Response of Cutaneous A- and C-Fiber Nociceptors in the Monkey to Controlled-Force Stimuli

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Slugg, R. M., R. A. Meyer, and J. N. Campbell. Response of cutaneous A- and C-fiber nociceptors in the monkey to controlled-force stimuli. J. Neurophysiol. 83: 2179–2191, 2000. The goal of this study was to determine the capacity of primary afferent nociceptive fibers (nociceptors) to encode information about noxious mechanical stimuli in primates. Teased-fiber techniques were used to record from 14 A-fiber nociceptors and 18 C-fiber nociceptors that innervated the hairy skin. Stimulus-response functions were examined with an ascending series of force-controlled stimuli. Stimulus-interaction effects were examined with use of a series of paired stimuli in which the interval between the stimulus pairs was varied systematically. Both A-fiber and C-fiber nociceptors exhibited a slowly adapting response to the stepped force stimuli. The response of the A fibers increased monotonically with increasing force, whereas the response of the C fibers reached a plateau at low force levels. The slope of the stimulus-response function for the A fibers was significantly steeper than that for the C fibers, and the total response was greater. The A fibers also provided more discriminative information regarding stimulus intensity. The C fibers demonstrated a significant fatigue in response when the interstimulus interval between the paired stimuli was ≤150 s, whereas the A fibers did not demonstrate a significant fatigue until the interstimulus interval was ≥30 s. This fatigue in response was not due to changes in tissue compliance. These results suggest that A- and C-fiber nociceptors have different mechanical transduction mechanisms. A-fiber nociceptors exhibit steeper stimulus-response functions and less fatigue than C-fiber nociceptors.

INTRODUCTION

The neurophysiological basis for pain from mechanical stimulation of normal or injured skin and in patients with nerve injury is not well understood. This is due in part to the lack of suitable mechanical stimulators for delivering controlled noxious stimuli. Many previous studies of nociception relied on hand-held stimuli such as forceps with strain gauges (Burgess and Perl 1967), arterial clamps (e.g., Willis et al. 1974), and von Frey probes (von Frey, 1896) to establish threshold for activation of nociceptors (e.g., Barasi and Lynn 1986; Bessou and Perl 1969; Kress et al. 1992; Lynn 1994; Meyer et al. 1981a; Perl 1968; Reeh et al. 1987; Tanelian and Beuerman 1984). Feedback controlled mechanical stimulator systems used in tactile research (e.g., Chubbuck 1966) can generate precise forces and/or displacements, but these devices usually have a very limited range of motion (e.g., 2 mm) and therefore do not produce pain or activate nociceptors unless the probe tip is very small or the underlying tissue is noncompliant (Green-span and McGillis 1991, 1994). To overcome these limitations, we recently developed a high-resolution mechanical stimulator for pain research based on a servo-controlled linear motor with a 20-mm range of motion. The position of the stimulator in space is controlled by a three-dimensional translation table (Schneider et al. 1995).

Only a few studies have investigated the stimulus-response functions of nociceptors to mechanical stimuli (Adriaensson et al. 1984; Campbell et al. 1979; Cooper et al. 1991, 1993; Garel et al. 1996; Handwerker et al. 1987; Khalsa et al. 1997; Koltzenburg and Handwerker 1994; van Hees and Gybels 1981). In this paper, we present the first systematic evaluation of mechanically-sensitive nociceptive afferents in monkey. We compare the stimulus-response functions of A- and C-fiber nociceptors to controlled-force stimuli and investigate whether the marked stimulus interaction that occurs when repeated heat stimuli are presented to the receptive field (e.g., LaMotte and Campbell 1978; Tillman 1992) also occurs for mechanical stimuli presented to a single location. Some results were presented previously in abstract form (Slugg et al. 1993, 1994).

METHODS

Neurophysiological preparation

A standard teased-fiber technique was used to record from single primary afferent nociceptors (e.g., Campbell and Meyer 1983). Briefly, monkeys were sedated with ketamine and then anesthetized to a level such that the corneal reflex was absent by intravenous administration of sodium pentobarbital (3 mg · kg⁻¹ · h⁻¹) and morphine sulfate (0.5 mg · kg⁻¹ · h⁻¹). Animals were intubated, and peak expired pCO₂ was maintained at 35–40 Torr using mechanical ventilation. The animals were paralyzed with pancuronium bromide (0.1 mg/kg) to minimize muscle artifacts during recordings as well as to facilitate respiratory control. The rectal temperature was controlled at 38°C by means of a circulating water heating pad. Five percent dextrose in 0.9% normal saline was administered intravenously in the course of the experiments to maintain hydration. Adequate depth of anesthesia was ensured by continuous monitoring of heart rate with an electrocardiogram. The heart rate was maintained throughout the experiment within 10% of the baseline heart rate that was recorded before any surgical stimulus. Any sudden increase in heart rate that was related temporally to a surgical or test stimulus was treated with an additional bolus of the intravenous anesthetics. When it became apparent that the animal was spontaneously breathing (~2–3 h after pancuronium bromide administration), the absence of motor responses to noxious stimuli was verified, and an additional bolus dose of pancuronium bromide administered.

The nerves used in this study were the medial antebrachial cutaneous and superficial radial on the forelimb and the sural, saphenous, and superficial peroneal on the hindlimb. None of the nerves had been used in previous studies, and the areas of the receptive fields were free from previous injury. A skin incision was made over the nerve of...
interest, the nerve was dissected free of connective tissue, and fascicles from the nerve were separated on a dissecting platform and placed on a gold electrode wire for extracellular recordings of action potentials from single nerve fibers. At the end of the recording session, the wound was irrigated repeatedly with saline solution and then sutured. Benzathine penicillin (450,000 U) was injected intramuscularly at the beginning of each experiment. Animal housing conformed to federal regulations and the facilities were accredited by the American Association for Accreditation of Laboratory Animal Care.

The analog action potential activity from the nerve filament was amplified and passed through a time-amplitude window discriminator. The discriminator delivered a pulse to the computer whenever a neural event met the waveshape criteria. Each discriminated action potential was time-stamped relative to stimulus delivery by the stimulus control software. To synchronize the neural activity with stimulus delivery, the conduction delay from the receptive field to the recording electrode was subtracted in the post hoc analysis. The conduction delay was determined by electrically stimulating the receptive field through a saline soaked cotton swab at twice the electrical stimulus threshold (Meyer et al. 1991).

Receptive fields were located by squeezing of the skin in the distribution of the nerve under study. Calibrated nylon monofilaments (von Frey type, Stoelting asthesiometer set) were used to locate sensitive spots in the receptive field and to determine their response thresholds. The boundary of the receptive field was determined with a suprathreshold von Frey filament. Radiant heat was used to test for thermal sensitivity in C-fiber nociceptors. All receptive fields were located on the distal hairy skin of either the upper or lower extremity.

Fibers were classified as nociceptors if they responded to pinching of the skin but did not respond to blunt pressure (2 N applied via a 1-cm-diam probe = 2.5 N/cm²) or light stroking. This criteria has been used to reliably identify A-fiber nociceptors in the past (e.g., Treede et al. 1995). No attempt was made to subclassify A fibers into type I and type II AMHs (Meyer et al. 1994; Treede et al. 1995) to avoid thermally induced sensitization. In a previous systematic study of AMH nociceptors from the hairy skin of monkey (Treede et al. 1998), very few type II AMHs had thresholds to mechanical stimuli in the range of the nociceptors reported in this study. Thus it is likely that most of the A-fiber nociceptors studied here were type I AMHs. Mechanical thresholds were determined with a set of von Frey filaments (force range: 0.3–168 mN; pressure range: 1.2–7.8 bar, diameter range: 178–523 μm). A-fiber nociceptors were studied only if their von Frey threshold was not lower than 1.9 bar (5 mN) to eliminate the possible inclusion of low-threshold slowly adapting mechanoreceptors in the study. C fibers with thresholds <1.9 bar were included if they had a heat response typical of nociceptors (i.e., heat threshold <49°C).

**Mechanical stimulator**

We developed a computer-based electromechanical stimulator system suited for neurophysiological and psychophysical studies of pain (Schneider et al. 1995). The stimulator consists of a servo-controlled linear motor capable of generating 10 N of force over a 22-mm range. Forces collinear to the interchangeable probe tip were calculated by combining the signal from three load cells (1.25-mN resolution, 2.5-N range) arranged in an equilateral triangle (see Schneider et al. 1995). Probe position was measured with an optical encoder (1-μm resolution, 25-mm range). Stimulus probes of differing size and shape were placed in a receptacle centered at the end of the motor’s shaft. The probe was positioned orthogonal to the skin by means of an articulated joint. A microprocessor-based digital control system allowed feedback control to be switched between force or position at the 1-KHz update rate. An IBM-compatible computer was used to command stimulus paradigms and to display real-time motor performance and neural spike-train data.

**Mechanical stimulation protocols**

**STIMULUS WAVEFORM.** All studies were conducted using a 0.4-mm-diam, stainless steel, cylindrical probe with a flat, right-angled tip. A typical stimulus waveform is shown in Fig. 1. Each stimulus trial started with a 4-s prestimulus period during which the probe was moved under position control from a position ~1 mm above the skin surface to a position at which skin contact was made as detected by an increase in the measured probe force. At this point, the stimulator control automatically switched to force control, and a 5-mN resting force was applied for the remaining time in the prestimulus period. The 3-s stimulus period consisted of a 0.2-s force-controlled ramp to the desired force and a 2.8-s constant force interval. A gradual creep in probe position typically was seen during the constant force stimulus (see Fig. 1, top). At the end of the stimulus, the probe was withdrawn in position-control mode to a position 1 mm above the skin surface.

Initial studies revealed that a rise period of 0.2 s resulted in the optimum physical performance of the motor (i.e., minimum overshoot, minimum oscillations of waveform) over a range of force intensities (0–200 mN), physical indentations (0–5 mm), skin types, (e.g., glabrous, hairy), and locations (e.g., volar forearm, digit). A controlled-force stimulus as opposed to a controlled position stimulus was chosen for several reasons. First the adequate stimulus for activating nociceptors is thought to be related to the applied force (e.g., Garell et al. 1996). Second, the location of the skin surface can vary between trials. Third the compliance of the tissue may change with repeated trials. And fourth, a small cardiovascular and respiratory related movement can be present.

Probe position and force data were recorded routinely in the data file at the beginning and end of the 3-s stimulation period for subse-

![Fig. 1. Typical stimulus waveform. Position (top) and force (bottom) measurements during a typical 80-mN stimulus applied to the volar forearm of the monkey. Each stimulus trial started with a 4-s prestimulus period during which time the probe approached the skin under position control, and skin contact was detected (vertical dash line). At this time, feedback switched automatically to force control, and skin contact was maintained with a 5-mN applied force. The three-second stimulus period consisted of a 0.2-s force-controlled ramp to the desired force, and a 2.8-s maintained force. At the end of the stimulus, the probe was withdrawn to a position ~1 mm above the skin surface.](http://jn.physiology.org/)

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forces higher than 200 mN were not used to minimize the potential for stimulus interaction. A 2-min interstimulus interval was used to minimize stimulus interaction. Frey stimulation. Some units were studied with both stimulus ranges. A lower force range was used for units that had a lower threshold to von Frey stimuli of 20–100 mN or 40–200 mN by 40-mN increments. In general, the stimulus minus the probe position at the beginning of the 3-s stimulus period for a given force was defined as the probe position at the end of the stimulus response paradigm. Two or more spots in each receptive field were investigated systematically by delivering pairs of stimuli to a given location of a given fiber. Likelihood ratio tests were used to test the hypothesis that the average curves for the A and C fibers are the same.

**RESULTS**

Fourteen A-fiber nociceptors and 18 C-fiber mechanoheat-sensitive nociceptors (CMHs) were included in this study. All receptive fields were located on the hairy skin of the forelimb or hindlimb. The static properties of these afferents are listed in Table 1.

**Stimulus response functions**

An ascending series of constant force stimuli was used to assess the stimulus response function of the nociceptors. The responses of a typical A- and C-fiber nociceptor (both with a von Frey threshold of 5 mN) to this ascending series are shown in Fig. 3. Both fibers exhibited a slowly adapting response to the stimulus. Although the C fiber had a larger response than the A fiber to the 20-mN stimulus (i.e., 5 action potentials for the C fiber vs. 2 action potentials for the A fiber), the A fiber responded more vigorously to the 100-mN stimulus than did the C fiber (43 vs. 15 action potentials). The total evoked response of the A-fiber nociceptor increased monotonically as the stimulus force increased (Fig. 3B). In contrast, the total response of the C fiber was not consistently affected by the stimulus force.

**Statistical analysis**

For data analysis, four different measures of response were used: the response during the 3-s duration of the stimulus, the maximum instantaneous frequency, and the mean instantaneous frequency during the first 1 s. The data comprised repeat measures of these four parameters at five forces between 20 and 100 mN (or between 40 and 200 mN). Some fibers were recorded more than once with the stimulator at a different location. For each response variable, a random-effects linear regression (Diggle et al. 1994) was used to estimate and compare the typical force response function for the A and C fibers. The average response was modeled as a quadratic function of force with intercept and slope that varied across fibers and across the repeated tests at different locations of a given fiber. Likelihood ratio tests were used to test the hypothesis that the average curves for the A and C fibers are the same.

### Table 1. Static properties of nociceptors in this study

<table>
<thead>
<tr>
<th></th>
<th>A Fibers</th>
<th>C Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conduction velocity, m/s</td>
<td>36 ± 4 (13–60)</td>
<td>0.91 ± 0.05 (0.6–1.2)</td>
</tr>
<tr>
<td>Mechanical threshold, mN</td>
<td>11 (5–65)</td>
<td>5 (2–65)</td>
</tr>
<tr>
<td>Mechanical threshold, bar</td>
<td>2.8 (1.9–5.1)</td>
<td>1.9 (1.3–5.1)</td>
</tr>
<tr>
<td>Receptive field size, mm²</td>
<td>75 ± 22 (3–314)</td>
<td>31 ± 4 (6–71)</td>
</tr>
<tr>
<td>Number of sensitive spots</td>
<td>11.9 ± 2.3 (3–31)</td>
<td>7.4 ± 0.8 (3–14)</td>
</tr>
</tbody>
</table>

Values are means ± SE with ranges in parentheses. n = 14 for A fibers and n = 18 for C fibers.
The evoked response of the C fiber reached a plateau for stimuli of $\geq 40$ mN (Fig. 3D).

The stimulus response functions for all of the units in this study are shown in Fig. 4. For some fibers, a stimulus response function was obtained at different locations in the receptive field. Receptive field locations with relatively low von Frey mechanical thresholds (2–12 mN; 8 A fibers, 11 C fibers) were studied with stimuli ranging from 20 to 100 mN (20-mN increments), whereas locations with higher mechanical thresholds (5–65 mN; 9 A fibers, 9 C fibers) were studied with stimuli ranging from 40 to 200 mN (40-mN increment). The intention was to use a stimulus range such that the lowest stimulus intensity was near the von Frey threshold.

There are several ways that neural responses in nociceptors may provide information to the CNS. Because it is not certain which feature of responsiveness is relevant, four measures were analyzed (Fig. 5): number of action potentials during the 3-s stimulus, number of action potentials during the first 1 s of the stimulus, average instantaneous frequency during the first 1 s of the stimulus, and peak instantaneous frequency. Regardless of which response parameter is considered, the magnitude of responses of A-fiber nociceptors was significantly greater than that of the C fibers (all $P$ values $<0.001$). This difference was associated with a significantly greater slope of the stimulus-response function of the A-fiber nociceptors (Fig. 5). Of note, the response of the C fibers reached a plateau, whereas the A fibers had a monotonically increasing response.

We sought to determine which of the four response measures would provide the most discrimination between different force levels. As a measure of discrimination, we determined the distance in standard deviation units between the responses to the middle (60 or 120 mN) and highest (100 or 200 mN) force. A discrimination score for each response measure was determined by calculating the difference in the response to these two forces for a given fiber and then dividing the mean of this difference across fibers by the standard deviation of this dif-
ference distribution. As indicated in Table 2, for a given force series (i.e., a column in Table 2), the discrimination scores were similar (i.e., within 35% of each other) for each response measure except for the peak instantaneous frequency measure whose score was substantially lower than the others in two of the four instances. The discrimination scores for the A fibers were substantially higher than the scores for the C fibers. This suggests that A-fiber nociceptors provide more discriminative information to the CNS than C-fiber nociceptors regarding the magnitude of intense mechanical stimuli over this force range.

Adaptation of response

The response of both the A fibers and the C-fiber nociceptors adapted during the 3-s-duration stimulus. To investigate adaptation, we plotted the instantaneous frequency as function of time (Fig. 6) for the first conditioning stimulus in the stimulus-interaction paradigm. The response to this stimulus was investigated because the stimulus was always above threshold and because it was always preceded by a 10-min stimulus-free interval so that stimulus interaction was negligible. As illustrated by the examples in Fig. 6, peak instantaneous frequency usually occurred at the beginning of the response, and the response adapted such that the instantaneous frequency at the end of the stimulus was significantly lower than at the beginning. The discharge pattern for many fibers (e.g., Fig. 6, A and B) contained two regions with different adaptation rates, a period of fast adaptation that occurred during the first part of the stimulus followed by a period of slow adaptation. This suggests that two different mechanisms are involved in the adaptation phenomena, one with a short time constant and one with a long time constant. The curves in Fig. 6 (—) were obtained by fitting the data assuming a two-compartment, exponentially decaying response. Indeed, the responses in Fig. 6, A and B, were fitted well by a curve with dual exponential decay.

FIG. 4. Stimulus-response functions for all of the A- and C-fiber afferents. Total evoked response is plotted as a function of stimulus intensity. In general, the response of the A fibers increased monotonically with increasing force, whereas the response of many of the C fibers reached a plateau level before the end of the ascending sequence. In addition, the A fibers tended to have a higher evoked response than the C fibers. Data are grouped according to the fiber type (top = A fibers, bottom = C fibers) and the stimulus sequence used (left = 20–100 mN ascending sequence, right = 40–200 mN ascending sequence). Heavy curve is the mean for each set of curves. A: A fibers that received 20- to 100-mN range of stimuli (n = 11). B: A fibers that received 40- to 200-mN range of stimuli (n = 17). C: C fibers that received 20-to 100-mN range of stimuli (n = 22). D: C fibers that received 40- to 200-mN range of stimuli (n = 20). Different scales are used for the A- and C-fiber responses. AP, action potentials.
decay. In contrast, the responses in Fig. 6, C and D, were fitted well by a curve where the two time constants were almost identical, suggesting that a single exponentially decaying process probably was involved for these fibers.

For most fibers, the response ended at the end of the stimulus. However, one C fiber showed an afterdischarge that lasted for $\approx 7$ s.

**Tissue compliance**

As the stimulus force increased, the position of the probe also changed. The skin displacement for a given stimulus force was defined as the probe position at the end of the stimulus minus the probe position at the start of the stimulus. The amount of displacement is related to the compliance of the tissue. For low compliance tissue (e.g., over bone), displacements of $< 1$ mm were observed at the highest forces. In contrast, for high compliance tissue (e.g., over muscle), displacements of $> 2$ mm were frequently observed at the highest forces. To estimate compliance, we computed the slope of the displacement versus force curve over the force interval of 40–80 mN. These forces were chosen because they are common to both stimulus ranges used in these experiments. The distribution of compliance is shown in Fig. 7A. A- and C-fiber nociceptors were found in both compliant and noncompliant tissue.

A mechanical stimulus to the skin leads to compressive, shear and tensile stresses in the vicinity of the nociceptor terminals. Although it is not clear which of these stresses is most important for activating nociceptors, Khalsa et al. (1997) suggested that skin tension is a critical factor. We did not control tension directly. However, the amount of tension is related directly to the amount of displacement that is produced for a given force. In very compliant skin (e.g., over muscle), there is much more displacement, and therefore more tension,

![Graphs showing the comparison of different measures of evoked response.](http://jn.physiology.org/)

**TABLE 2. Comparison of discrimination scores for different response measures**

<table>
<thead>
<tr>
<th>Response Measure</th>
<th>A Fibers 20–100 mN</th>
<th>A Fibers 40–200 mN</th>
<th>C Fibers 20–100 mN</th>
<th>C Fibers 40–200 mN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action potentials in 3 s</td>
<td>1.18</td>
<td>1.74</td>
<td>0.49</td>
<td>0.90</td>
</tr>
<tr>
<td>Action potentials in 1 s</td>
<td>1.08</td>
<td>1.98</td>
<td>0.41</td>
<td>0.65</td>
</tr>
<tr>
<td>Mean instantaneous frequency</td>
<td>0.84</td>
<td>1.87</td>
<td>0.61</td>
<td>0.71</td>
</tr>
<tr>
<td>Peak instantaneous frequency</td>
<td>0.92</td>
<td>0.87</td>
<td>$–0.19$</td>
<td>0.94</td>
</tr>
</tbody>
</table>
than in noncompliant skin (e.g., over bone). To determine whether the neural response was a function of compliance, we grouped the data into two groups: data from high-compliance versus low-compliance tissue. The criteria level was set at a compliance level of 10 \( \mu \text{m/mN} \), which divides the population approximately in half (see Fig. 7A). The average responses of A fibers tended to be higher in low-compliance tissue (Fig. 7B). In contrast, the average responses of C fibers were significantly higher (\( P < 0.05 \), t-test on sum of response to force sequence) in high-compliance tissue (Fig. 7C). These results are consistent with the conclusion that tension may be a greater factor for C-fiber nociceptors than for A-fiber nociceptors.

Stimulus interaction

A significant fatigue in response is observed when repeated heat stimuli are applied to C-mechanoheat nociceptors (e.g., LaMotte and Campbell 1978; Tillman 1992). To see if a similar phenomenon existed for mechanical stimuli, a stimulus-interaction paradigm was designed to examine the effect of a conditioning stimulus on the response to a test stimulus delivered at specified intervals (see Fig. 2B). The responses of a typical A- and C-fiber nociceptor to this stimulus-interaction paradigm are shown in Fig. 8. For the A fiber, the response to each of the conditioning stimuli was similar both in terms of the time course and magnitude of evoked response (Fig. 8A). In addition, interaction effects (fatigue) were present only when the interstimulus interval (ISI) was \( \leq 30 \) s.

As with the A fiber, the response of the C fiber to each of the conditioning stimuli was similar in terms of both time course and magnitude of the evoked response (Fig. 8B). However, stimulus-interaction effects were present for all ISIs \(< 300 \) s.

Similar results were obtained from the population of A and C fibers that were tested with the stimulus-interaction paradigm. The response to the conditioning stimulus did not vary significantly with time during the stimulus-interaction protocol for either the A or the C fibers. Thus the 10-min interval between conditioning-test pairs provided sufficient time for complete recovery of the response.

To combine data across fibers, the amount of fatigue for each ISI was determined by computing the ratio of the response to the test stimulus divided by the response to the immediately preceding conditioning stimulus. This interaction ratio was dependent on the ISI for the A fibers (Fig. 9, top) and for the C fibers (Fig. 9, bottom). The maximum interaction (i.e., smallest interaction ratio) was observed for the shortest ISI (i.e., 15 s), and the amount of interaction decreased (i.e., the interaction ratio approached unity) as the ISI became longer. To avoid stimulus-interaction effects, ISIs \( > 30 \) s for the A fibers and 150 s for the C fibers are required. These restrictions apply
to mechanical stimuli applied to a single locus in the receptive field. The interstimulus interval can be considerably shorter if the mechanical stimuli are not applied to the same site in the receptive field (Slugg et al. 1995).

Because the stimulus-response functions (Fig. 5) were obtained with an ISI of 120 s, some stimulus interaction was present for the C-fiber data. Therefore the possibility exists that the difference in slope between the A- and C-fiber nociceptors described earlier was solely due to the more prominent fatigue evident in the C fibers for the 2-min ISI used in the ascending series. In Fig. 10, we compare the total responses of the A and C fibers to the different forces in the ascending series. On this figure, we also plot the response that was obtained to the first stimulus in the stimulus-interaction paradigm; this stimulus was equivalent to the highest stimulus in the ascending series and was presented 10 min after the completion of the ascending series. This response corresponds to the expected response to the ascending series if stimulus interaction did not occur. As expected, the mean response of the C fibers to this stimulus was significantly lower during the ascending series compared with 10 min later. Thus the fatigue in the C fibers tended to flatten their stimulus response curve. However, this fatigue was not sufficient to make up for the marked difference in slope between the A- and C-fiber stimulus response functions. A new discrimination score was computed for the total response to the 3-s stimulus using the response to the first conditioning stimulus (instead of the response to the last stimulus in the ascending series as done in Table 2). The discrimination scores for both the A fibers (1.31 for low series, 1.93 for high series) and the C fibers (1.00 for the low series, 1.23 for the high series) improved (see for comparison first row in Table 2). However, the discrimination scores for the A fibers were still better than for the C fibers.

A proportion of the decrease in responsiveness of both the A- and C-fiber nociceptors appeared to be due to a decrease in the high-frequency discharge that occurred at the onset of the stimulus (e.g., Fig. 8). To quantitate the amount of fatigue that was observed in the first, high-frequency part of the stimulus and the second, low-frequency part of the stimulus, the 3-s duration stimulus period was divided into the initial 1-s interval and the subsequent 2-s interval. The evoked response in each interval was calculated for the conditioning stimulus and the test stimulus. An interaction ratio (as described in the preceding text) was computed for each stimulus part. For the A fibers, the interaction ratio at an ISI of 15 s for the first 1 s (0.68 ± 0.03; mean ± SE) was significantly lower than the interaction ratio for the last 2 s (0.81 ± 0.03, P ≤ 0.01, paired t-test). In contrast, no significant difference was observed for the C fibers.

The stimulus interaction does not appear to be due to changes in biophysical properties of the skin because displacements for a given stimulus were similar throughout the testing. These data strongly suggest that the prominent interaction effects are not due to a change in the transmission of the stimulus because the biophysical properties of the skin do not change throughout the protocol.

**DISCUSSION**

The objective of this study was to characterize the responses of A- and C-fiber nociceptors in monkey to controlled-force stimuli applied to their receptive fields. We found that the response to stepped force stimuli for both types of fibers is slowly adapting, the stimulus response functions of A-fiber nociceptors are steeper than those of C-fiber nociceptors, greater discriminable information about stimulus intensity was...
provided by the A fibers, and fatigue effects are greater in C-fiber nociceptors than in A-fiber nociceptors.

### Slowly adapting response

Both A- and C-fiber nociceptors exhibited a slowly adapting response to stepped force stimuli. For many fibers, the adaptation appeared to have two components: a fast adaptation during the first part of the stimulus followed by a slow adaptation for the rest of the stimulus. This suggests that two different mechanisms may account for the adaptation. For some of the fibers, the high-frequency discharge occurred during the 200-ms initial ramp phase of the stimulus after which a marked decrease in discharge frequency was observed. Thus one explanation for the fast adaptation at the onset of the stimulus could be that the initial response of the nociceptor is dependent on the rate-of-change of the stimulus, a well-known property of low-threshold mechanoreceptors. Unfortunately, the paradigms used in this study did not allow us to examine this possibility systematically. Garell et al. (1996), in their study of feline nociceptors, did not observe an adapting response similar to what we observed in the present study. Although species differences might play a role, differences in experimental design such as ramp rate and ISI likely account for this discrepancy.

Rate sensitivity fits anecdotally with the psychophysical observation that a slap stimulus to the skin is painful, whereas comparable forces applied gradually do not necessarily induce pain. In addition, Koltzenburg and Handwerker (1994) found that nociceptor response increased with the velocity of a projectile stimulus. Similarly C-fiber nociceptors (e.g., Meyer and Campbell 1981b) and innocuous thermal receptors (e.g., Konietzny and Hensel 1977; Molinari and Kenshalo 1977) exhibit both a rate- and temperature-sensitive response to thermal stimuli.

### Stimulus response functions

An important finding of the present study was the marked difference in stimulus response functions between A- and C-fiber nociceptors. The A- and C-fibers were similar with regard to responses to low-intensity stimuli. However, the A fibers had a relatively steep stimulus response function, whereas the response of many of the C fibers reached a plateau at the higher stimulus intensities. A similar saturation in the stimulus-response functions of C-fiber nociceptors has been noted in rat (Khalsa et al. 1997) and cat (Garell et al. 1996). A discriminative analysis that considered both the slope of the stimulus-response function and the standard deviation of the response revealed that the A-fiber nociceptors provide more information about noxious mechanical stimuli to the CNS than individual C-fiber nociceptors. Garell et al. (1996) came to similar conclusions in a study of feline nociceptors. A direct comparison of the magnitude of the response and
shape of the stimulus-responses functions between this and other studies is difficult because of different stimulus conditions. For example, in the Garell et al. (1996) study, the ISI was 7–8 s in contrast to the 2-min ISI used for comparable experiments in this study. In addition the stimulus duration and force range were greater in the Garell study, but the displacement range was restricted to 2 mm. It would appear that response saturation occurred with much higher forces in the Garell study. Whether this is a species difference or relates to stimulus variables remains an open question.

**Tissue compliance**

Both A- and C-fiber nociceptors innervate tissues that vary widely in compliance. Highly compliant tissue is present when receptive fields are located in skin that overlies muscle, for example, and relatively noncompliant tissue is present when receptive fields overlie bone. By studying the stimulus-response functions of nociceptors in compliant and noncompliant tissue, we get some hint as to the importance of different mechanical stresses in generating neural activity. The A-fiber nociceptors had a tendency to respond more vigorously when their receptive fields were in regions of noncompliant tissue. In contrast, C-fiber nociceptors responded significantly more vigorously when their receptive fields were in areas of compliant tissue (see Fig. 7). These data support the hypothesis that A- and C-fiber nociceptors have preferential sensitivities to different types of stresses. The relative role of compressive versus tensile and shear stresses varies with tissue compliance. For example, in compliant tissue, the amount of displacement for a given force applied orthogonal to the skin is greater. Thus tensile stresses are greater than in relatively noncompliant tissue where the same force leads to much less displacement. In noncompliant tissue, compressive stress likely plays a greater role in relation to the total stress generated by the stimulus. Our observations that A fibers responded more in noncompliant tissue and C fibers responded more in compliant tissue suggest that compressive stress plays a more important role for A-fiber nociceptors, whereas tensile stresses play a more important role for C-fiber nociceptors. This is consistent with the observation of Khalsa et al. (1997) that C-fiber nociceptors respond better to tensile loading than to compressive loading.

**Stimulus interaction**

Fatigue (the decrement in response that results from a prior stimulus) was found in this study to be a property of both the A- and C-fiber nociceptors. Fatigue was more prominent in the C fibers than in A fibers. In addition, the time for full recovery of the initial response was longer for C fibers (5 min) than for A fibers (1 min). This fatigue could result from changes in stimulus transmission, stimulus transduction, spike initiation, and spike propagation.

**Transmission** refers to the transfer of energy from the stimulus contact point (the skin surface in this instance) to the...
rical stimuli found in this study (0.70 action potentials, study. The initial response to the heat stimulus (16.9 the interaction to mechanical stimuli observed in the present Fig. 11, the interaction to heat stimuli from a previous study pronounced than the fatigue to repeated mechanical stimuli. In receptor membrane. One line of evidence that suggests that physical energy of the stimulus into an ionic current in the transduction refers to the conversion of the stimulus to the next may modify stimulus transmission and account for the interaction phenomena. This explanation of course would not explain the differences between A and C nociceptors (assuming that the terminals were similarly located in the skin). However, no change in tissue compliance was evident during these experiments, and therefore a change in stimulus transmission does not appear to account for the fatigue.

Fatigue also could result from changes in the stimulus transduction apparatus. Transduction refers to the conversion of the physical energy of the stimulus into an ionic current in the receptor membrane. One line of evidence that suggests that transduction may be the site of fatigue is the observation that fatigue to repeated heat stimuli also is present but is more pronounced than the fatigue to repeated mechanical stimuli. In Fig. 11, the interaction to heat stimuli from a previous study that used a similar paradigm (Tillman 1992) is compared with the interaction to mechanical stimuli observed in the present study. The initial response to the heat stimulus (16.9 ± 5.8 action potentials, n = 13) was not significantly different from the initial response to the mechanical stimulus (18.3 ± 1.1 action potentials, n = 33). Of particular note is the observation that the interaction ratio for heat stimuli presented 30 s apart (0.39 ± 0.03) was significantly smaller than that for mechanical stimuli found in this study (0.70 ± 0.03, P = 0.001).

Another potential site for fatigue is the point of spike initiation. Cross-modality interaction effects would support this hypothesis. Preliminary studies in our laboratory have demonstrated that mechanical stimulation lead to a reduction in the response to a subsequent heat stimulus (Peng et al. 1997).

Another possibility is that alteration in the propagation of the action potential could account for the temporal interaction effects. A slowing of propagation after a spike train is readily apparent in C-fiber nociceptors (e.g., Meyer et al. 1985) and has been used by microneurographers to mark fibers (e.g., Schmelz et al. 1995). Indeed, a failure of propagation has been reported in A- and C-fiber nociceptors when the parent axon was stimulated electrically at relatively high frequencies (50 Hz for A fibers, 10 Hz for C fibers) for relatively long durations (i.e., 20 s) (Raymond et al. 1990). Recovery of propagation took 3–60 s. Although peak frequencies reached these levels in this study, they were not maintained for long durations, and thus propagation failure of the parent axon probably is not a factor for the fatigue to repeated mechanical stimuli. Whether propagation failure occurs in the fine branches of the terminal arborization remains to be determined.

We wondered whether the prominent fatigue in C fibers could account for the differences in stimulus-response functions between the A and C fibers because the time between stimuli for the stimulus response protocol was only 2 min, which is not long enough for full recovery in the C fibers. In other words, had the stimuli been presented with a sufficiently long ISI to allow full recovery of response in both the A and C fibers, perhaps the slope of the stimulus response functions would not have been different. Because fatigue is related to the intensity of the preceding stimulus (LaMotte and Campbell 1978; Peng et al. 1997), the fatigue for an ascending series is greatest at the higher force levels. This would tend to flatten the stimulus-response curves for the C fibers. However, even if the response of the C fibers to the ascending series was replotted to incorporate fatigue (for example, by using the response to the maximum stimulus presented at a 10-min ISI), the stimulus-response function still would plateau at higher stimulus intensities, and the response still would be lower than that obtained for A fibers (Fig. 10).

Role of nociceptors in pain sensation

For heat stimuli, the threshold for activation and the response to suprathreshold stimuli of C-fiber nociceptors closely parallels the pain threshold and stimulus response function for human subjects (e.g., LaMotte and Campbell 1978; Meyer and Campbell 1981a). In contrast, for mechanical stimuli, the threshold for activation of C fibers is significantly lower than the threshold for pain (Cooper et al. 1993; Greenspan and McGillis 1991), and the response of many of the C fibers has reached a saturation level at force levels that are below the pain threshold. For example, the 200-mN mechanical stimulus in this study produces a mean response of 16 action potentials in the C-fiber nociceptors, but this stimulus is below the pain threshold in humans (>400 mN) (Greenspan and McGillis 1991). In contrast, an initial 45°C 3-s laser thermal stimulus evokes a weaker discharge (mean = 10 action potentials) in C-fiber nociceptors (Campbell and Meyer 1983), but this stimulus reliably induces a distinct pain sensation (LaMotte and Campbell 1978). What accounts for this disparity?

Adriaensen et al. (1984) suggested that the concurrent activation of low-threshold mechanoreceptors (LTMs) centrally suppressed the pain that would otherwise be evoked by activity in the nociceptors. Heat stimuli hurt more because no coactivation of LTMs occurs with a heat stimulus.

Another possible explanation relates to the observation that the laser heat stimulus activates nociceptors over an 8-mm diameter region, whereas the number of nociceptors activated by the punctate mechanical stimulus is considerably less. The disparity may relate to the psychophysical property of spatial...
summation which has been shown to be important for heat pain sensation (Douglas et al. 1992; Price et al. 1989).

Another possibility is that mechanically sensitive afferents are not concerned with pain sensation (Meyer et al. 1994). Garell et al. (1996) found that nociceptors with high mechanical thresholds (i.e., mechanically insensitive afferents, MIAs, von Frey threshold >6 bar) showed less response saturation and appeared to have the capacity to encode unambiguously mechanical stimuli into the noxious range. However, in a study of oral mucosal afferents in goat (Cooper et al. 1991), a group of nociceptors with especially high mechanical thresholds did not encode the magnitude of pressure stimuli. MIAs were not encountered in the present study because an electrocortaneous search strategy (Meyer et al. 1994) was not employed and intense mechanical stimuli were not used for searching.

In this study, mechanical stimuli were delivered to the most sensitive region within the receptive field with a small, punctate probe. A saturation in the stimulus response function of C-fiber nociceptors may not occur if the same stimuli were presented to less-sensitive areas of the receptive field or if larger probe diameters were used. Thus the response of the C-fiber population may not exhibit saturation until much higher force levels if spatial recruitment is taken into account. Future studies are needed to determine the stimulus-response function of nociceptors for stimuli presented to any position within the receptive field.

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REFERENCES


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