Responses of Cutaneous A-Fiber Nociceptors to Noxious Cold

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Simone, Donald A. and Keith C. Kajander. Responses of cutaneous A fiber nociceptors to noxious cold. J. Neurophysiol. 77: 2049–2060, 1997. Responses of cutaneous nociceptors to natural stimuli, particularly mechanical and heat stimuli, have been well documented. Although nociceptors are excited by noxious cold stimuli, there have been few studies of their stimulus-response functions for cold stimuli over a wide range of stimulus temperatures. Furthermore, the proportion of nociceptors excited by noxious cold is not clear. In the present study, we examined responses of mechanosensitive Aδ-nociceptors and low-threshold mechanoreceptors to a wide range of cold stimuli that included stimulus temperatures <0°C. Electrophysiological recordings were made from single primary afferent fibers in the saphenous nerves of anesthetized rats. Cutaneous sensory receptors were classified according to their conduction velocity and subgrouped functionally according to their responses evoked by mechanical, heat, and cold stimuli (0°C). Responses evoked by a wide range of cold stimulus intensities that included stimuli considered innocuous and noxious (painful) were then assessed. Stimuli of 20 to −20°C were delivered to the receptive field via a 1-cm² contact thermode from a base temperature of 32°C. Stimuli were applied in descending order of 2°C decrements. Stimulus ramp rate was 5°C/s, and stimulus temperatures were applied for a duration of 10 s. A total of 90 A fibers was studied, of which 61 were nociceptors and had conduction velocity in the Aδ-range (2–30 m/s). Nociceptors were classified initially as mechanical, mechanothermal, and mechanocold nociceptors. The remaining 29 fibers were low-threshold mechanoreceptors with conduction velocity in the Aδ- or Aδ-range (>30 m/s). These were subgrouped according to their adaptive properties as slowly or rapidly adapting, and according to whether they were excited by hair movement (hair follicle afferent fibers). All nociceptors were excited by noxious cold. Only 30% of nociceptors were considered sensitive to cold on initial classification with the use of a cold stimulus of 0°C. However, all nociceptors were excited by stimulus intensities <0°C. Response thresholds for cold ranged from 14 to −18°C (−4.6 ± 1.07°C, mean ± SE). The total number of impulses, discharge rate, and peak discharge increased monotonically as intensity of cold stimuli increased. Power functions were used to determine the rate at which the number of impulses increased as stimulus intensity increased. The slopes of power functions ranged from 0.12 to 2.28 (mean 1.07 ± 0.13). Most mechanoreceptors were not excited by cold stimuli. The only types of mechanoreceptors that responded reliably to cold stimuli were the slowly adapting mechanoreceptors. Responses usually occurred during the temperature ramp when the skin temperature was decreasing. There was no evidence that mechanoreceptors encoded the intensity of cold stimuli at intensities above or below 0°C, because evoked responses did not increase with intensity of cold stimuli. It is concluded that the proportion of cutaneous Aδ-nociceptors excited by noxious cold stimuli has been underestimated in previous studies. All nociceptors were excited by stimulus temperatures <0°C and encoded the intensity of cold stimuli. It is therefore likely that cutaneous Aδ-nociceptors contribute to the sensation of cold pain, particularly pain produced by stimulus temperatures <0°C.

INTRODUCTION

Cooling the skin by as little as 1°C from normal skin temperature evokes a nonpainful “cool” sensation. Further reduction in skin temperature to ~15°C evokes a sensation of cold pain. In contrast to our understanding of the mechanisms underlying other modalities of pain sensation, such as heat pain and inflammatory pain (for reviews, see Levine and Taiwo 1994; Raja et al. 1988; Treede et al. 1992), the peripheral neural mechanisms that contribute to the sensation of cold pain are poorly understood. It is known, however, that innocuous cool sensation and cold pain are mediated by separate populations of primary afferent fibers. Cool sensation is signaled by activity in cold-specific Aδ-fibers (and to a lesser extent by cold-specific C fibers) whose discharge rate peaks within a relatively restricted range of cold temperatures that are innocuous (~20–30°C) (Beitel and Dubner 1976; Darian-Smith et al. 1973; Douglas et al. 1960; Dubner et al. 1975; Hellon et al. 1975; Hensel 1974; Hensel and Iggo 1971; Hensel and Zotterman 1951a; Iggo 1969). There are, however, some cold fibers that exhibit maximal discharges at colder temperatures (as cold as −5°C) (Duclaux et al. 1980).

The cutaneous receptors and afferent fibers that contribute to the sensation of cold pain are less clear. The sensation of pain evoked by cold can have various qualities including cold aching, burning, and pricking (Chery-Croze 1983a,b; Kilo et al. 1994; Kunkle 1949; Lewis and Love 1926; Wolf and Hardy 1941; Yarnitsky and Ochoa 1990). This suggests that the sensation of cold pain may be mediated by multiple classes of nociceptors. Although it has been well documented that a portion of Aδ- and C fiber nociceptors is excited by noxious cold (Bessou and Perl 1969; Burgess and Perl 1967; Georgopoulos 1976, 1977; LaMotte and Thalhammer 1982; Perl 1968), the exact proportion excited by cold is unknown because the stimulus temperatures and species used in previous studies varied. For example, Perl (1968) reported that cutaneous nociceptors in monkeys were not excited by cold stimuli down to 15°C, whereas LaMotte and Thalhammer (1982) reported that 78% of Aδ- and C nociceptors were excited by ice applied to their receptive fields (RFs). In a comprehensive study of sensory receptors in the rat hindpaw, Leem et al. (1993) reported that 10% of Aδ- and 8% of C nociceptors were excited by cold stimuli with the use of a maximum intensity of 12°C.

Few studies have systematically examined stimulus-response functions for cutaneous nociceptors over a wide range of controlled cold stimulus temperatures. Georgopoulos (1976, 1977) found that 29% of Aδ- and 48% of C nociceptors in monkeys were excited by noxious cold stimuli. Stimu-
lus-response functions were approximately linear, with slopes of power functions ranging from 0.9 to 1.58 (mean 1.15). In those studies, controlled cold stimuli with a peak temperature of 8°C (as well as ice) were used.

Responses of nociceptors to stimulus temperatures <0°C have not been studied systematically. Therefore the range of cold stimulus intensities over which nociceptors encode is unknown. For example, it is not known whether cutaneous nociceptors continue to encode stimulus intensity at temperatures <0°C or which classes of nociceptors are excited when the skin begins to freeze. Interestingly, Lewis and Love (1926) reported that freezing of the superficial skin was associated with intense pinching pain sensation. This finding suggests that cold intracutaneous temperatures excite nociceptors, although their location (cutaneous or deep) and receptor type are unknown. For example, a role for nociceptors located in venous walls has been suggested because cold saline injected into veins in humans evoked a sensation of cold pain (Fruhstrofer and Lindblom 1983), and nociceptors located in venous walls were excited by cold (Klement and Arndt 1992).

Because afferent receptors are grouped functionally according to the types of natural stimuli that excite them, it is important to assess their responses over a wide range of stimulus intensities so that appropriate classification can be made. In the present study, we examined responses of mechano-sensitive Aδ-nociceptors and low-threshold mechanoreceptors to a wide range of cold stimulus temperatures, including temperatures <0°C. A preliminary report has appeared (Simone and Kajander 1993).

**METHODS**

**Subjects**

Adult male Sprague-Dawley rats (250–350 g) were used in this study. Animals were housed in pairs and maintained on a 12-h light:dark schedule. Food and water were provided ad libitum. All procedures were approved by the Animal Care Committee at the University of Minnesota.

**Surgical preparation**

Animals were anesthetized initially with a mixture of acepromazine (1 mg/kg im) and ketamine hydrochloride (100 mg/kg im). The right jugular vein was catheterized and deep anesthesia was maintained with supplemental doses of pentobarbital sodium (~6 mg·kg⁻¹·h⁻¹) given as needed to maintain areflexia. A tracheotomy was performed to maintain an unobstructed airway throughout the experiment. A feedback-controlled heating pad maintained the animal’s core temperature close to 37°C.

**Electrophysiological recording**

Action potentials were recorded extracellularly from the left or right saphenous nerve with the use of conventional microdissection and recording techniques. An incision was made in the skin overlying the nerve. The skin was sewn to a metal ring to form a pool for the nerve. The skin was sewn to a metal ring to form a pool for the nerve. The nerve was dissected from connective tissue and placed on a dissection platform. The epi- and perineurial sheaths were opened, and small fascicles were cut and proximal ends were placed on the platform for fine dissection. Fascicles were teased into fine filaments and placed on a silver wire electrode. Only single units that could be easily discriminated were studied. An amplitude window discriminator was used to separate action potentials of the fiber under study from those of other fibers and from background noise. However, recordings usually consisted of one afferent fiber.

**Identification and classification of primary afferent fibers**

The RF of all afferent fibers was located with the use of mechanical stimulation that consisted of gently stroking the skin with a cotton swab or the experimenter’s fingers, and mildly pinching the skin with the experimenter’s fingers. Once a single unit was identified, the RF was marked with the use of a calibrated von Frey monofilament that was above threshold. The border of the RF was marked on the skin with the use of a felt-tip pen. Afferent fibers were grouped as Aδ or Aβ according to their conduction velocity, and classified functionally according to responses evoked by mechanical and thermal stimuli.

**Conduction velocity.** The conduction velocity of each fiber was determined by stimulating the fiber electrically at the RF and measuring the conduction latency and conduction distance. Two fine needle electrodes (30 gauge) were inserted into the skin just outside the border of the RF and at its opposing ends. Conduction latency used for determining the conduction velocity was evoked by electrical stimulation at 1.5 times threshold for activation of the fiber. Fibers with conduction velocities >30 m/s were considered Aβ, whereas those that conducted between 2 and 30 m/s were classified Aδ.

**Functional classification.** Afferent fibers were classified functionally according to responses evoked by mechanical and heat stimuli. Innocuous mechanical stimuli consisted of stroking the skin lightly with a cotton swab, innocuous stretching, and application of von Frey monofilaments. In addition, fine forceps were used to move one or several hairs; this process was observed with the use of a dissecting microscope. Noxious mechanical stimuli included pinching the skin mildly with the experimenter’s fingers or pressure applied to the skin with a blunt glass probe (1 mm diam). Heat stimuli were applied with the use of a feedback-controlled Peltier device with a contact area of 1 cm². Heat stimuli of 39–51°C, each 5 s in duration, were delivered in ascending order of 2°C increments from an adapting temperature of 32°C (rate of temperature rise 20°C/s). In some instances, heat stimuli of 53°C were applied one or two times for a duration of 30 s.

**Low-threshold mechanoreceptors.** Low-threshold mechanoreceptors were divided into rapidly adapting (RA), slowly adapting (SA), and hair follicle units that were also RA. SA and RA fibers were discriminated by their responses to constant pressure applied to their RF with a suprathreshold von Frey monofilament. SA mechanoreceptors were subdivided into type I if they lacked spontaneous activity and if their evoked discharge consisted of an irregular pattern of discharge, and type II if they exhibited spontaneous discharge if they were excited by skin stretch, and if their evoked discharge was fairly regular (constant discharge rate). Hair follicle afferent fibers were classified as such if they were excited by movement of single hairs. Further subclassification of hair afferent fibers was not made.

**nociceptors.** Nociceptors were initially classified as Aδ-mechano-nociceptors (AM) or as Aδ-mechanoheat nociceptors (AMH) according to their responses to mechanical and heat stimuli. Nociceptors excited only by noxious mechanical stimuli were considered AM. AMHs were excited by noxious mechanical and by noxious heat stimuli and were further subdivided into type I and type II. AMHs were considered type I if they had a response threshold for heat that was >51°C and if they became sensitized to heat after a strong and prolonged heat stimulus (53°C for 30 s). Nociceptors with a response threshold ≤51°C were classified as AMH type II.
Cold stimuli

The same Peltier device that was used to apply heat stimuli was used to deliver cold stimuli. Cold stimuli of 20 to −12°C were delivered in descending order of 2°C steps from an adapting temperature of 32°C. Occasionally, stimulus temperatures to −20°C were applied. Stimuli were each 10 s in duration and applied every 3 min. Between presentations of successive stimuli, the temperature of the thermode was maintained at 32°C. In delivering the cold stimuli, a constant ramp rate of 5°C/s was used to get to the desired stimulus temperature and to return to the adapting temperature (32°C) because this was the fastest stimulation rate we could deliver reliably. In addition to the descending series of cold stimuli, we applied a stimulus of 0°C for 30 s.

Testing sequence

Once a unit was identified and its RF was mapped, the following quantitative measures were obtained. First, mechanical threshold was determined with the use of von Frey monofilaments. Threshold (mN) was defined as the smallest force that evoked a response ~50% of the time. Next, the ascending series of heat stimuli (39−51°C) was applied. Cold stimuli were applied after the heat stimuli. The first cold stimulus given was 0°C for 30 s and was applied 5−10 min after the last heat stimulus. The purpose of this cold stimulus was to approximate ice placed on the RF, as has been done in earlier studies. If there was no response to 0°C during the first 10 s of the stimulus, a ascending series of cold stimuli was applied beginning with −2°C. If a response occurred during the first 10 s of the 0°C stimulus, the ascending series of cold stimuli began with a higher stimulus temperature, typically 20°C. A minimum of 5 min lapsed between the first 0°C stimulus and the beginning of the descending series of cold stimuli.

Some nociceptors were exposed to the descending series of cold stimuli twice to determine the stability of cold-evoked responses. When this was done, a minimum of 10 min separated the end of the first stimulus series and the beginning of the next.

Nociceptors that were not excited by the ascending series of heat stimuli were exposed to an injurious heat stimulus (53°C for 30 s) at the end of the experiment to determine whether they became sensitized to heat stimuli and thereby met the criteria for classification as AMH type 1.

Data analyses

Electrophysiological responses were recorded on video tape and analyzed off-line with the use of DataWave Systems computer interface and software. Discriminated responses were stored as events on a laboratory computer. For thermal stimuli, the beginning of the ascending or descending ramp and the time at which the desired stimulus temperature was reached were stored on a separate channel. Stimulus temperature (temperature of the interface between the skin and the thermode) was digitized and stored.

To assess responses evoked by cold, the number of impulses, the discharge rate (from the first to the last evoked impulse), and the peak discharge rate (reciprocal of the smallest interval between two consecutive spikes) evoked by each cold stimulus were determined for each afferent fiber. Discharge rate was not calculated for responses that consisted of one impulse. Power functions were generated to determine stimulus-response relationships for each fiber as follows. Evoked responses were determined by subtracting any ongoing activity from the response evoked during the cold stimuli. The cold stimuli were normalized by defining the “zero point” as the lowest stimulus temperature that did not evoke a response. Stimulus intensities were defined as the difference from the zero point. Because our stimuli were always delivered in 2°C increments, the threshold stimulus was assigned an intensity of 2°C.

After log-log transformation, the slopes of the stimulus-response functions were determined with the use of linear regression. Comparisons were made between the mean response thresholds to cold for the various subgroups of nociceptive afferent fibers with the use of Student's t-tests. Similarly, t-tests were used to determine differences between fiber types in stimulus-response functions for cold. Comparisons were made between mean slopes of power functions that related the number of impulses to stimulus intensity. The significance level was set at 0.05.

RESULTS

Recordings were made from a total of 90 A fibers, of which 61 were classed as nociceptors and 29 as low-threshold mechanoreceptors. All RFs were located on hairy skin of the hindpaw. Because mechanical stimuli were used as the search stimuli, all fibers were mechanosensitive. Each of the nociceptive afferent fibers conducted in the Aβ-range, whereas low-threshold mechanoreceptors conducted in the Aδ- or Aβ-range.

Functional classification of Aδ-nociceptors

On the basis of responses evoked by mechanical, heat, and the 0°C cold stimulus, 67% of Aδ-nociceptors were classed as AM, 15% as AMH (5 type I and 4 type II), and 18% as mecanocold. Because all mechanosensitive nociceptors were excited by cold stimuli at temperatures <0°C, the classification was modified to include sensitivity to cold stimuli and consisted of mecanocold nociceptors (85%) and mecano-heat-cold nociceptors (15%). The mecano-heat-cold nociceptors were subgrouped as type I and type II according to their responses evoked by noxious heat (as described above). None of the nociceptors exhibited spontaneous discharge. General characteristics of all the fiber types studied, including conduction velocity, mechanical threshold, and heat threshold, are summarized in Table 1.

Responses of Aδ-nociceptors to noxious cold

There were no statistical differences among the various nociceptor subtypes in either their response thresholds for cold or in their stimulus-response functions for noxious cold. Responses to cold have therefore been combined for all nociceptors.

RESPONSE THRESHOLD. Aδ-nociceptors exhibited a wide range of cold thresholds, ranging from 14 to −18°C (mean −4.6 ± 1.07°C). Figure 1 shows the distribution of threshold temperatures for all nociceptors. As shown in Fig. 1A, 79% had response thresholds of ≤0°C. Responses evoked by the threshold temperature usually consisted of one or two spikes, and occurred at any time during the stimulus.

Figure 1B shows the proportion of nociceptors excited by the various stimulus temperatures. This distribution is based on the proportion of fibers with thresholds at or below the indicated stimulus temperature. Only 11% of the nociceptors were excited by stimulus temperatures ≥10°C, whereas 30% of nociceptors were excited by 0°C. Nearly 90% of Aδ-nociceptors were excited by a stimulus temperature of −12°C, and, 100% of the nociceptors were excited by −18°C. Thus there is a monotonic increase in the recruitment of Aδ-nociceptors with increasing intensity of cold stimuli.
TABLE 1. General characteristics of primary afferent fibers

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean Conduction Velocity, m/s</th>
<th>Median Mechanical Threshold, mN</th>
<th>Mean Heat Threshold, °C</th>
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<tbody>
<tr>
<td>Nociceptors</td>
<td></td>
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<tr>
<td>AMC</td>
<td>52</td>
<td>14.5 ± 1.25 (2.1–30.0)</td>
<td>19.6 (0.63–721.2)</td>
<td>NR</td>
</tr>
<tr>
<td>AHMC type I</td>
<td>5</td>
<td>11.3 ± 2.56 (6.8–18.3)</td>
<td>43.2 (19.6–95)</td>
<td>&gt;51</td>
</tr>
<tr>
<td>AMHC type II</td>
<td>4</td>
<td>11.1 ± 4.8 (4.7–25.3)</td>
<td>40.4 (13.5–61.8)</td>
<td>48.5 ± 0.50</td>
</tr>
<tr>
<td>Mechanoreceptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RA</td>
<td>10</td>
<td>28.1 ± 2.93 (23.1–43.8)</td>
<td>0.61 (0.61–8.25)</td>
<td>NR</td>
</tr>
<tr>
<td>SA</td>
<td>6</td>
<td>28.0 ± 3.23 (21.1–52.1)</td>
<td>1.58 (1.58–2.53)</td>
<td>NR</td>
</tr>
<tr>
<td>Hair</td>
<td>13</td>
<td>24.0 ± 1.85 (10.2–33.0)</td>
<td>NT</td>
<td>NR</td>
</tr>
</tbody>
</table>

Values for mean conduction velocity are means ± SE, with ranges in parentheses. AMC, mechanocold; AMHC, mechano-heat-cold; RA, rapidly adapting; SA, slowly adapting; NR, no response to 51 or 53°C; NT, not tested.

RESPONSES TO SUPRATHRESHOLD COLD STIMULI. Responses to a series of stimulus temperatures were obtained for 27 nociceptors. Because of the wide range of response thresholds, we were unable to obtain stimulus-response functions for nociceptors with a high response threshold (low stimulus temperature). We therefore included only those nociceptors in which responses to at least four different cold stimuli were obtained. The evoked number of spikes, discharge rate, and peak discharge rate each increased monotonically as stimulus temperature decreased. These features are illustrated in Fig. 2, which shows the responses of a single Aδ-nociceptor evoked by a series of cold stimuli. This unit had a conduction velocity of 8.1 m/s, and was originally classed as AM because it did not exhibit any response to noxious heat or to 0°C.

Figure 3 shows the mean number of spikes evoked by stimulus temperatures between 16 and —20°C. The numbers of impulses evoked at or near threshold and down to —2°C were low, usually one or two spikes. However, stimulus temperatures of —4°C and below evoked greater responses. The mean number of impulses ranged between 3 (for —4°C) and 14 (for —20°C). These results show that Aδ-nociceptors are more sensitive to extremely cold stimulus temperatures than to moderately cold stimulus temperatures.

The mean discharge rate also increased as stimulus temperature decreased, and is illustrated in Fig. 4. However, average discharge rates were low. Stimulus intensities down to —10°C evoked mean discharge rates that were typically <1 Hz. Greater discharge rates were evoked by the colder stimuli, but did not exceed 2.5 Hz.

Figure 5 shows that the mean peak discharge rate evoked by cold stimuli increased as stimulus temperature decreased. Although the peak discharge rates were considerably higher than the discharge rates described above, the pattern was the same in that the greatest peak rates were evoked by the extremely cold stimuli. Mean peak discharge rates ranged from 0.1 Hz for 10°C to 8.1 Hz for —20°C.

Power functions were derived to determine whether the number of impulses evoked by cold stimuli increased linearly, or in a positively or negatively accelerating fashion as stimulus temperature decreased. Power functions relating log number of impulses to log stimulus intensity were determined for 24 fibers. The slope (mean ± SE) of the power functions was 1.07 ± 0.13, and ranged from 0.12 to 2.28. Of the 24 power functions generated, 11 were positively accelerating, 11 were negatively accelerating, and 2 were approximately linear (slopes of 0.98 and 1.05).

Stability of cold-evoked responses

To determine whether responses evoked by cold stimuli are reproducible, six nociceptors were exposed to the cold stimulus series twice. A 10-min period separated the end of the first stimulus series and the beginning of the next. Responses evoked by the two series of cold stimuli were very similar. Response thresholds remained unchanged for two nociceptors, increased (became colder) by 2–4°C for three nociceptors, and decreased (became warmer) by 2°C for one nociceptor. Stimulus response functions evoked by each stimulus set were nearly identical. A representative example is provided in Fig. 6, which shows for a single nociceptor the number of impulses evoked by each stimulus temperature for each stimulus series. These results suggest that responses to the series of cold stimuli are stable for at least two trials.

Latency to peak discharge rate

It is possible that the successive increase in responses evoked by progressively colder stimuli, particularly the evoked number of impulses, may not be attributed to the actual stimulus temperature but rather to the duration of cold exposure. For each successive 2°C decrease in stimulus temperature there is an 0.8-s increase in total cold exposure that occurs during ramping to and from the stimulus temperature as a result of the relatively slow ramp rate (5°C/s). We therefore determined whether the peak evoked discharge rate was a function of stimulus temperature or was dependent on the duration of cold. For each nociceptor, the latency of the peak discharge was determined relative to the time at which each stimulus temperature was achieved. The time at which the stimulus temperature was achieved was considered the zero point, and latencies were expressed from this time. It was found that the peak discharge rate evoked by each stimulus temperature always occurred close to the zero point (within ~2 s), as is shown in Fig. 7. This suggests that peak discharge is the result of stimulus temperature, because latencies to peak discharge would have been longer for progressively colder stimuli if stimulus duration was a crucial factor in the evoked discharge.

Responses of mechanoreceptors to cold

Recordings were made from 29 mechanoreceptors of which 6 were classed as SA (type I), 10 as RA, and 13 as hair afferent fibers. Responses evoked by cold stimuli were...
FIG. 1. Response thresholds for cold stimuli and the proportion of Aδ-nociceptors excited by various intensities of cold stimuli. A: distribution of response thresholds (°C) for all nociceptors (n = 61). Threshold for most nociceptors was near 0°C and below. B: proportion of nociceptors excited by the various intensities of cold stimuli. It can be seen that as the intensity of cold increases, there is recruitment of additional nociceptors. Approximately 30% of Aδ-nociceptors were excited by 0°C, and stimulus temperatures of −12 to −18°C excited 90–100% of nociceptors.

A

![Histogram of response thresholds](image1)

B

![Bar graph of proportion of fibers](image2)

Dependent on the type of mechanoreceptor and whether the stimulus was in the descending phase of the temperature ramp.

**SA MECHANORECEPTORS.** The only type of mechanoreceptor that responded to cold stimuli reliably was the SA type I, and each of the six studied were excited by cold. However, responses were usually weak and occurred almost exclusively during the descending phase of the stimulus. Once the plateau temperature was reached, responses usually ceased. Response thresholds of the two most sensitive units were 30 and 22°C. Thresholds of the remaining mechanoreceptors ranged between 16 and −8°C. The number of spikes evoked during the descending phase of the cold stimuli increased monotonically for only one mechanoreceptor, whereas the rest did not show any evidence of encoding cold intensity. Figure 8 shows responses of the most sensitive SA type I mechanoreceptor to cold stimuli and illustrates that most responses occurred during the descending phase of the stimulus. The mean number of spikes evoked by the cold stimuli for all SA mechanoreceptors is shown in Fig. 9. It can be seen that mean responses did not increase significantly as stimulus temperature decreased.

**ALL OTHER MECHANORECEPTORS.** RA and hair follicle afferent fibers rarely exhibited responses to cold stimuli. Only 4 of 10 RA mechanoreceptors were excited by cold stimuli. Response thresholds for two of these mechanoreceptors were
C, and thresholds were 0 and 0.18°C for the remaining two. However, responses were evoked by only one or two of the cold stimuli within the series and usually consisted of one or two impulses. None of the RAs encoded the intensity of cold. Similarly, responses of hair follicle afferent fibers to cold stimuli were rare and weak. Of 13 hair afferents, 6 exhibited a response to cold and response thresholds ranged from −2 to −16°C. Responses were similar to those of RA mechanoreceptors in that they were weak and were usually evoked by only one or a few of the stimuli. None of the hair afferent fibers exhibited encoding of cold intensity.

**DISCUSSION**

The most important finding of the present study was that all mechanosensitive Aδ-nociceptors were excited by noxious cold stimuli. This is in agreement with our previous preliminary report suggesting that all mechanosensitive nociceptors were excited by noxious cold stimuli (Simone and Kajander 1996). Because the response threshold of most nociceptors was <0°C, the physiological relevance of the stimuli used and the specificity of the responses to cold temperature could be questioned. We believe the range of cold stimuli used is physiologically relevant because humans often come in contact with cold objects whose temperatures are within the range of temperatures used in this study. For example, ice represents a cold stimulus with a temperature of ~0°C. Moreover, many individuals live in cold climates where air temperatures <0°C are common. Regarding the specificity of responses to stimulus temperature, it is possible that responses evoked by the coldest stimuli were not due to absolute temperature, but rather to other factors related to injury discharge associated with freezing the skin. For example, freezing would provide a mechanical transient due to abrupt tissue expansion during ice crystallization that could excite nociceptors. When crystallization begins, it is associated with a rapid rise in temperature as many water molecules crystallize simultaneously, thereby converting potential energy to kinetic energy. A rise in temperature at the thermode-skin interface was observed occasionally, but this was rare and occurred only for stimulus temperatures below −12°C. Responses evoked under these conditions were not included in our analyses. The possibility that evoked responses were due to freezing is further minimized for the following reasons. First, nearly all nociceptors exhibited graded responses to cold stimuli and were recruited monotonically with increasingly cold stimuli. If responses were due to freezing and not to stimulus temperature, nociceptors would not have been excited by nonfreezing stimuli, and responses would have been steplike rather than monotonic with response thresholds at the lowest temperatures. This was not the case for most nociceptors. Second, spontaneous discharge rarely developed after the descending series of

**FIG. 2.** A: responses of a single Aδ-nociceptor evoked by cold stimuli of −12 to −18°C. Numbers indicated refer to the peak stimulus temperatures, which were each maintained for a duration of 10 s. Response threshold for cold was −12°C. The total evoked number of impulses, the mean discharge rate, and the peak discharge rate increased with stimulus intensity. B: constant conduction latency for this fiber is also illustrated and shows 5 overlapping traces. Arrow: stimulus artifact.
cold stimuli, and responses evoked by two descending series of cold stimuli were similar. Nociceptors often exhibit spontaneous activity and sensitization after injury or inflammation (Levine and Taiwo 1994; Raja et al. 1988; Treede et al. 1992). Sensitization to cold stimuli was not observed when cold stimuli were given a second time. It should be pointed out, however, that the capacity of nociceptors to become sensitized to cold is not clear. In a study of the responses of four nociceptors to repeated application of noxious cold (−5°C for 3 min) applied repeatedly, Saumet et al. (1985) reported that responses increased with repeated application of cold stimuli. This suggests that some nociceptors can become sensitized to cold stimuli. Third, most low-threshold mechanoreceptors responded poorly or not at all to noxious cold. If responses of nociceptors to extremely cold temperatures were due to movement associated with crystallization, it is likely that all mechanosensitive afferent fibers would exhibit discharge. Thus the evidence outlined above supports the notion that stimulus temperature was the critical factor that excited nociceptors rather than injury associated with freezing, and demonstrates that Aδ-mechanosensitive nociceptors are likely to contribute to the sensation of cold pain and provide a warning signal before cold stimuli freeze and damage the skin.

Classification of Aδ-nociceptors

Aδ-nociceptors have typically been classed as AM or AMH, with very few reported as being sensitive to noxious cold. It has been reported that ~29% of Aδ-nociceptors in monkey were excited by noxious cold (Georgopoulos 1976, 1977). In the most comprehensive study of cutaneous sen-
sory receptors in the skin of the rat hindpaw, Leem et al. (1993) classified ~70% of Aδ-nociceptors as mechanical nociceptors, 20% as mechanoheat nociceptors, and 10% as mechanocold nociceptors. Those proportions, which were obtained with the use of cold stimuli to 12°C, are consistent with those found in the present study with the use of a cold stimulus of 0°C. However, the proportions of nociceptors subgrouped into the various functional categories changed dramatically when cold stimuli <0°C were used. First, none of the nociceptors were excited by mechanical stimuli alone. All mechanical nociceptors that were not excited by noxious heat were excited by noxious cold. Second, all nociceptors excited by mechanical and heat stimuli were also excited by noxious cold. With the use of the cold stimuli employed in the present study, it was found that 85% were classed as mechanocold nociceptors and 15% as mechano-heat-cold nociceptors. Thus the proportion of nociceptors excited by noxious cold has been underestimated in other studies because stimulus temperatures <0°C have not been used.

**Encoding of cold intensity by Aδ-nociceptors**

The number of impulses, discharge rate, and peak discharge rate each increased as intensity of cold increased. The number of impulses and the peak discharge rate increased at a greater rate than the overall discharge rate. This suggests that these parameters, the total number of impulses and peak discharge, are most relevant in encoding stimulus intensity.

There have been few studies that have systematically examined responses of nociceptors to noxious cold. In a previous study conducted in monkeys, it was shown that some cutaneous nociceptors encoded intensity of noxious cold...
that the sensation of pain produced during prolonged cold stimulation was greatest during vasoconstriction (Greenfield et al. 1950; Krehe et al. 1984; Kunkle 1949; Minut-Sorokhtina and Glebova 1976). This is interesting because it introduces the possibility that excitation of nociceptors during cold stimulation may result from changes in blood flow rather than absolute temperature. For example, factors associated with vasoconstriction and vasodilatation that could excite nociceptors include contraction of smooth muscle and release of vasoactive substances, respectively. However, Krehe et al. (1984) measured blood flow, heat flux, intracutaneous temperature, and pain magnitude simultaneously in humans during local cooling and concluded that the critical parameter for the magnitude of cold pain was low intracutaneous temperature. This finding is consistent with the present results demonstrating that the peak evoked discharge of nociceptors to cold stimuli occurs approximately at the time at which maximum decrease in temperature occurs. It should be pointed out, however, that the duration of cold stimuli used in the present study was relatively short compared with those used in earlier studies (e.g., Chéry-Croze 1983a; Krehe et al. 1984; Rainville et al. 1992; Wolf and Hardy 1941).

Excitation of low-threshold mechanoreceptors by noxious cold

The only type of mechanoreceptors that were excited by noxious cold stimuli were SA mechanoreceptors. It has been

Vascular reactions associated with cold skin

Intense cooling of the skin produces vascular responses including vasoconstriction followed by vasodilatation. In addition, during prolonged cooling, vasoconstriction and vasodilatation alternate (Lewis 1930). Subsequent studies determined
shown that SA mechanoreceptors are responsive to innocuous cooling (Burton et al. 1972; Darian-Smith et al. 1973; Ducaux and Kenshali 1972; Hensel and Zotterman 1951b). In rat, a proportion of both SA type I (5%) and SA type II (26%) mechanoreceptors was excited by cooling stimuli between 12 and 27°C (Leem et al. 1993). We found that all SA mechanoreceptors tested (n = 6) were excited during cooling. Thresholds ranged between 30 and 16°C for four SA mechanoreceptors, whereas the remaining two had thresholds of −8 and −10°C, and responses occurred primarily during active cooling of the skin and not when the skin was maintained at a constant cold temperature. It should be pointed out that responses of mechanoreceptors evoked by extremely cold stimulus temperatures may not be specific to absolute temperature, but may be due to small mechanical changes in the skin that may occur as a consequence of vasoconstriction.

The functional significance of mechanoreceptor responses to cold stimuli is unclear. Microneurography studies in humans have shown that intraneural stimulation of mechanoreceptors, including SA mechanoreceptors, evokes tactile sensations or sensation of joint movement (Macefield et al. 1990; Ochoa and Torebjörk 1983; Vallbo 1981). It is unlikely that mechanoreceptors have any role in signalling cold or cold pain sensation. However, they may contribute to central modulation of the responses of C fibers to innocuous cool stimuli. In humans, it has been demonstrated that normally innocuous cool stimuli evoke a sensation of burning pain during block of conduction in A fibers (Aβ and Aδ) (Wahren et al. 1989; Yarnitsky and Ochoa 1990). This implies that at least some heat-sensitive C nociceptors are excited by innocuous cold stimuli but this activity is inhibited by simultaneous activation of cold-specific receptors at the central level, and perhaps by cold-sensitive mechanoreceptors. Studies of cold-evoked responses of C nociceptors in rats and in monkeys are currently in progress and suggest that ~20% of C polymodal nociceptors in monkeys are excited by innocuous cold stimuli and have response thresholds between 25 and 30°C (unpublished observations). It is likely that at least one mechanism underlying cold hyperalgesia associated with large fiber neuropathy is the loss of inhibitory modulation at the central level provided by cold-sensitive A fibers (Ochoa and Yarnitsky 1994).

**Functional role of Aδ-nociceptors in cold pain sensation**

The contribution of cutaneous nociceptors to cold pain sensation cannot be assessed definitively without correlative behavioral and physiological measures. However, certain inferences can be made on the basis of previous psychophysical studies of the sensation of cold pain and on sensations evoked by activation of certain types of nociceptors. The sensation of pricking pain evoked by cold has been described by Lewis and Love (1926) in their early studies of vascular reactions of the skin to freeze injury. Lewis and Love reported that stimulus temperatures of −2.2°C and colder produced pricking pain. Wolf and Hardy (1941) found that the sensation of "pins and needles" often followed the aching pain produced by cold stimuli <12°C. It has been reported that cold stimuli that produce freezing of the superficial skin produce a sensation of pricking (Kreh et al. 1984). The sensation of pricking pain is thought to be signaled by activity in Aδ-nociceptors. In humans, the latency to the detection of "first pain" evoked by noxious heat was consistent with conduction latency of Aδ-fibers (Campbell and LaMotte 1983). Moreover, microneurography and intraneural microstimulation of identified cutaneous primary afferent fibers in humans demonstrated that electrical stimulation of nociceptive Aδ-fibers evoked a sensation of sharp pain, whereas stimulation of nociceptive C fibers produced a sensation of dull or burning pain (Ochoa and Torebjörk 1981, 1989). It is therefore likely that pricking pain produced by cold stimuli is mediated, at least in part, by excitation of Aδ-nociceptors.

C-fiber nociceptors may also contribute to the sensation of cold pain, because a portion of C nociceptors in animals (Bessou and Perl 1969; Georgopoulos 1976, 1977; Kumazawa and Perl 1977; LaMotte and Thalhammer 1982) and...
humans (Campero et al. 1996; Torebjörk 1974; Torebjörk and Hallin 1974) was excited by noxious cold. In preliminary studies of a small sample of C and Aδ-nociceptors in rat, we found that the response thresholds for cold stimuli and the responses evoked by suprathreshold stimuli did not differ from each other (Simone and Kajander 1996). This suggests that Aδ- and C nociceptors in rat hairy skin share a common functional role in cold nociception. However, functional differences between Aδ- and C nociceptors may occur in primates. On the basis of preliminary results of studies currently in progress, there appears to be a clear difference in response thresholds between Aδ- and C nociceptors: response thresholds for most Aδ-nociceptors were <0°C, whereas response thresholds of most C nociceptors were >0°C (Simone et al. 1994). This supports the notion that C nociceptors contribute to the sensation of dull pain produced by cold stimuli ≥0°C and that Aδ-nociceptors contribute to pricking pain evoked by cold stimuli <0°C and by skin freezing. Correlative psychophysical measures of the magnitude and quality of cold pain sensation are needed to determine the functional contribution of Aδ- and C nociceptors to sensation of cold pain.

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