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RESEARCH ARTICLE

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Recordings of polymodal single C-fiber nociceptive afferents following mechanical and argon-laser heat stimulation of human skin

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Abstract Recordings were made in the peroneal nerve of healthy volunteer subjects from C-mechano-heat (CMH) nociceptors (*n*=25) with their receptive fields in the skin on the dorsum of the foot. The investigation focused on afferent single C-fiber activity induced by short (200 ms) high-intensity argon-laser light pulses projected to localized spots of the skin. Cutaneous heat stimulation with the argon laser, 2–3 times the activation threshold, induced inter-burst spike frequencies in the nerve, reaching 50 Hz, while mechanical stimulation 10–20 times threshold only evoked frequencies reaching 10 Hz. The decrease in conduction velocity of action potentials in the C-fiber afferents following mechanical and heat stimulation was closely related to the degree of activation. Following a laser pulse of 200 ms, a spike pattern with highly reproducible inter-spike intervals was evoked with a fast saturation. On the contrary, a high variability in the number of action potentials evoked by both heat and mechanical stimuli was found, depending on the location of stimuli within the receptive field. A relation between the conduction velocity and the peak firing within the spike train following laser stimulation was detected. Heat and mechanical stimulation activated single C-fibers in matching spots within the same skin areas, in line with the assumption that the two modalities in the CMH-fibers share matching morphological cutaneous substrates. No correlation was found in thresholds or excitability to mechanical and heat stimulation, respectively. This suggests that subsets of receptors exist within nerve endings of the cutaneous receptive fields, with the ability to generate action potentials independent of heat and mechanical stimuli. Unexpectedly, no signs of sensitization or other inflammatory responses were observed after repeated laser pulses; on the contrary, a rapidly developing fatigue was observed when single spots were repeatedly stimulated. However, no fatigue was observed if neighboring spots were stimulated, indicating a localized generator of the fatigue. In each subject, a good correlation was observed between the reported pain sensation and the activity evoked in the afferent C-fibers by the laser. However, the magnitude of the reported pain sensation to comparable degrees of C-fiber activation showed a high variability between different subjects. A fairly good subjective estimate of the afferent-fiber activation was observed when skin spots from 3- down to 1-mm diameter were stimulated. In a few individuals, no painful sensation was reported when the stimulated spots were reduced to 1-mm diameter, despite the occurrence of multiple spikes in single C-fiber afferents, amplifying the importance of spatial summation in the perception of pain.

Key words C-fiber · Pain · Skin · Argon laser · Psychophysics

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Introduction

Intra-neuronal recordings from fibers of cutaneous mechano-heat (CMH)-nociceptors in man have shown different categories with respect to their activation by heat, mechanical, or chemical stimulation. In man, approximately 50% of them consist of polymodal fibers (Schmidt et al. 1995). Populations of nociceptors with more specific sensitivity to either mechanical stimuli or heat as well as silent receptors have been described under normal conditions. The receptive field characteristics are well described in mechanical stimulation of polymodal nociceptors (Torebjörk and Hallin 1974a; Treede et al. 1992). Estimates of the extent of the receptive fields to noxious heat and mechanical stimulation of the CMH-nociceptors have previously been investigated and found to coincide in experimental animals (Iggo and Ogawa 1971; Treede et al. 1990). However, the exact characterization of the field for heat activation in the polymodal C-fiber afferents have not been done thus far in human subjects.

The laser technique has been described as a good tool for studying peripheral heat-sensitive nociceptors (Bromm et al. 1984; Arendt-Nielsen and Bjerring 1988). The first method introduced was the CO_2 laser (Mor and Carmon 1975) followed by the argon laser (Arendt-Nielsen and Bjerring 1988). Both methods were used for objective as well as subjective pain measurements, and the specificity of the former was tested with microneurography (Bromm et al. 1984). The advantage of the CO_2 laser is a low reflection of light from the skin (Bargeron et al. 1981), disadvantages are that the infrared light is absorbed in superficial skin layers (Cummins and Nauenberg 1983) and that wavelengths of the CO_2 laser cannot be transmitted through an optical fiber (Bourgelais and Itzkan 1983). Argon-laser light penetrates deeper into the skin and is effectively absorbed by haemoglobin (Parrish and Anderson 1983; Cummins and Nauenberg 1983), which has the advantage of a more localized activation of nociceptors with lower energy and, hence, a smaller risk of inducing skin damage than the CO_2 laser. Disadvantages with the argon laser is the high reflection from the skin (Parrish and Anderson 1983; Arendt-Nielsen and Bjerring 1988) and that it can not be used to activate nociceptors in dark skin, which is not the case with the CO_2 laser.

So far, the neuronal responses in CMH-nociceptors to high-intensity argon-laser stimulation has not been evaluated in man. The possibility of focussing the light onto small and well-characterized spots of skin makes the method a possible candidate for mapping heat receptive fields. Thus, the aim of this study was to investigate human CMH-nociceptor activity in the peripheral nerve following argon-laser stimulation. The laser method proved to be able to characterize the distribution of heat-sensitive spots within electrically and mechanically characterized receptive fields of single C-fiber afferents. Psychophysical pain estimates of different intensities and different skin areas were also possible to quantify with the use of the argon laser. Data in the present study have been presented in a preliminary form (Olausson 1996).

Materials and methods

Subjects

Healthy Caucasian voluntary subjects, ten female and 15 male, aged 22–43 years were included in the study after informed consent. The study was performed according to the Declaration of Helsinki, and the study protocol was accepted by the local ethics committee.

Experimental procedure

The subjects laid supine on a comfortable bed. To prevent damage to the eyes from the argon laser, all people in the investigation room wore protective glasses during the experiment. A thermocouple was applied on the skin on the investigated right foot. The location of the peroneal nerve distal to the knee joint and behind the fibula head was localized by transcutaneous electrical stimulation. After localizing the nerve, an uninsulated tungsten micro electrode (used as reference) was positioned above the bone of the fibula head and within 2–3 cm from the recording electrode in the nerve. An insulated tungsten micro electrode with a 0.2-mm diameter shaft and tip diameter of less than 1 μm and typical impedances of 0.5–4 MOhm at 1000 Hz (Frederic Haer, USA) was then positioned subcutaneously and electrically stimulated with low voltage pulses (1 Hz, 0.2 ms, 0.5–1.5 V). With this electrode, recordings of single-unit C-fiber activity were made when the peroneal nerve was reached. Subjects were instructed to give a sign if a projected sensation into the skin of the foot or distal leg was evoked by the electrical pulse. If, in a cutaneous fascicle, activity in the nerve could be recorded from the electrode by stroking the skin. By arousing the subject, sympathetic efferent activities could also be observed. The receptive field for the respective unit was detected by giving transcutaneous electrical stimulation (0.3 Hz, 0.5 ms, 50–150 V) to the skin area. If an action potential with stable latency was evoked, two uninsulated tungsten electrodes (0.2-mm diameter) were positioned intracutaneously 5–10 mm from each other and electrically stimulated (0.25 or 0.33 Hz, 0.5 ms) with a constant current stimulator (Grass S88, USA).

Recording procedure

The nerve signal was amplified 50 000–250 000 times and filtered with a band width of 100–8000 Hz. The amplified and filtered signal was fed to a microcomputer (IBM PC 486), A/D converted, and sampled (12 bits) with a specially designed program (SC, Department of Physiology, Umeå). During stimulating sessions, the nerve signal was continuously recorded for off-line analysis and selected parts displayed consecutively, following a time delay after an electrical trigger signal for on-line analysis (see Fig. 1) using the paradigm described by Torebjörk and Hallin (1974b). With this method, activation of a C-fibers can be detected by a slowing of the conduction velocity of succeeding action potentials evoked by electrical stimulation. The triggering pulse to the laser as well as the calibrated measure of the light emitted from the laser were recorded on line. In some experiments, calibrated nylon filaments were coupled to a force transducer with the signal amplified (bridge amplifier) and fed into the computer.

Stimulating procedure

Electrical cutaneous stimulation

Short trains (3–5 pulses of 5 Hz) of 0.5-ms pulses were delivered onto the skin with a stainless steel electrode with a blunt tip of 0.5-mm diameter. Cutaneous spots where electrical stimulation induced single-unit activity were indicated with water-soluble blue or green ink.

Mechanical cutaneous stimulation

Calibrated nylon mono filaments (von Frey-type, Simms-Weinstein aesthesiometer, Stoelting, Chicago) with blunted tips and diameters of 0.2–0.8 mm were used on the mechanical receptor properties.

Argon laser stimulation

An argon laser (LEXEL 95–4 Ion Laser, USA) with 75% of its power in the blue (488.0 nm) and green (514.5 nm) spectra were used. The output energy of the laser beam could be adjusted between 0.5 mW and 4 W. The laser beam was transmitted through a fiber-optic cable and a flexible lens system, which projected the light onto the skin. To study the impact of spatial summations, the diameter of illuminated skin spots could be adjusted from 1 to 3 mm. The lens arrangements made a homogenous intensity of light within the stimulated skin spot. Single square-wave light pulses with a duration of 200 ms were given. The energy output from the laser was recorded by a lens system, linking a fraction (8%) of the emitted light onto a light detector (Keynce RV2–52, Japan) with its optimal sensitivity

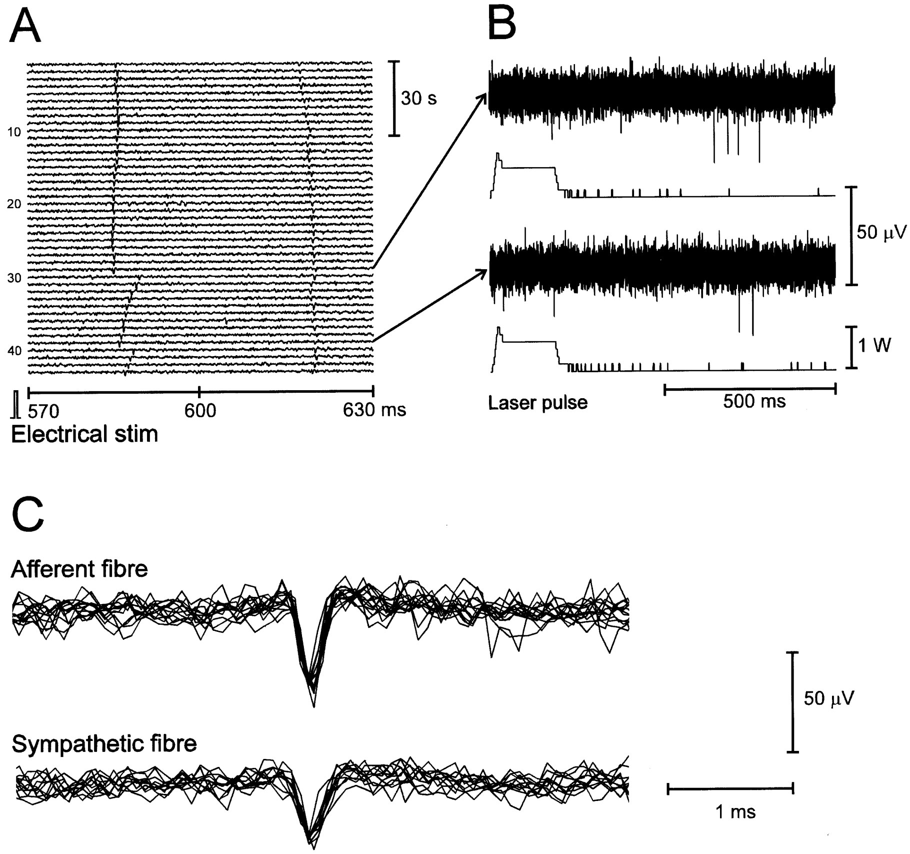

Fig. 1A–C Original recordings of the nerve signal from the peroneal nerve. **A** Intracutaneous electrical stimulation evoked single action potentials with a constant latency if the fiber did not receive supplementary activation, and latency shifts were observed when the receptor was activated. **B** Nerve recordings following two successive laser pulses to the same spot within the receptive field. Four action potentials were evoked by the first pulse and two by the second, indicating a fatigue mechanism. The latency shift (see **A**) following the first four action potentials is about twice that of the two successive action potentials. **C** Superimposed action potentials in an afferent and a sympathetic fiber evoked by electrical stimulation. A stable latency (see **A**) of the action potential following electrical stimulation was observed in the afferent C-fibers, while spontaneous latency shifts were observed in the sympathetic fiber due to spontaneous activity

for green light. The light detector was calibrated against a calorimetric instrument (Ophir, Israel), which measured the absolute output energy from the laser. No direct temperature measurements were made within the skin during argon-laser stimulation.

Receptive field mapping and threshold testing

The receptive fields of single C-fiber afferents were characterized with respect to the cutaneous area where stimulation with either electrical, mechanical, or heat stimulation could activate the fiber. The mapping procedure started with electrical stimulation in distinct spots, separated by about 1–2 mm from each other in a skin area extending about 3 cm from the receptive field center. These spots were then used as targets for the succeeding receptive-field mapping. To avoid fatigue or sensitization, repeated stimulation of single spots were avoided except in investigations of fatigue.

Mapping of the mechanical receptive fields was made with forces exceeding the threshold by at least four times. If the selected spots were insensitive to the mechanical stimulus of four-times threshold, successively higher indentation forces were used, up to 910 mN. Spots within the electrically defined receptive fields were mechanically tested at least twice before being considered non-responsive. The threshold was considered to be the lowest force activating the fiber three times in succession in the most sensitive spot within the mechanically defined receptive field. In a few units, assessment of mechanical thresholds was not preceded by electrical stimulation.

After mechanical stimulation, the electrically excitable spots were tested with a 200-ms heat pulse with a beam diameter of approximately 1 mm. The threshold to heat was assessed in a way similar to mechanical stimuli. Pulses of 0.3–0.4 W was initially given to the skin. If the unit did not respond, the light intensity was successively increased in steps of 0.05 W. The receptive field was then tested with stimuli 2–3 times the threshold. Spots not responsive to stimuli three times threshold were considered non-responsive to heat. Higher intensities was sometimes given, but a repeated high-intensity stimuli needed for the mapping procedure could not be performed because of the painful response of the subjects.

At the end of the experiment, the shapes of the receptive fields were traced onto a transparent plastic sheet. The traces were then digitized, and the area measured with a graphic program (Adobe, Photoshop 2.5.2).

Psychophysical pain rating

The pain evoked by cutaneous laser stimulation was ranked by the subjects according to a verbal analogue scale, with scaling steps from 0 to 10: 0 corresponding to no pain and 10 to the worst imaginable pain from the skin. The subjects were all well familiarized with the method before the study.

Analysis and statistics

The off-line analyses were computerized (IBM PC 486) using a specially designed program (ZOOM, Department of Physiology, Umeå), which facilitated selective spike analysis and reorganization of the data into a suitable format. Investigations of the nerve signal 3–4 s before the first detected latency shift in conjunction with the output signal from the laser, both spontaneous and evoked C-fiber activity, could be accurately detected. By selecting these periods and rearranging the nerve signal now triggered by the onset of the laser pulse, laser-evoked single C-fiber activity could be detected. Included in the analyses were only those action potentials of consistent amplitude and shape, which could be reliably differentiated from the background noise and from other spontaneously occurring activity (e.g., sympathetic skin efferents). Since a continuous recording of the nerve signal was made during the experimental session, evoked and spontaneously occurring spikes could be picked up due to their appearance and the induced conduction-velocity reduction of electrically evoked action potentials.

Parametric statistics with Student's *t*-test (two tailed) and ANOVA were used for dependent and independent observations. Differences with *P*-values less than 0.05 were considered significant.

Results

The investigated fibers (*n*=25) were all characterized as polymodal mechano-heat sensitive nociceptors with receptive fields on the skin on the dorsum of the foot. None of the fibers showed spontaneous activity. The recorded action potentials were predominantly unipolar negative potentials, but sometimes small positive potentials were found, giving them a bi- or tri-phasic shape. The temperature of the skin during the recordings was 26–32°C. No complications during the experiments or late side effects related either to the microneurography or to the laser stimulation were reported by the subjects.

General characteristics

The CMH-fibers were identified by their almost constant conduction velocity (Fig. 1A) following consecutive cutaneous electrical pulses (mean 0.96 m/s, range 0.66–1.15 m/s). A slowing of the conduction velocity was induced by activation of the fiber either by supplementary heat (Fig. 1B,C) or mechanical stimulation of the receptive field. The conduction-velocity change was observed as a latency shift of the electrically evoked action potential. The sympathetic efferent C-fibers (Fig. 1C) were separated from the CMH fibers by their inability to respond to any type of natural cutaneous stimulation and by their activation during mental arithmetic or following an arousal stimulus, such as a sudden loud noise (Hallin and Torebjörk 1974a). In contrast to CMH fibers, the sympathetic fibers showed spontaneous activity, which was reflected as small spontaneous latency shifts in the response to electrical stimulation (Fig. 1A). In no recording could the argon-laser pulse be related to any direct activation of faster conducting fibers (A-delta, A-beta).

Natural heat stimulation

The response to a 200-ms laser pulse was either a single spike or a burst of action potentials (range 2–20) with a mean duration of 160 ms (range 27–437 ms). A highly significant correlation was observed between the duration of the bursts and the number of spikes in each burst (r=0.90, P=0.001). A high variability in the evoked response was observed, depending principally on the location of the heat stimuli within the receptive field, which could to some extent explain the fairly bad relation between the number of action potentials and the energy delivered to the skin (r=0.48, P=0.001).

The activation of the afferent C-fiber could be detected both by the latency shift in the electrically evoked action potential due to a decrease in the conduction velocity following preceding activation (Torebjörk and Hallin 1974b; Schmelz et al. 1995) and by the identification of individual spikes in the original nerve recording. A highly significant relation (r=0.87, P<0.001) was found between the number of action potentials and the slowing of the conduction velocity (delta velocity) following heat stimulation (Fig. 2A).

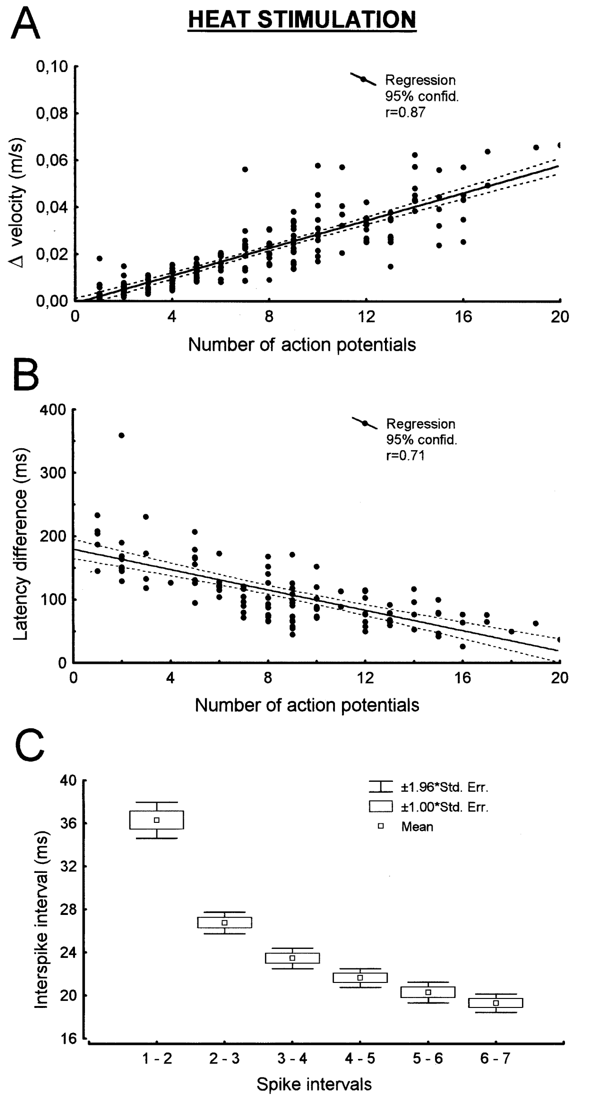

Fig. 2A–C Results from pooled data following cutaneous argon-laser stimulation. **A** A highly significant relation was found (r=0.87, P<0.001) between the number of action potentials evoked by laser stimulation (to the receptive field) and the slowing of conduction velocity (delta velocity) in the nerve of electrically evoked action potentials. **B** A significant relation was detected (r=0.71, P<0.001) between the number of action potentials evoked by laser light of 200-ms duration and the latency difference between the electrically evoked action potential and the spike-train following the heat-pulse onset. **C** Box-plot of the inter-spike intervals in trains of action potentials evoked by laser light of 200-ms duration. The data are presented as means (*small square*) and standard error of the mean [sem, *large squares* (1 sem) and *whiskers* (1.96 sem)]

The mean onset latency to the first action potential following laser stimulation was delayed by 123 ms (mean,

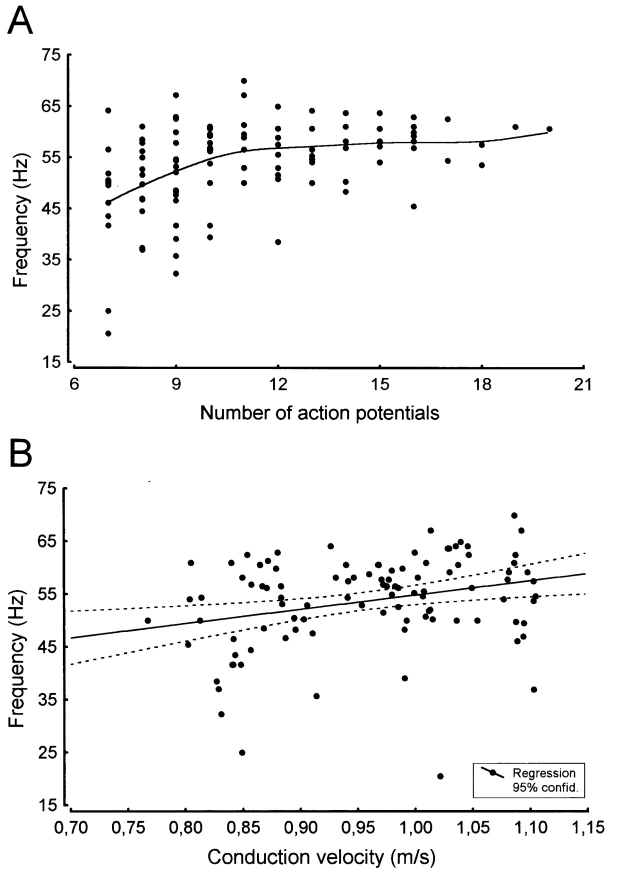

Fig. 3A, B Results from pooled data following cutaneous argon-laser stimulation. **A** Scatterplott of the relation between the number of action potentials (*n*>6) within each spike-train following a 200-ms-long laser pulse and the peak frequency. The *line* indicates a linear least-square estimation of the frequencies. **B** The relation between conduction velocity of the C-fibers and their peak frequency following argon-laser stimulation (*r*=0.29, *P*=0.002)

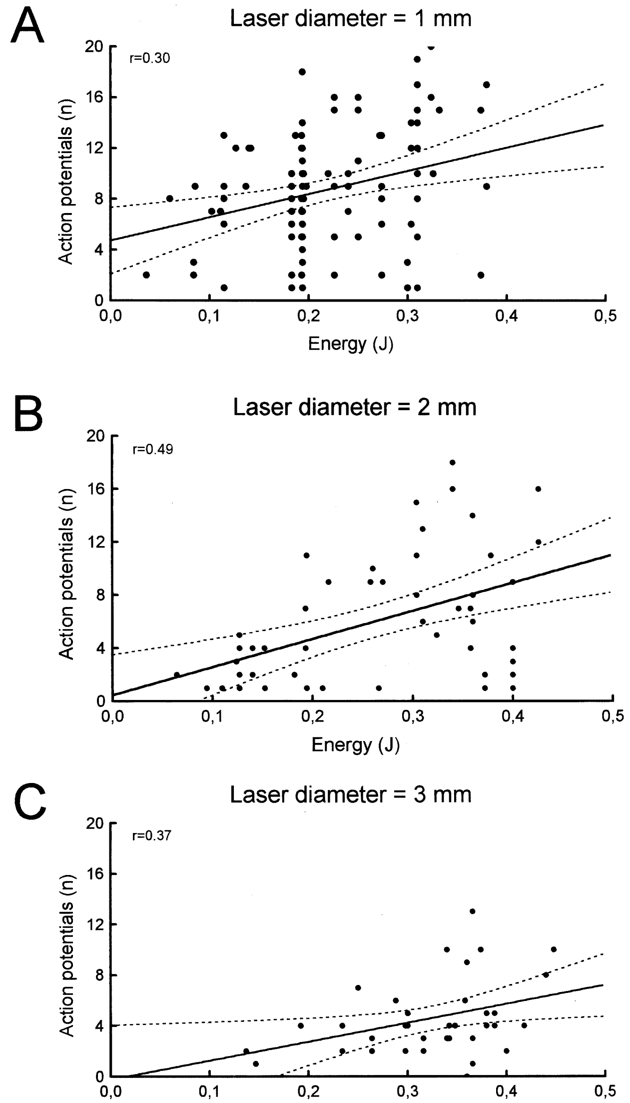

Fig. 4A–C Relationship between the heat energy given to the receptive field by the laser and the evoked number of action potentials. The data in diagram in **A–C** is subdivided according to the diameter of the laser beam (1–3 mm, respectively) projected on the skin. A significant shift to the right of the *regression line* was observed by increasing the area of the stimulated skin. *P*<0.001 between **A** and **B**, and *P*=0.0024 between **B** and **C**, ANOVA

range 26–359 ms) compared with the electrically evoked spike. As expected, a negative correlation was found between the onset time to the first action potential and the degree of activation (evaluated by number of action potentials) evoked by the laser pulse (*r*=0.71, *P*=0.001; Fig. 2B).

When the degree of C-fiber activation was related to the inter-spike intervals in the spike trains, successively shorter inter-spike intervals (*P*<0.001) were detected between the first and second (28 Hz, range 11–45 Hz) and the second and third spike (37 Hz, range 13–51 Hz). No significant shortening of the inter-spike intervals was detected following the fifth spike (Fig. 2C). By relating the number of spikes in each spike train to the peak frequency within the train (Fig. 3A), a saturation was detected at frequencies around 55 Hz. A relation was also detected between the peak frequency and the conduction velocity of the fibers (Fig. 3B, *r*=0.29, *P*=0.002).

Figure 4 shows the responses of all the afferents to energy delivered to a 1-, 2-, or 3-mm diameter skin spot. When stimulating a 1-mm diameter spot, the lowest energy needed to activate the fiber was less than 40 mJ. By increasing the energy, more action potentials were evoked (*P*<0.001); however, a high variability in the fiber response to quantitatively similar heat stimulation was observed. By increasing the stimulated area, the thresholds were increased, reflected in a parallel shift to the right of the linear regression curve (*P*<0.001). Despite the difference in thresholds to stimulation in 1-, 2-, or 3-mm diameter spot, no significant difference in the pattern of the inter-spike intervals was detected when small or large spots were stimulated.

When stimulating localized areas (1 mm in diameter) of the skin within the receptive fields, spots with various sensitivities to heat stimulation were detected. The variability in response to identical heat pulses was highly de-

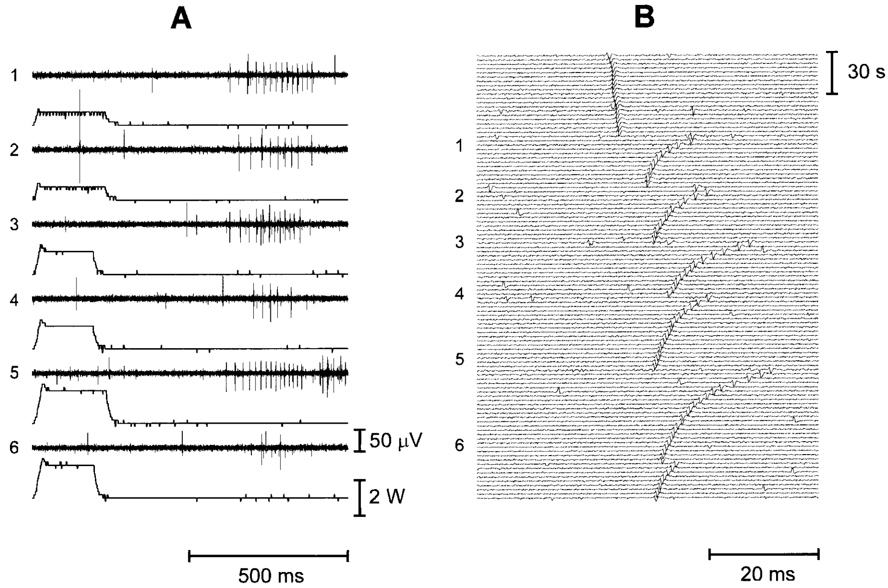

Fig. 5A, B Recordings from an afferent C-fiber in the peroneal nerve with a receptive field on the non-hairy skin of the foot. Activation of the fiber induced by laser pulses of 200-ms duration projected to a 1-mm diameter skin spot within the receptive field. **A** Neuronal response to laser pulses of increasing intensity (the amplitude of the square wave is given under each nerve recording, indicating the output energy from the laser reaching the skin). A fatigue to successive stimuli of the same intensity was detected (recording 1–2, 3–4, and 5–6 from the top). By increasing the stimuli (3rd and 5th recording), the fatigue was overwhelmed. The interval between each laser pulse was approximately 30 s. **B** The activation of the fiber is related to the latency shift of the action potential, as described in Fig. 1

pendent on the position of the laser beam, and small changes in position (often less then 1 mm) resulted in a quantitatively different response. By repeated stimulation of a single spot with the same intensity, a decrease in responsiveness was already observed in the fiber after the first burst of action potentials, with fewer action potentials and a longer delay to the response onset to the following stimulus (Fig. 5A and B). By increasing the stimulus intensity further, the number of action potentials increased; however, the responsiveness to subsequent stimuli was even further diminished.

Natural mechanical stimulation

Six units responsive to heat stimulation were also systematically characterized regarding their response to mechanical stimulation. The neuronal C-fiber response to 85 mN or 180 mN stimuli usually consisted of irregularly occurring spikes with mean receptor onset delay (latency difference between electrically and mechanically evoked action potentials) of 228 ms (range 58–412 ms). The effect on the conduction velocity of the fibers following mechanical stimulation was basically similar to the effects evoked by heat and could be related to the number of spikes preceding the electrically evoked spike (Fig. 6A). The inter-

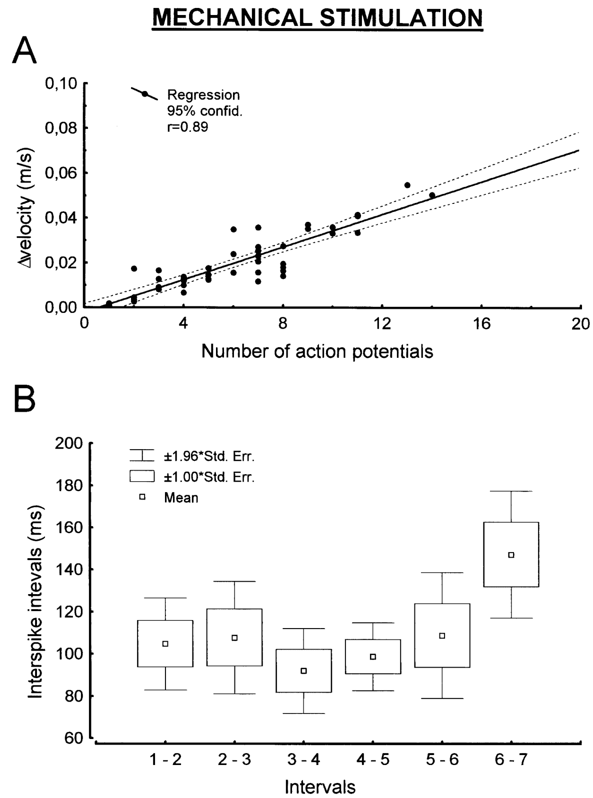

Fig. 6A, B Results from pooled data following cutaneous mechanical stimulation **A** A highly significant relation was found (r=0.89, P<0.001) between the number of action potentials evoked by mechanical stimulation of the receptive field and the slowing of conduction velocity (delta velocity) in the nerve of electrically evoked action potentials. **B** Box-plot of the inter-spike interval in trains of action potentials evoked by mechanical receptive field stimulation. The data are presented as in Fig. 2C

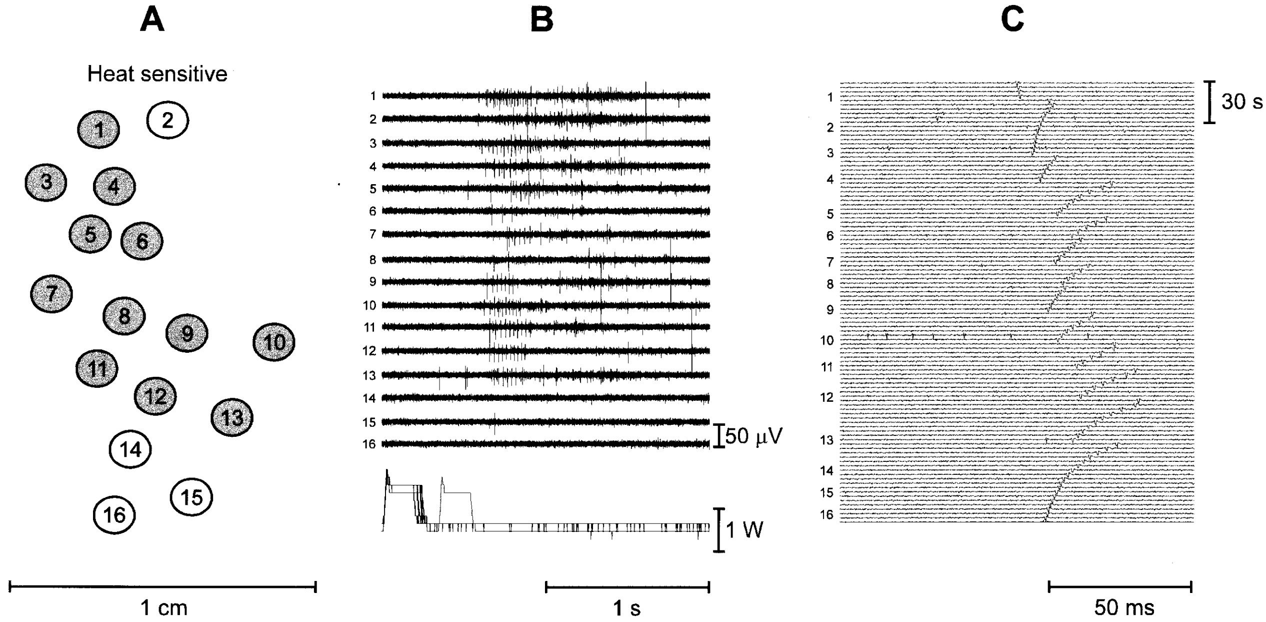

Fig. 7A–C Recordings from an afferent polymodal C-fiber unit tested with cutaneous argon-laser stimulation. **A** Receptive field delineated by electrical cutaneous stimulation. *Spots* sensitive to heat (argon laser) are *shaded*. **B** Original nerve recordings following argon laser stimulation of spots within the receptive field. The *numbers* indicate the location on the skin of the preceding laser stimulus. At the *bottom* of the figure, the output energy projected to the skin is indicated. Note that recording no. 4 was preceded by a double laser pulse. **C** Latency shifts of electrically evoked action potentials (as in Fig. 5B) following heat activation

spike intervals of action potentials following mechanical stimulation are indicated in Fig. 6B. The mean inter-burst frequencies was 9.6 Hz (range 2.6–23 Hz) following mechanical stimulation of 85–910 mN. The mechanical stimulation was not reported by the subjects as being painful.

Receptive fields

Electrical cutaneous stimulation (n=14) revealed skin receptive fields with mean areas of 74.3 mm^2 (range 32.1–296.4 mm^2). Mechanical stimulation (n=17) with forces 4–20 times the threshold (85–910 mN) revealed mechano-sensitive receptive fields with mean areas of 36.6 mm^2 (range 17.1–58.6 mm^2). Heat stimulation (n=17) with single laser pulses of 2–3 times threshold and a beam diameter of 1 mm revealed mean receptive field areas of 37.8 mm^2 (range 15.4–68.8 mm^2). Receptive fields to electrical stimulation (mean 74.3, range 32.1–296 mm^2) were significantly larger than those to mechanical (P=0.002) and heat (P=0.001) stimulation. The extension of the areas activated by heat and mechanical stimulation were always within the receptive field limits defined by the electrical stimulation. The receptive fields for mechanical and heat stimulation overlapped to a great extent, and there was a significant correlation between the heat and mechanical receptive field sizes (r=0.59, P=0.016, n=17). At the borders of the receptive fields, spots only sensitive to heat or mechanical stimulation were sometimes detected, but no significant difference in the receptive field size was found for the two modalities (P=0.92).

The typical response of one single polymodal fiber to mechanical and heat stimulation is shown in Figs. 7 and 8, respectively. The innervation territory of the fiber was defined by electrical stimulation. By testing the skin within the electrically defined field, spots sensitive to both mechanical and heat stimulation were found. Spots sensitive to mechanical stimulation were also commonly excited by heat. The receptive fields were investigated in the order indicated by the number of the spots. From this and other recordings, it appeared that no systematic pattern in the responsiveness of the unit to stimuli at consecutively tested spots could be detected. As exemplified in the figure, the response to stimuli at one spot could be followed by an increase or decrease in responsiveness by stimulation of a neighbouring spot. However, if the same spot was stimulated by the laser twice in succession, fatigue was always observed (Figs. 1 and 5).

In five polymodal fibers with well-characterized receptive fields, the evoked response (e.g., number of action potentials) to quantitative mechanical and heat stimulation were compared at numerous spots (n=38) within their receptive fields. No significant correlation (r=0.12, P=0.51) in activity to mechanical and heat stimulation was detected.

Thresholds to cutaneous mechanical and heat stimulation

The thresholds to mechanical and heat stimulation was evaluated by investigating the entire receptive field, selecting the most sensitive spot for threshold investigations. The median threshold to mechanical stimulation

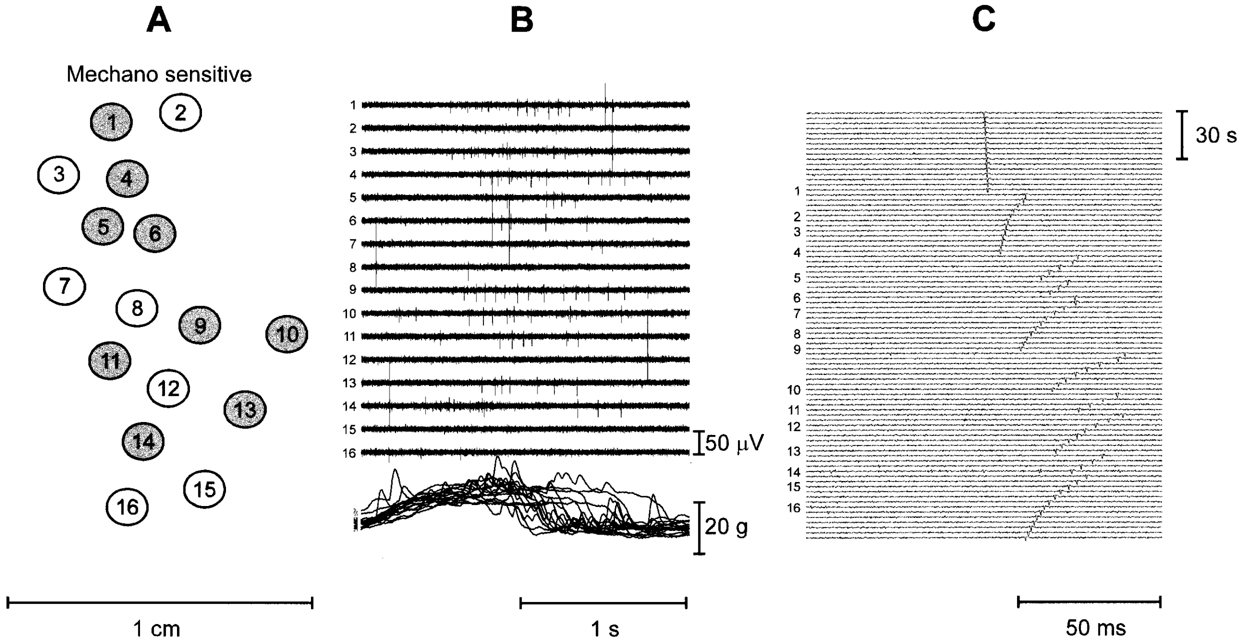

Fig. 8A–C Recordings following mechanical testing from the same unit as in Fig. 7. **A** The *shaded spots* were sensitive to mechanical stimuli. **B** The force and the duration of the mechanical stimuli is indicated at the *bottom* of the figure. **C** Latency shifts of electrically evoked action potentials following mechanical stimuli to the receptive field

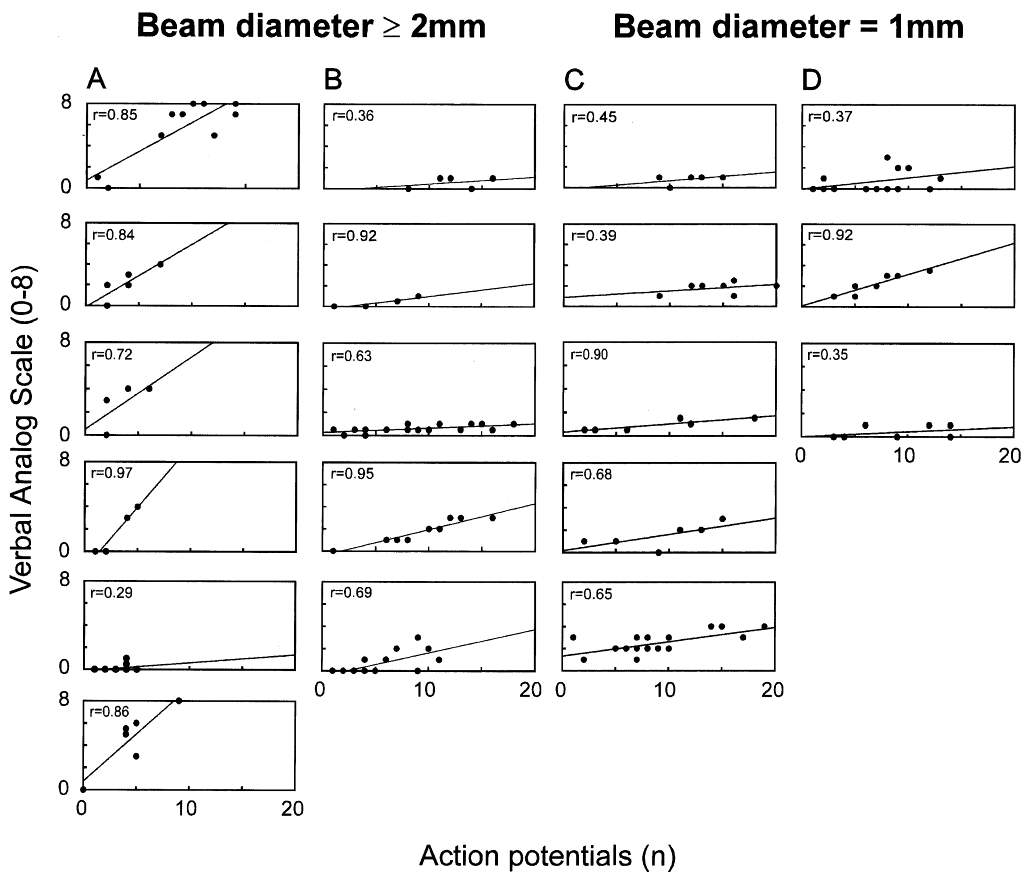

Fig. 9A–D Diagrams of subjective pain sensation evoked by cutaneous argon-laser stimulation as a function of the neuronal activity in single fibers. Each diagram represents the stimulus response function for one subject. **A** and **B** show the responses when skin spots of 2- to 3-mm diameter were stimulated. **C** and **D** show the responses following stimuli of 1-mm diameter skin spots. Note that, in **B** and **C**, each row represents the individual results from four subjects of stimuli to skin-spots of different diameters with nylon filaments was 22 mN (range 14–640 mN, n=17). The mean threshold to 200-ms, 1-mm diameter laser pulses was 102 mJ (range 36–210 mJ, n=17). No significant relation between the thresholds to mechanical and heat stimulation were detected. The thresholds for both mechanical and heat stimulation were tested at least twice during the experiment, and no threshold change to either mechanical or heat stimulation was detected during the 1- to 2-h duration of the investigations.

Psychophysical estimates related to C-fiber activity

A distinct burning or pricking-painful sensation was commonly reported following high intensity, single argon-laser pulses. In the entire material, no significant relation was detected between energy delivered to the skin by the laser and the reported pain sensation (r=0.16, P=0.09). However, a fairly good linear correlation was found for most subjects between the reported magnitude of pain and the number of action potentials evoked in the CMH afferents (Fig. 9, column A and B). As it appears from the figure, the psychophysical response pattern showed pronounced differences between subjects.

By reducing the stimulated spots in the skin to a diameter of 1 mm, the ability of some individuals to discriminate the intensity was preserved, but in a few the correlation was augmented (Fig. 9, column C and D). Only light- or medium-intensity pain was reported by stimulating small-diameter spots (1 mm).

Discussion

This is the first study to use an argon laser and C-fiber recordings in humans. The data show that the extent of the receptive fields on the skin of polymodal nociceptors to mechanical and heat stimulation are similar under normal conditions. The psycho-physical data indicate a good relation between the laser-evoked nerve activity and the subjective pain estimates, while large differences appeared in the responses between subjects.

The marking technique of C-fiber afferents previously described by Torebjörk and Hallin (1974b) was, in this study, proven to give both a qualitative and quantitative estimate of the activity generated by heat and mechanical stimulation, in agreement with recent studies (Schmelz et al. 1995). A good correlation between the number of action potentials and conduction velocity change was achieved following both heat and mechanical stimulation. The possibilities of on-line mapping of the receptive-field properties was fundamentally dependent on the marking procedure, since the method was accurate enough to detect the occurrence of single action potentials, which were also partly dependent on the long distance between the stimulating and recording site, which amplifies the latency shifts to electrical stimulation.

Natural heat and mechanical stimulation

The argon laser induces a short, lasting heating of the skin. Since no direct temperature measurements were made in the stimulated skin, the thresholds needed to activate the polymodal nociceptors could not determined. Argon-laser light with its blue and green spectrum is absorbed (by haemoglobin) deeper in the skin than CO_2-laser light (which is absorbed by water superficially in the skin), and, with the quantal square-wave heat stimulus delivered to the skin, a ramp-like temperature increase could be expected. From previous experiments in man (Torebjörk et al. 1984) and monkey (Tillman et al. 1995b), the heat thresholds for the polymodal C-fibers on the surface of uninflamed skin are estimated to be 40°C, which is also suggested to be the approximate threshold of the units in this study (see Tillman et al. 1995a).

The latency from the beginning of the laser pulse to the first action potential recorded was linearly related to the degree of C-fiber activation. The higher the energy delivered to the skin, the shorter the onset time to the first spike, reflecting a more rapid heating. The data also indicate that a critical temperature must be reached to activate fibers, since more energy was needed when larger skin areas were stimulated. Data from studies with the yag-laser (Ellrich et al. 1996), where short (0.3 ms) high intensity light was used, suggest that the initiation time in the receptor is at least 30 ms, in agreement with the onset latencies of action potentials, which were related to the degree of C-fiber activation in this study.

From previous experiments in man, the receptor-saturation temperature could be expected to be above 50°C (Torebjörk et al. 1984). It is suggested in the present study that this temperature is rapidly reached following the onset of the laser pulse. Supporting this assumption is the relative uniformity of the spike train following laser stimulation, with a fast saturation. Further evidence of an initial fast saturation is that the evoked trains of spikes appeared the same whether a larger or smaller skin area was stimulated. The stereotypic neuronal response to laser stimuli at larger and smaller areas of the skin also suggests that the origin of the spike train can be referred to the most sensitive spot within the stimulated skin (Treede et al. 1990).

A significant correlation between the energy delivered to the skin and the neuronal response was detected. This relation was, however, unexpectedly poor. Factors such as different receptor sensitivity within the receptive field and variability of sensitivity in different fibers may explain some of the variability in the neuronal response.

Action-potential frequencies typically around 10 Hz in the intra-neuronal responses to mechanical stimulation were observed in this study, in agreement with previous findings (Torebjörk and Hallin 1974a; Koltzenburg and Handwerker 1994). The response to mechanical and heat stimulation was qualitatively different, with higher frequencies in the spike trains following local heating. Since mechanical stimulation with nylon filaments seldom evokes any pain sensation, it has been suggested that the pain sensation is frequency dependent: this assumption is in agreement with recent findings with forceful mechanical stimulation, giving high-frequency bursts of action potentials (exceeding 10 Hz), which have been related to subjective pain sensation (Kilo et al. 1994).

Since a similar pattern of inter-spike intervals to the laser stimuli was evoked from different parts of the innervated skin, it is suggested that the sensory unit does not possess the ability to distinguish differences in the location of the heat stimuli within the receptive field. This also applies to the neuronal response to mechanical stimulation (Torebjörk and Hallin 1974a; Koltzenburg and Handwerker 1994).

Receptive fields and thresholds

The size and configuration of the receptive fields to mechanical and heat stimulation showed large variability between different units. Most commonly, single confluent areas were found, but also fibers with one or more satellites were detected. The satellites shared the receptor characteristics of the core area. Neither were satellites sensitive exclusively to mechanical or heat stimuli found nor could stimulus modality activate all electrically excited spots. One of the reasons for this might be that the stimuli were not strong enough, especially with respect to the heat stimuli, which often were only 2–3 times threshold. Against this speaks the good overlapping of mechanically and heat receptive fields. A fairly good correlation between the receptive field size to mechanical and heat stimulation was detected, in agreement with previous studies in monkey by Treede and co-workers (1990). However, they found smaller receptive fields to mechanical stimulation than in our study, which is suggested to be related to the lower mechanical pressure used in their study (200 mN compared with 910 mN in the present study). Other possibilities are species-related differences or mechanically insensitive spots previously described in man (Schmelz et al. 1994).

No correlation was found in thresholds or excitability to mechanical and heat stimulation, respectively. This finding is in line with the suggestion of different transduction mechanisms for the two modalities, as shown in previous studies (Treede et al. 1990). This suggests that general excitability mechanisms in the receptor are of less importance for the response to qualitatively different stimuli (heat and mechanical) and, rather, that subsets of receptor mechanisms specific for the different modalities exist.

Sensitization and fatigue

Sensitization of the fibers was expected to be induced by the laser stimulation. However, neither signs of increased responsiveness nor decreased thresholds was detected following the testing procedures. No other signs of inflammation due to the laser heat, such as tissue swelling or increased blood flow, were observed (unpublished data).

The mechanisms of fatigue were not systematically studied in this investigation. However, the data suggest a mechanism demanding more than 400 ms, since no fatigue to single laser pulses was observed in the spike trains, i.e., no decrease in the inter-spike intervals occurred at the end of the spike train following a single laser pulse of 200-ms duration. The mechanisms of fatigue is also suggested to be strictly localized and mediated by the local receptor membrane, since a stronger stimuli at the same spot or a stimuli at another spot within the receptive field could activate the fiber more effectively.

Psychophysical estimates

A single or short train of action potentials in the CMH fiber did not commonly evoke a painful sensation (Vallbo et al. 1979), in line with the assumption that a single action potential in a few fibers is not enough to evoke pain. By increasing the stimulus strength and subsequently the number of action potentials, an increase in pain was reported. However, the psycho-physical estimates of the evoked pain sensation was poorly related to the energy given to the skin. One of the reasons might be the high variability in sensitivity at different spots within the receptive field, and a better relation was found when the perceived sensation was related to the number of spikes evoked by the heat stimulation, in agreement with previous studies (Torebjörk et al. 1984). A correlation between the subjective estimates and the number of action potentials was found if spots of 1-mm diameter were selected. The correlation was commonly improved if the stimulated diameter was 2 mm or larger. This supports the importance of spatial summation in the nociceptive system for adequate quantification of nociceptive stimuli. This finding is in agreement with previous psycho-physical studies of subjective estimates of pain (Arendt-Nielsen and Bjerring 1988; Grönroos et al. 1996) in that the accurateness in the subjective report of pain to the stimulus intensity given is dependent on the area of skin stimulated.

In summary, this study shows similarities in polymodal C-fiber nociceptors in their responses to mechanical and heat stimulation with respect to the extent of the receptive fields. Dissimilarities in the activation pattern indicate different transduction mechanisms, and whether these differences have any implications for perception of different qualities can not be determined by this study, since the mechanical stimuli used was not painful. The argon-laser method proved to be well tolerated by the subjects, caused no burns, and was also highly reproducible in activating the C-fibers, making it a good tool for further investigations of function of heat-sensitive nociceptors in man.

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