, U test) accounted for a significant difference in the number of action potentials evoked by the heat stimulus (P < 0.01, U test). Heat threshold, latency-to-peak discharge, conduction velocity, mechanical threshold, and receptive field size, however, were not significantly different between these subgroups.

The distinguishing characteristic between the types I and II heat responses was the latency to the peak discharge (either <1.2 or >3 s). In addition to the 35 afferents presented in Fig. 3A, 8 other fibers were classified as having a type I or II heat response based on less intense heat stimuli. Of the 43 heat-responsive cutaneous A-fiber nociceptors, 26 (60%) were type I afferents, whereas 17 (40%) were type II afferents. These incidences were not significantly different (χ² = 1.88, df = 1).

The population responses of A-fiber nociceptors exhibiting either of the two heat response types are shown in Fig. 3, B and C. The slowly increasing discharge of the type I afferents contrasted with the marked adaptation of the type II afferents, many of which stopped discharging before the end of the constant heat stimulus. The adaptation of the type II afferents was fit by a single exponential with a time constant of 2.4 s, which turned into a plateau of <1 Hz after 5 s.

**Mechanical thresholds of afferents with a type I or type II heat response**

The distributions of mechanical thresholds for the cutaneous A-fiber nociceptors in this study are shown in Fig. 4. Fibers that did not respond to the 18-bar probe were considered to be unresponsive to mechanical stimuli (15/50 = 30%), although a few of these subsequently responded to pinching of the skin with forceps (3/10). Fibers with a mechanical threshold >6 bar (dashed line) were considered to be MIAs because ~95% of the A-fiber nociceptors in hairy skin in previous studies, which used mechanical stimuli to identify the receptive field, had thresholds <6 bar (Meyer et al. 1991). With the electrical search technique used in this study, more than one-half of the cutaneous A-fiber nociceptors were MIAs (29/50 = 58%).

Notably, however, more than one-half of nociceptors with a type I heat response were mechanically sensitive (15/26 = 58%), whereas the majority of type II responses was observed in MIAs (13/17 = 76%). This difference in incidence was significant (P = 0.05, χ²-test). Seven of the type II nociceptors were unresponsive to the highest von Frey probe used, and five of them could only be activated by heat; the other two responded to squeezing with small forceps. Consistent with the above analysis, the median mechanical threshold for fibers with a type II heat response was significantly higher than for fibers with a type I response (15 bar vs. 4.9 bar; P = 0.002, U test, Table 2).

**Conduction velocities of afferents with a type I or type II heat response**

All A-fiber nociceptors with a type II heat response had conduction velocities <30 m/s and thus all of them were considered Aδ-fibers (Fig. 5B). In contrast, the conduction
FIG. 3. Response of the A-fiber nociceptor population to the 53°C, 30-s heat stimulus. A: scatter plot of peak discharge latency vs. response latency for mechanically insensitive afferents (MIA; ●, n = 16) and mechanically sensitive afferents (MSA; ○, ●, n = 19). Mechanical sensitivity was classified according to previously published operational criteria (Meyer et al. 1991). Heat response was classified in a manner similar to that used previously for mechanically sensitive nociceptors only (Treede et al. 1995). Thus receptors that had a long peak discharge latency were considered to have a type I heat response (○, ●). Receptors that had a short response latency and a peak discharge near the stimulus onset were considered to have a type II heat response (○, ●). The type II heat response was found more frequently in the MIA group (P < 0.05, χ²-test).

B: average peristimulus frequency histogram (obtained with 0.2-s binwidth) of the response to the 53°C, 30-s stimulus for A-fiber nociceptors that had a type I heat response (n = 21).

C: average peristimulus frequency histogram for A-fiber nociceptors that had a type II heat response (n = 12).

velocities of the type I afferents ranged from 8.2 to 70 m/s (Fig. 5A) and thus bracketed the Aβ- and Aδ-fiber classification. The mean conduction velocity of the type II afferents (14.2 ± 5.2 m/s) was significantly slower than the mean conduction velocity of the type I afferents (25.4 ± 15.7 m/s, P < 0.05, U test, Table 2).

Stimulus duration and heat thresholds of types I and II afferents

The median heat threshold for type II afferents for 1-s duration stimuli (48°C) was significantly lower than that of the type I afferents (>53°C, P < 0.002, U test). Most (16/17) of the type II afferents responded to 1-s stimuli ≤49°C, whereas only 4 of the 26 type I afferents responded to such stimuli.

This threshold difference, however, strongly depended on stimulus duration. Three type I afferents and seven type II afferents had threshold determinations with 1-s, then 30-s, then again with 1-s duration stimuli. In contrast to the results with 1-s duration stimuli, the thresholds for the types I and II afferents were similar when the 30-s duration stimuli were used (Fig. 6, A and B). Expressed as temperature increase above the 38°C base, the median threshold decrease for 30-s versus 1-s stimulus duration was 53% for type I afferents versus 18% for type II afferents (P < 0.02, U test). The threshold of four type II afferents studied with a 38°C base temperature was contrasted with the threshold determined with a 35°C base temperature. In contrast to a previous study in C-fiber nociceptors (Tillman et al. 1995), there was no consistent effect of baseline temperature on threshold (Fig. 6C).

It might be argued that the type I A-fiber nociceptors are actually high-threshold mechanoreceptors (Perl 1968) and that the responses to the long-duration heat stimuli represent sensitization. However, the threshold of the three type I afferents shown in Fig. 6A was stable over multiple runs (Fig. 7A). Moreover, the threshold to the 1-s duration stimulus was not altered by these tests (Fig. 6A). Thus the long-duration threshold sequence did not lead to heat sensitization. The slow response of the type I afferents to the 53°C, 30-s stimulus therefore reflects a slow transduction mechanism and not sensitization. Repeated testing of the type II afferents also revealed that their heat thresholds were stable (except for 1 fiber: Fig. 7B, ⋯).

Fatigue of type II afferents to repeated heat stimuli

Type II afferents exhibited a marked fatigue to repeated presentations of the same heat stimulus. As shown in Fig. 8A, the response to a second and third presentation of a 49°C stimulus was 40 ± 10% (SE) and 35 ± 13%, respectively, of the first response when the repetition interval was 30 s. For three fibers, the repetition interval was systematically varied (Fig. 8B). The amount of fatigue decreased as the repetition interval increased, but pronounced fatigue was still present at a repetition interval of 10 min (70 ± 7%).

Stimulus response functions for type II afferents

The stimulus response functions of four type II afferents were determined with the use of an ascending series of 1-s
Responses to a second burn

The intense heat stimulus (53°C, 30 s) was applied for a second time to the receptive fields of 15 A-fiber nociceptors. At least 25 min passed between these stimuli. In the nine type I afferents, the number of action potentials elicited increased from 104 ± 64 (SE) to 148 ± 48 (NS), response latency decreased from 7.0 ± 2.2 s to 4.7 ± 1.3 s (NS), and peak latency decreased significantly from 15.2 ± 2.9 s to 7.7 ± 1.7 s ($P < 0.01$, Wilcoxon matched-pairs tests). In other words, type I afferents continued to show a type I heat response in spite of some signs of sensitization. In the six type II afferents, the response to the second burn was identical to that to the first burn in the three mechanically sensitive fibers (thresholds 4.2–5.1 bar), but no response to the second burn was observed in the other three type II afferents that were MIAs (threshold >18 bar). In two nociceptors it was possible to apply a second burn to a part of the receptive field that was not tested with heat before. One fiber showed a type I heat response for both areas and the other a type II response for both areas. These data indicate that the type of heat response is a property of the afferent fiber under study.

Responses of low-threshold mechanoreceptors to a burn

The receptive fields of 13 low-threshold mechanoreceptors (6 Aβ and 7 Aδ) were also exposed to the 53°C, 30-s heat stimulus. Five of these fibers showed an OFF response to mild heat stimuli, which turned into no response ($n = 2$), an OFF response ($n = 1$), or a paradoxical ON response to the burn ($n = 2$). One fiber had a highly sensitive pacinian-like fiber (17 m/s conduction velocity), which responded with one or two action potentials to the onset of all heat stimuli including the 38°C base. None of these characteristics resembled those of the A-fiber nociceptor population described above. Seven low-threshold mechanoreceptors did not respond to any of the heat stimuli.

**DISCUSSION**

A-fiber nociceptors are of two distinct types with regard to the response to heat stimuli. Type I afferents have a wind-up response to heat stimuli as manifest by the delay in peak latency of several seconds after stimulus onset. Type II afferents have an adapting response to heat stimuli similar to that seen in C-fiber nociceptors. Whereas we previously noted this difference in mechanically sensitive fibers (Treede et al. 1995), the present study establishes that this same dichotomy of heat response types applies to MIAs.

Notably, however, the mechanical sensitivity of types I and II A-fiber nociceptors was markedly different. Type II afferents had significantly higher mechanical thresholds than did type I afferents. A large proportion (76%) of type II afferents met the criterion for being mechanically insensitive (Meyer et al. 1991). Moreover, a substantial number of them (41%) had no response to the highest von Frey force used in this study.

This finding resolves one of the paradoxes regarding peripheral neural mechanisms of pain sensation. The conduction velocity and heat response characteristics of type II A-fiber nociceptors suggest strongly that these afferents signal the first pain sensation evident with stepped heat stimuli. In earlier studies, however, there were only anecdotal reports about type II afferents (Adriaensen et al. 1983; Bromm and Treede 1984; Dubner et al. 1977; Perl 1968). Even our systematic study of mechanically sensitive afferents (Treede
et al. 1995) identified only 28% of the hairy skin A-fiber nociceptors as type II, significantly less than type I ($\chi^2 = 8.70, P < 0.01$). Given that first pain sensation is an easily demonstrated sensation in hairy skin (1st pain sensation is not evoked by stimulation of the glabrous skin of the hand and type II afferents are not evident in this skin) (Campbell and LaMotte 1983), the dilemma was how this sensation could be evoked so easily if the substrate for this sensation, the type II afferents, was so few in number.

It appears from the current study that type II afferents were infrequently observed previously because of their relative mechanical insensitivity. In this study we used an electrocutaneous search technique to obtain an unbiased sample of the total A-fiber population and to identify the receptive fields independent of the adequate stimulus. With this technique the incidence of types I and II afferents approached equality (60 vs. 40%, NS). As discussed in the following section, the true incidence of type II afferents may be even higher.

**Search technique**

The search technique used in this study differs from many previous studies in two aspects: 1) the receptor characteristics were determined for all A-fibers identified after electrical nerve stimulation, leading to an unbiased sampling of all fibers (see Fitzgerald and Lynn 1977; Leem et al. 1993 for the rat); and 2) an electrocutaneous search technique allowed the receptive field to be identified independent of the adequate stimulus (see Schmidt et al. 1995 for human C-fiber nociceptors).

Adequate sampling of all sizes of myelinated afferents is indicated by the fact that we found about the same percentage of A$\delta$-fibers (149/263 = 57%) as was reported in histological studies of the human radial nerve (56 ± 9%, SD) (O’Sullivan and Swallow 1968). We were able to identify and study the receptive fields of 77 of 114 A$\delta$-fibers (68%) and 79 of 149 A$\beta$-fibers (53%). Several factors contribute to the relatively high proportion of receptive fields not identified. 1) Several afferents may have been disconnected from their terminal because of the extensive surgical preparation that was necessary for placing stimulation and recording electrodes. In fact, several receptive fields were immediately adjacent to the well, and other fibers could not be followed electrically outside the well. 2) Some fibers had receptive fields that were inaccessible for the laser stimulator. 3) Fibers that innervated previously damaged skin were not studied beyond detecting their eRF. For the low-threshold mechanoreceptor population the standard mechanical search procedure is adequate. In our study, rapidly adapting receptors outnumbered slowly adapting ones (62 vs. 38%) by a similar ratio as in previous studies in monkey hairy skin (69 vs. 31%) (Merzenich and Harrington 1969).

The value of the electrocutaneous search technique is emphasized by comparing the current results with some earlier studies. With the use of mechanical stimuli as the means to search for the receptive field of nociceptors in monkey hairy skin, only 8 of 124 A-fibers had thresholds $\leq$ 100 mN (equivalent to 6 bar, the point chosen as the boundary between MIAs and mechanically sensitive afferents) (Meyer et al. 1991). In contrast, 58% of the cutaneous nociceptors were in the MIA range in the present study. Other studies in rat and rabbit (Fitzgerald and Lynn 1977; Lynn and Carpenter 1982) explored a threshold range up to 50–100 mN. Thresholds in the only extensive study in humans (Adriaensen et al. 1983) covered a range of 20–220 mN, but again 88% of the values were $< 100$ mN. In spite of anecdotal reports of mechanically unexcitable A-fiber nociceptors with a type II heat response (Meyer et al. 1991; Perl 1968), previous studies did not find the significant difference in mechanical thresholds between types I and II afferents (Dubner et al. 1977; Treede et al. 1995). Therefore adequate sampling of the population of A-fiber nociceptors with a type II heat response requires the electrocutaneous search technique.

The electrocutaneous search also enabled us to identify the receptive fields of deep units in the superficial radial nerve. All four deep units were unresponsive to heat stimuli. One unit exhibited a slowly increasing discharge to constant blunt pressure (see Garell et al. 1996) and may account for the slowly increasing pain in humans during skin fold stimulation at forces $\approx 4$ N (Adriaensen et al. 1984).

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**TABLE 2.** Response properties of A-fiber nociceptors in monkey hairy skin

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Type II</th>
<th>Type I</th>
<th>Heat Insensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conduction velocity (m/s)</td>
<td>14.2 ± 5.2 (17)</td>
<td>25.4 ± 15.7° (26)</td>
<td>18.9 ± 11.9 (7)</td>
</tr>
<tr>
<td>From nerve electrode</td>
<td>10.4 ± 4.8 (17)</td>
<td>20.9 ± 14.9° (25)</td>
<td>13.6 ± 5.4 (7)</td>
</tr>
<tr>
<td>From receptive field</td>
<td>15 (16)</td>
<td>4.9° (26)</td>
<td>&gt;18 (7)</td>
</tr>
<tr>
<td>Mechanical threshold</td>
<td>280 (26)</td>
<td>143 (26)</td>
<td>&gt;720 (7)</td>
</tr>
<tr>
<td>Force (mN)</td>
<td>510 (16)</td>
<td>51 (26)</td>
<td>&gt;720 (7)</td>
</tr>
<tr>
<td>Receptive field size (mm²)</td>
<td>9.8 ± 10.6 (11)</td>
<td>15.5 ± 18.3 (25)</td>
<td>13 and 55 (2)</td>
</tr>
<tr>
<td>Heat threshold (°C)</td>
<td>47.2 ± 2.6 (13)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>48 (16)</td>
<td>&gt;53° (26)</td>
<td>NA</td>
</tr>
<tr>
<td>Response to 53°C, 30 s</td>
<td>0.22 ± 0.18 (13)</td>
<td>5.0 ± 5.4° (22)</td>
<td>NA</td>
</tr>
<tr>
<td>Response latency (s)</td>
<td>0.45 ± 0.30 (13)</td>
<td>16.1 ± 8.0° (22)</td>
<td>NA</td>
</tr>
<tr>
<td>No. of action potentials</td>
<td>42 ± 61 (13)</td>
<td>121 ± 183 (22)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are means ± SD; number of nociceptors is in parentheses. Type I: A-fiber nociceptor with a slow heat response; type II: A-fiber nociceptor with a fast heat response. NA, not applicable. * Type II vs. type I: $P = 0.05$, Mann-Whitney $U$ test; † median values only; ‡ type II vs. type I: $P = 0.002$, Mann-Whitney $U$ test; ‡ receptive field areas for mechanically sensitive afferents only; and † tested with 1-s duration heat stimuli.
Traditionally, A-fiber nociceptors were considered to be mainly high-threshold mechanoreceptors (Lynn and Carpenter 1982; Perl 1968). The current data argue against this classification because only three fibers were found that fit this category. The majority (86%) of A-fiber nociceptors in this unbiased sample from monkey hairy skin responded to heat stimuli. This percentage was even higher than the proportion of nociceptors that responded to mechanical stimuli (74%). Most of the nociceptors with a type I heat response would have been classified as high-threshold mechanoreceptors if the long-duration heat stimulus was not used. The current data show that the adequate heat test stimulus may be of an intensity that does not cause primary afferent sensitization. In addition to their mechanical and heat sensitivity, A-fiber nociceptors were shown to respond more vigorously to chemical stimuli than C-fiber nociceptors (Davis et al. 1993). Thus A-fiber nociceptors predominantly are polymodal nociceptors.

If 30 m/s (see RESULTS for rationale) is considered the boundary between Aδ- and Aβ-fibers, 82% of myelinated nociceptive afferents were in the Aδ-range. Here again, however, there was a significant difference between the types I and II afferents; the mean conduction velocity of the type I afferents approached the boundary between Aδ and Aβ, and 31% of the type I afferents were Aβ-fibers. In contrast, in this sample all type II afferents were Aδ-fibers. Because Aδ-fibers tend to have high electrical thresholds and the mechanical thresholds of type II afferents were also high, type II afferents may constitute a high percentage of those Aδ-fibers for which a receptive field was not found.

Reliability and validity of the types I and II classification

We previously reported that cutaneous injury can sensitize the type I heat response and desensitize the type II response (Treede et al. 1995). One may thus hypothesize that 1) the type I response is a fatigued type II response or 2) the type II response is a sensitized type I response. A fatigued type II heat response loses its initial peak and might thus look similar to a type I heat response (see Fig. 2 in Treede 1995). However, most of the type I afferents did not respond to heat stimuli before the 53°C, 30-s stimulus used to classify the type I heat response independently for two different parts of the receptive field, resulting in the same classification for both parts. All these findings taken together indicate that the type of heat response is a property of the primary afferent nerve fiber.

Characteristics of the type I heat response

An important finding in this study is that the heat threshold for type I afferents is not as high as originally reported. Whereas most type I afferents do not respond to a 53°C, 1-s heat stimulus, the three afferents that were tested with an ascending threshold sequence that used long-duration (30-
FIG. 6. Heat thresholds at different stimulus durations and baseline temperatures. Heat thresholds were determined with ascending series of stimuli. A run was terminated when ≥1 action potential was elicited or 49°C was reached. There was a 2-min, stimulus-free interval between runs. ●, MIAs; ○, MSAs. A: heat thresholds at different stimulus durations for A-fiber nociceptors with a type I heat response (n = 3, 1 MIA, 2 MSAs). Although the type I afferents typically had high heat thresholds when tested with 1-s duration stimuli, the thresholds for the 30-s duration stimuli were substantially lower. Expressing threshold as temperature increase above baseline (38°C), the thresholds differed by a factor of 2. The unchanged heat threshold for 1-s duration obtained after the long heat stimuli indicates that this threshold difference was not caused by sensitization. B: heat thresholds at different stimulus durations for A-fiber nociceptors with a type II heat response (n = 7, 4 MIAs, 3 MSAs). The thresholds for the 30-s duration stimuli were only slightly lower than heat threshold obtained with 1-s duration. C: heat thresholds at different baseline temperatures (1-s stimulus duration) for A-fibers with a type II heat response (n = 4, 2 MIAs, 2 MSAs). Heat thresholds at the 35°C base were not significantly different from the thresholds obtained before and after at the 38°C base.

s) stimuli had much lower thresholds. In addition their threshold response was reproducible over multiple runs. Thus type I afferents appear to have a slow response not only to the intense heat stimulus used to classify them but also to less intense stimuli. These results also indicate that a sensitizing stimulus is not needed for these afferents to respond to heat. Possible explanations of the slow heat response in type I afferents include the following: 1) temperature conduction within the skin could lead to a long response latency if the fiber terminals are situated deep in the skin; 2) the apparent heat response is actually a response to chemical mediators released from nonneuronal cells by the intense, tissue-injuring heat stimulus; and 3) type I afferents have a slow heat transduction mechanism.

DEPTH OF RECEPTOR. The 53% difference in heat threshold between stimuli of 1- and 30-s duration would be compatible with a depth of ~700 μm if these afferents had the same

FIG. 7. Reliability of A-fiber nociceptor heat thresholds. Heat thresholds were determined with ascending series of stimuli from a 38°C base. A run was terminated when ≥1 action potential was elicited or 49°C was reached. There was a 2-min, stimulus-free interval between runs. ●, MIA; ○, MSA. A: heat thresholds for type I afferents were determined with 30-s duration stimuli (n = 3, 1 MIA, 2 MSAs). B: heat thresholds for type II afferents were determined with 1-s duration stimuli (n = 8, 5 MIAs, 3 MSAs). Heat thresholds with this paradigm did not vary substantially with repeated runs for A-fiber nociceptors with either type of heat response except for one type II afferent (⋯⋯). Thus heat thresholds of A-fiber nociceptors in monkey hairy skin in this study were as reliable as those of C-fiber nociceptors in a previous study (Treede et al. 1990).
transduction mechanism as C-fiber mechanohot nociceptors (CMHs), i.e., depended only on local temperature at the nerve terminal (see Tillman et al. 1995). Intracutaneous temperature measurements, however, showed that the skin reaches thermal equilibrium ≈5–10 s after stimulus onset (Treede et al. 1995), whereas most (15/22) of the nociceptors with a type I heat response exhibited their peak discharge much later than 10 s, and three fibers did not even start to generate action potentials until after 10 s. Thus receptor depth would not account for the late peak in discharge frequency. Although the radial nerve, which was used for most of the experiments in this study, innervates some subcutaneous tissue (4/79 = 5% fibers in this study), the mechanical receptive field structure of fibers with a type I heat response was not compatible with such a deep location. The majority (15/26) of type I afferents responded to von Frey probes of 1.3–5.1 bar (2.4–51 mN). In contrast, the four deep receptors with receptive fields that did not move with the skin had very high mechanical thresholds to von Frey probes and did not respond to heat stimuli at all.

**SENSITIZATION RESPONSE.** The long latency of the heat response may indicate involvement of a chemical mediator that is released from damaged tissue. Whereas the 53°C, 30-s stimulus does cause tissue damage, this is not the case for the 42.5–45.5°C stimuli to which the three fibers shown in Fig. 7A exhibited a reproducible response. Moreover, the threshold of these fibers to subsequent 1-s stimuli was not lowered (see Fig. 6A), and thus these long-duration stimuli of threshold intensity did not lead to sensitization. Also, psychophysiologically, the induction of primary hyperalgesia to heat in human hairy skin requires stimuli of 50°C for 60 s or 48°C for 120 s, whereas 50°C for 20 s is ineffective (Davis et al. 1995; LaMotte et al. 1983). Therefore to explain the type I heat response by a chemical mediator this chemical must be released by very mild heat stimuli that do not produce sensitization or primary hyperalgesia.

**SLOW TRANSDUCTION MECHANISM.** The long response latency most likely is caused by a slow transduction mechanism within the nerve terminals. An element of this slow transduction mechanism is a wind-up of the action-potential response. If the opening of a cation channel leads to the initial response to heat, the initial influx of cations may perpetuate a further opening of the same or other cation channels. Alternatively, heat transduction in these terminals may involve a slow intracellular signal cascade.

**Characteristics of the type II heat response**

The heat response of type II A-fiber nociceptors is similar to the heat response of C-fiber nociceptors in many ways. Type II afferents exhibit a rapid adaptation of their response to the 53°C, 30-s stimulus (time constant of 2.4 s) and a sustained response in the adapted state of <10% of the peak. Polymodal C-fiber nociceptors exhibit a similar rate of adaptation (τ = 2.5 s) (Treede 1995) and amount of sustained...
response (Meyer and Campbell 1981a). In addition, both C-fiber and A-fiber nociceptors with a type II heat response exhibit fatigue to repeated heat stimuli; for both, the response is suppressed by about a factor of three at an interstimulus interval of 30 s. Whereas C-fiber nociceptors in glabrous skin fully recover between 4 and 10 min (LaMotte and Campbell 1978) and those in hairy skin are 90% recovered after 10 min (Tillman 1992), A-fiber nociceptors with a type II response were only 70% recovered after 10 min (this study). Adaptation and fatigue may occur in the process of transduction of heat into the generator potential and/or in the process of transformation into trains of action potentials. Recently, dissociated dorsal root ganglion neurons, which are the somata of the primary sensory neurons that innervate the skin, were used to study heat transduction mechanisms with whole cell voltage-clamp techniques (Baumann and Martenson 1994; Cesare and McNaughton 1996; Kirschstein et al. 1997). There is preliminary evidence that the heat-evoked inward currents show adaptation when a heat pulse of 49°C is applied for 3 s (P. McNaughton, personal communication). On the other hand, nociceptive A-fibers have a limited capacity to transmit high action-potential rates on electrical stimulation when compared with cold fibers of the same conduction velocity (Raymond et al. 1990). Slowing of conduction velocity and conduction failure may contribute to the overall adaptation.

It was recently suggested that the heat thresholds of A-fiber nociceptors depend on the rate of temperature rise (Yeomans and Proudfoot 1996). In contrast, the first action-potential threshold of C-fiber nociceptors depends only on local temperature at the nerve terminal, not on its rate of change (Tillman et al. 1995; Yarmitsky et al. 1992). For C-fiber nociceptors in monkey hairy skin, differences in the heat thresholds obtained with different stimulus parameters (base temperature, rate of temperature change, and duration) could be explained by an average depth of their terminals of 150 μm and by the different spatial temperature gradients created by these stimuli (Tillman et al. 1995). The 18% difference in heat threshold between stimuli of 1- and 30-s duration would be compatible with a depth of ~150 μm for the terminals of type II A-fiber nociceptors. From a lower base temperature (35°C instead of 38°C), we would expect a heat threshold that is ~1°C higher because a higher skin surface temperature is necessary to reach the same temperature at 150 μm. Such a difference in surface temperature at threshold between the two base temperatures, however, was not observed for type II A-fiber nociceptors (this study) in contrast to C-fiber nociceptors (Tillman et al. 1995). Thus from the lower base temperature a lower intracutaneous temperature was sufficient to reach threshold. Because the size of the temperature step was larger from the lower base temperature, a rate dependence of A-fiber nociceptor heat threshold could explain this behavior.

The heat response of type II A-fiber nociceptors increased with stimulus intensity, and thus these afferents may play a role in the perceived intensity of noxious heat stimuli. In addition, the threshold distribution of these afferents in monkey matches those of pricking pain and (Aδ-fiber mediated) laser-evoked potentials in humans (Treedee et al. 1994). Thus these fibers likely signal pricking pain to heat.

Heat transduction or chemical transduction?

The polymodality of nociceptors suggests that tissue damage and release of inflammatory mediators could be the common pathway for the transduction mechanism of nociception. In this view, nociceptors are considered to be chemoreceptors. However, the current data provide evidence against this concept. Although the slow response of type I afferents to the intense heat stimulus (53°C, 30 s) would be compatible with the concept of chemical mediators, a similar response could be obtained with stimulus intensities that do not damage the skin. The type II heat response is too fast for this mechanism and is likely related to the heat-evoked inward currents that were identified in some acutely dissociated dorsal root ganglion neurons (Cesare and McNaughton 1996; Kirschstein et al. 1997). The range of thresholds of A-fiber nociceptors is very broad, both for mechanical and for heat stimuli. But, even for those A-fibers that had very high thresholds for one stimulus modality, actual tissue damage does not seem to be the adequate stimulus because I) about one-half of the fibers that did not respond to our strongest mechanical stimulus (7/15) had a type II heat response and 2) most of the fibers with a type I heat response had moderate mechanical thresholds (<6 bar (15/26)).

In conclusion, A-fiber nociceptors are polymodal and have two distinct types of response to heat. Type I afferents have a slow, wind-up response to heat. These fibers sensitize to injurious heat stimuli, have rapid conduction velocities (at times in the Aβ-range), and generally have mechanical thresholds <6 bar (100 mN). Type I afferents may be primarily responsible for signaling sharpness (Garell et al. 1996) and/or pricking pain to mechanical stimuli and were shown previously to account for the heat hyperalgesia to a burn injury (Meyer and Campbell 1981b). The type II afferents have responses to heat that resemble those of C-fiber nociceptors: early peak frequency and a slowly adapting response. These fibers have slower conduction velocities, always in the Aδ-range, and are characteristically insensitive to mechanical stimuli (thresholds >6 bar). The short response latency, low heat threshold, and graded heat response of type II afferents suggest that their primary function may be to signal heat-induced pain. With little question these fibers signal first pain sensation. Both types I and II afferents respond to chemical stimuli (Davis et al. 1993; Ringkamp et al. 1997) and both likely participate in chemogenic pain. Because A-fiber nociceptors appear to exhibit a higher degree of stimulus specificity than do C-fiber nociceptors, they may provide the most important contribution to discrimination of the quality of a noxious stimulus. A population code for stimulus quality was studied most extensively in the gustatory system, where each primary afferent can be activated by prototypical stimuli for each of the four taste qualities (sour, salty, sweet, and bitter), but each fiber exhibits its lowest threshold to one of the qualities. The differential sensitivity spectra of nociceptive afferents suggest a similar population code for pain quality.

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HEAT RESPONSES OF MYELINATED NOCICEPTORS


