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SENSATIONS EVOKED BY INTRANEURAL MICROSTIMULATION OF C NOCICEPTOR FIBRES IN HUMAN SKIN NERVES

By JOSÉ OCHOA and ERIK TOREBJÖRK

From the Department of Neurology, Good Samaritan Hospital and Oregon Health Sciences University, Portland, OR 97210, USA and the Department of Clinical Neurophysiology, Academic Hospital, Uppsala, Sweden

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SUMMARY

1. Seventy-one C polymodal nociceptors supplying glabrous and hairy skin in limbs of awake human volunteers were identified on the basis of cutaneous stimulus–response characteristics recorded intraneurally by microneurography (MNG). The large majority of such units were primarily detected during intraneural microstimulation (INMS) on the basis of subjective quality and cutaneous localization of evoked painful sensation. Electrophysiological studies were supplemented with rigorous psychophysical testing during microstimulation delivered at intraneural C recording sites.

2. The conduction velocity of single C nociceptor units could be shown to become transiently slowed following repetitive INMS at threshold intensity for conscious sensation. Such 'marking' witnessed that particular C units, identified by recording, had been effectively activated during INMS and psychophysical testing.

3. Cognitive attributes of sensations evoked from C recording sites by INMS at threshold intensity for perception were estimated psychophysically for subjective quality, temporal attributes and localized projection. There was remarkable matching of physiological unit type (C polymodal nociceptor) with subjective quality of evoked sensation (dull or burning pain). Further, there was remarkable spatial matching of receptive field of given C nociceptors with projected field of the pain sensation evoked from the C recording site by INMS delivered at threshold intensity for conscious sensation.

4. Dissociated A nerve fibre blocks caused by compression-ischaemia did not abolish the sensation of burning pain projected to hairy skin, evoked by INMS delivered at C recording sites.

5. While the double matching of (a) subjective quality and spatial localization with (b) objective physiological unitary type and receptor location, coupled with the results of A blocks, provide evidence that C nociceptor fibres can be fairly selectively activated during INMS, the results also attest that C polymodal nociceptors from human skin evoke delayed dull or burning pain, accurately projected to a defined locus in skin, even after spatial summation is reduced to a minimum.

INTRODUCTION

Research on fundamental mechanisms of somatic sensation became significantly enhanced in the 1960s with the application of invasive electrophysiological techniques, previously reserved for animal experimentation, to the study of receptor response properties of single myelinated and unmyelinated afferents in awake human subjects (Vallbo & Hagbarth, 1968; Hallin & Torebjörk, 1970; Torebjörk & Hallin, 1970; Van Hees & Gybels, 1972; Vallbo, Hagbarth, Torebjörk & Wallin, 1979). When, in the early 1980s, the basic microneurographic (MNG) method was supplemented with intraneural microstimulation (INMS) of myelinated fibres of identified mechanoreceptors, it first became possible to question the human brain directly with regard to laws for decoding physiologically specified afferent messages into subjective quality, magnitude and localization of sensation. It was learned from such studies that, for tactile sensations, those cognitive attributes can be resolved from the simplest input initiated in certain types of single mechanoreceptor units in the hand (Torebjörk & Ochoa, 1980; Vallbo, 1981; Ochoa & Torebjörk, 1983; Schady, Ochoa, Torebjörk & Chen, 1983*a*; Schady, Torebjörk & Ochoa, 1983*b*; Vallbo, Olsson, Westberg & Clark, 1984). Thus, the combined MNG–INMS strategy has emerged as a powerful approach for investigation of somatic sensation (Torebjörk, Vallbo & Ochoa, 1987). Natural outcomes of those studies are the questions of whether C nociceptors can be similarly explored in humans and, if so, whether their excitation evokes sensations which conform to rules found to govern the ability of the human brain to qualify, localize and estimate magnitude of elementary tactile sensations. While the methodology was even more challenging than for the study of tactile mechanoreceptors, experimentation was feasible (Torebjörk & Ochoa, 1989*a*). Indeed, while evidence of excitation of individual C nociceptor axons in isolation cannot be substantiated, controlled activation of subpopulations of unmyelinated fibres can be documented, as can be selective activation of human A nociceptors (Torebjörk & Ochoa, 1989*b*).

Here we report on subjective attributes of sensations evoked by activation of bundles of C nociceptor units innervating human skin. The results may be regarded as relevant for the understanding of pain as a normal sensory submodality and also of pain as a clinical symptom of dysfunction of nociceptor systems (Cline, Ochoa & Torebjörk, 1989). A preliminary report has been presented on the present work (Ochoa & Torebjörk, 1988).

METHODS

Material

Microneurography and INMS were carried out on five healthy subjects, ranging in age from 33 to 51 years. Fourteen experiments were performed in the median nerve at elbow level or 10–15 cm proximal to the medial epicondyle in the upper arm. Eight experiments were performed in the ulnar nerve, and one experiment in the superficial radial nerve at wrist level. A few experiments used the posterior cutaneous nerve of the forearm ($n = 2$), or the peroneal nerve at knee level ($n = 1$), or just above the ankle ($n = 2$). A total of seventy-one C polymodal nociceptor units with receptive fields in glabrous (thirty-seven units) and hairy (thirty-four units) skin of the hand, forearm, leg or foot were sampled in this study.

General procedure

After informed consent the subject reclined on a chair with the arm or leg comfortably supported. A microelectrode was inserted manually through the skin into an underlying nerve trunk, which was localized by palpation or electrical stimulation. A reference electrode was inserted into subcutaneous tissues 1–2 cm outside the nerve trunk. Position of the microelectrode tip within a cutaneous fascicle was ascertained by recording afferent activity from the skin, or by evoking sensations projected to the skin while stimulating electrically through the electrode.

Electrodes and equipment

Lacquer-insulated tungsten electrodes, 200 μm in diameter, of the type designed for human microneurography (Vallbo & Hagbarth, 1968), were used for neural recording and stimulation.

Nerve signals were amplified, filtered and displayed on a storage oscilloscope (Tektronix), audiomonitored, and stored on tape (Sangamo Sabre IV or Tandberg 115) for subsequent analysis. The electrodes could be connected through a switch in the pre-amplifier to either the input of the recording system or the output from a constant voltage Grass S48 stimulator with stimulus isolation unit.

Intraneural microstimulation (INMS) and recording (MNG)

The intrafascicular electrode was primarily used to deliver trains of weak electrical stimuli while gently adjusting its position, and while attending to evoked sensations reported by the subject. Having reached an intrafascicular site where INMS evoked a weak, monofocally projected sensation, psychophysical studies were performed. The apparatus was eventually switched to recording mode and the search for sensory units recordable from the very site of INMS was started, based on the administration of natural stimuli to the cutaneous territory of the nerve (Torebjörk & Ochoa, 1989*a*).

Alternatively, the intrafascicular electrode was used primarily in the recording mode to search for units through conventional *natural stimulus–electrical impulse response* strategy. This second approach was used infrequently. With either approach, the subjective sensory data collected from microstimulation were matched against the electrophysiological data collected from recording of classified units.

Stimulus parameters for INMS

Square-wave pulses of 0·25 ms duration were used. Regular trains were given at frequencies of 3, 5 or 30 Hz. Train duration was usually 2–5 s to allow sufficient time for sensory detection. Longer trains (up to 40 s) were occasionally delivered to study changes in excitability of the stimulated fibres. Intervals between trains were 30–60 s. The amplitude of the stimulating pulses was gradually raised from 0 to a level where the subject felt a first threshold sensation. This usually occurred by 0·18–0·22 V.

Classification of units recorded through microneurography

For the purposes of the present study, aiming at nociceptor units, natural mechanical stimuli (stroking, scratching, pinpricking), heat (contact of a glowing match) and occasionally histamine (intradermal injection of approximately 0·01 ml of 1:1000 solution) were given to the cutaneous field, where sensations were projected. Having localized the unitary receptive field (RF) and classified the receptor according to established criteria (Vallbo *et al.* 1979), the receptive field was mapped with calibrated von Frey hairs at 5 × threshold, and its area was outlined with ink on the skin. Needle electrodes were optionally inserted in the receptive field to stimulate the fibre electrically, using square-wave pulses of 0·25 ms duration at amplitudes of up to 100 V with a constant frequency of 0·3 Hz. Conduction velocity was then calculated from measurement of latency and conduction distance between stimulating and recording sites. Proof that the nerve fibre of a recorded C unit was afferent rather than sympathetic was obtained by documenting transient increases in latency to electrical stimulation following activation of the unit by natural stimulation of the receptive field with noxious stimuli, and by lack of response to arousal stimuli which normally elicit sympathetic reflex responses (Hallin & Torebjörk, 1974; Torebjörk, 1974) (see Fig. 2*C*).

Psychophysical studies

The subjects had no clues as to exactly when intraneural stimuli were given, or what stimulus parameters were used during INMS sessions. They were asked to describe in their own words the qualities and temporal profiles of sensations evoked by INMS, and to map directly on a real size picture of the hand the sites and sizes of the skin areas where sensations were projected (projected field, PF). If the subjects had difficulties in naming the sensations, they were presented with a multiple choice questionnaire, composed from typical verbalizations collected in previous studies. They were asked to choose alternatives from each of five categories: category I: superficial, deep; category II: stationary, migratory; category III: intermittent, sustained; category IV: painful, non-painful; category V: tapping, flutter, vibration, tickle, pressure, tension, movement, cold, warm, hot, burning, sharp pain, dull pain, itch, electrical (Ochoa & Torebjörk, 1983).

'Marking' fibres of C polymodal nociceptor units stimulated by INMS

In an attempt to raise evidence to support the notion that nociceptive fibres situated very close to the intraneural tip of the electrode can be stimulated, and also recorded from experiments were performed to 'mark' objectively recorded fibres by using the intraneural stimulation technique. A C unit is 'marked' when its nerve fibre exhibits a sudden, reversible, shift in latency following repetitive activation by INMS. An example of this effect described by Torebjörk & Ochoa (1989*a*) is shown in Fig. 2*D*.

Dissociated A fibre blocks achieved by compression-ischaemia, and pain induced by INMS

In order to examine the fate of pain sensations induced by INMS at C recording sites, under conditions when only subpopulations of nerve fibres remain able to conduct impulses centrally, two MNG–INMS experiments were performed in the ulnar nerve at wrist level before and during selective A fibre blocks. Such blocks were caused by compression-ischaemia applied to the upper arm, proximal to the site of MNG–INMS, by means of a sphygmomanometer cuff inflated well above systolic pressure, to 200 mmHg, for periods of 30–40 min. The progression of the block was monitored by testing sensory submodalities of light touch, cold, warm and heat. An A fibre block, inclusive of A δ-fibres, was accepted as established when sensations of light touch and cold were lost, but warm sensation and delayed heat pain remained viable.

Dissociated A fibre blocks achieved by compression–ischaemia and dull compared with burning pain induced by natural stimulation of glabrous compared with hairy skin

To study qualitative aspects of pain sensation induced by noxious stimuli, in the absence of contribution from myelinated afferents, dissociated preparations were achieved through compression-ischaemia block of A fibres, as described above. Noxious heat stimuli were given to hairy as opposed to glabrous skin, before and during established A block, via 12·5 cm^2 Marstock thermodes (Fruhstorfer, Lindblom & Schmidt, 1976) while asking the subjects to describe whether the perceived pain was dull or burning in quality. In addition, painful punctate mechanical stimuli were delivered by a needle or a sharp wooden stick.

Test for C locognosia

The subject's ability to locate a hot stimulus from the input of C fibres alone was tested by a method modified from Noordenbos (1972). Eight experiments were performed on both hands in four subjects. Block of impulse conduction in A fibres was achieved through compression-ischaemia, as described above. Noxious heat stimuli were applied to the glabrous skin of the hand by means of a metal probe, heated to about 50 °C.

The circular contact area of the probe, measuring 1 cm^2, was painted with ink, to stamp red marks on the skin. Red goggles disabled the subject from seeing the red marks. While blindfolded, the subject was touched on palm or fingers for 3 s with the hot probe. Because of the A fibre block, the subject could not perceive touch, feeling only heat pain. Upon eye opening, while still wearing the red goggles, the subject marked with a black pen the site felt to have been stimulated. On average, six stimuli were given on each hand before the sphygmomanometer cuff was released. The distance between the centre of each red mark and the corresponding black mark was used as a measure of the subject's 'locognosia' (Hamburger, 1980).

In four control experiments, performed on both hands in two subjects, the capacity to localize a tactile stimulus in the hand was tested without nerve block. The same metal probe was used as in the A fibre block experiments, except that it was not heated. In all other respects, the same experimental protocol was observed as described above.

RESULTS

In addition to contributing novel information on the cognitive attributes of painful sensations evoked by INMS at C nociceptor recording sites, the observations to be described provide indirect evidence for fairly selective excitation of C nociceptor afferents through the present method.

Selection of material

As shown previously, intraneural microstimulation at 5 Hz in cutaneous fascicles of the median nerve at threshold for conscious detection gave rise to tactile sensations in about eighty per cent (80 %) of the tests in normal subjects, and to pain or (rarely) itch in the remainder (Schady *et al.* 1983*b*). Recordings from sites in which stimulation elicited tactile sensations often revealed signals from low threshold mechanoreceptors (Torebjörk & Ochoa, 1980; Ochoa & Torebjörk, 1983; Schady & Torebjörk, 1983) whereas signals from nociceptor units usually were not recordable (Torebjörk & Ochoa, 1989*a*). In this study, interest was focused on those preparations in which pain was reported as the threshold sensation during intraneural microstimulation. Such pain may be pricking in quality and projected to a punctate area of skin, or it may be described as burning or dull and projected to significantly larger areas of skin (Torebjörk & Ochoa, 1980; Schady *et al.* 1983*b*). As reported separately, the subjective experience of focal pricking or stinging pain was found to correlate with excitation of A nociceptor units (Torebjörk & Ochoa, 1989*b*). The data presented in this report derive from experimental preparations in which electrical intraneural microstimulation at threshold for conscious sensation gave rise purely to cutaneous pain of dull or burning quality. Thus, experiments yielding pain contaminated with tactile sensations were rejected, as were preparations yielding dull (or burning) pain together with pricking pain. Furthermore, only experiments which allowed identification of sensory units in focus for recording, and hence in the immediate vicinity of the tip of the electrode, were accepted. Such preparations were obtained in twenty-eight among hundreds of experimental attempts. It turned out that, in those experiments, recordings typically revealed signals from C nociceptor units.

It should be emphasized that harvesting C nociceptors in the glabrous skin of the human hand is a difficult aim in human microneurography (Torebjörk & Ochoa, 1989*a*). The detection of these units through prediction from monofocal dull or burning pain evoked by liminal intraneural microstimulation was much more efficient than when searching with the conventional strategy involving natural cutaneous stimulation and intraneural recording of afferent C impulses. The present material is listed in Table 1.

TABLE 1. Quality and localization of cutaneous pain evoked by INMS relative to receptive fields of recorded C nociceptors

Nerve	Projected sensory field (PF)	Skin type	Quality of evoked pain	Pain PF within 10 mm of C nociceptor RF
Median elbow	Thumb pulp	Glabrous	Dull	+
Median elbow	Thumb pulp	Glabrous	Dull	+
Median elbow	Index fingertip	Glabrous	Dull	+
Median wrist	Index finger	Glabrous	Dull	+
Median elbow	Middle finger	Glabrous	Dull	+
Median elbow	Middle finger	Glabrous	Dull	+
Median elbow	Middle finger	Glabrous	Burning	+
Median wrist	Ring finger	Glabrous	Dull	+
Median elbow	Palm thenar	Glabrous	Dull	–
Median* elbow	Palm thenar	Glabrous	Dull	+
Median* elbow	Palm thenar	Glabrous	—	–
Median elbow	Palm thenar	Glabrous	Dull	+
Median elbow	Palm	Glabrous	Burning	–
Median elbow	Palm	Glabrous	Dull	+
Median elbow	Middle finger	Hairy	Burning	+
Ulnar wrist	Little finger	Glabrous	Burning	+
Ulnar wrist	Palm hypothenar	Glabrous	Dull	+
Ulnar wrist	Palm hypothenar	Glabrous	Burning	+
Ulnar wrist	Palm hypothenar	Glabrous	Dull	–
Ulnar wrist	Palm hypothenar	Glabrous	Burning	+
Ulnar wrist	Palm hypothenar	Glabrous	Dull and itch	–
Ulnar wrist	Hand hypothenar	Transitional	Burning	+
Ulnar wrist	Hand dorsum	Hairy	Burning	–
Superficial radial wrist	Hand dorsum	Hairy	Burning	+
Posterior cutaneous forearm	Forearm dorsum	Hairy	Burning	–
Posterior cutaneous forearm	Forearm dorsum	Hairy	Burning	–
Common peroneal knee	Leg lateral lower	Hairy	Burning	–
Superficial peroneal ankle	Foot dorsum metatarsal	Hairy	Dull and itch	–
Superficial peroneal ankle	Third toe proximal phalanx	Transitional	Dull	+

* Data obtained from the same stimulation and recording site.

Intraneural microstimulation

Dull and burning pain from glabrous vs. hairy skin

Pain evoked as threshold sensation during weak INMS delivered in cutaneous nerve fascicles was regularly projected superficially to the skin, as opposed to the deep cramp-like pain evoked by activation of nerve fascicles supplying muscle (Torebjörk & Ochoa, 1980; Ochoa & Torebjörk, 1981; Torebjörk, Ochoa & Schady, 1984*a*).

The quality of pain projected to glabrous skin was often described as dull (thirteen experiments) or less frequently as burning (five experiments). By contrast, pain projected to hairy skin of the dorsum of the hand, forearm or lateral calf was typically reported as burning (all six experiments). Pain projected to transitional skin was reported as burning in one experiment (hypothenar region) and as dull in another (proximal phalanx of third toe). Dull pain and itch were projected simultaneously to the proximal hypothenar glabrous skin in one experiment, and to the dorsum of the foot in another. Thus, a difference in quality of pain was found; it was often described as dull (without temperature component) when projected to glabrous skin of the hand, whereas a burning pain quality was usually reported when projected to hairy skin on the hand, forearm or leg.

Size of projected fields of dull or burning pain

The subjective areas of projection of threshold pain sensations to the glabrous skin of the hand ranged from 20 to 120 mm^2, with a mean of 35 mm^2. Upon increasing the amplitude of the intraneural stimulus without changing its frequency, subjects reported that the area and/or the magnitude of dull pain sensation increased. This was in contrast to other types of cutaneous sensations, like pricking pain or tactile sensations, for which sensory projections usually were smaller and seldom increased in size with increase in stimulus amplitude (Ochoa & Torebjörk, 1983; Schady *et al.* 1983*b*). Areas of dull pain projected to the glabrous skin of the hand were generally smaller than areas of burning pain projected to hairy skin, particularly to the leg and foot, but this feature was not quantified in detail.

Temporal profile, temporal summation and decline of dull or burning pain

Without exception, both dull and burning pain were sustained sensations, without intermittency. In other words, subjects could not detect the frequency of intraneural stimulation. At very low (1 Hz) frequency of stimulation usually no sensation was felt during a 5 s train. At 3 Hz a gradual build-up of pain was noticed, often following a long latency of the order of 2–3 s relative to the onset of the stimulus train. With higher frequencies (5–30 Hz) the build-up of pain was faster, and the magnitude of pain increased proportionally to the stimulus frequency. Prolonged stimulation at high (30 Hz) frequency resulted in decline of the pain sensation, possibly due to excitation failure of thin fibres, as documented in previous work (Torebjörk, Schady & Ochoa, 1984*b*).

Persistence of burning pain evoked by INMS during A fibre block

In two experiments performed on the ulnar nerve at wrist level, a progressive block was achieved through compression-ischaemia by a sphygmomanometer cuff inflated proximal to the site of intraneural stimulation. Dissociated A fibre block was regarded as established when the subjects could no longer feel tactile or cold stimuli, while they could still feel warmth and delayed pain. Under these circumstances, subjects characterized the persistent burning pain evoked by intraneural stimulation and projected to hairy skin in the dorsum of the hand as identical in quality to the burning pain elicited by heat or even by mechanical stimuli applied with a needle to the skin of the numb hand.

Qualities of pain from suprathreshold natural stimulation of glabrous and hairy skin of the hand during A fibre block

In order to understand better why pain from intraneural microstimulation was often reported as dull when projected to glabrous skin, and burning when projected to hairy skin, five experiments were undertaken in which noxious heat from 12·5 cm^2 Marstock thermodes or punctiform noxious mechanical stimuli from needles or sharp wooden sticks were delivered either to glabrous or to hairy skin during dissociated A fibre blocks achieved by compression-ischaemia. At a time when touch and cold sensations were blocked, the sensation evoked by heat stimulation (47 °C) clearly remained as a burning pain sensation in both hairy and glabrous skin. Pain from focal mechanical stimulation was less consistently qualified; it was described as either dull or burning in glabrous skin, and reported as burning in most instances in hairy skin.

Microneurographic recording

Matching quality and projection of pain sensations evoked by intraneural stimulation, with type and innervation territory of recorded nociceptor units

When a dull or burning pain was evoked as the threshold sensation during INMS at very low stimulus amplitude (usually below 0·22 V) subsequent recording was undertaken to identify the fibres in close proximity to the electrode tip. Such recordings usually disclosed multiunit activity from several afferent units of different types, in addition to impulses in efferent sympathetic fibres. The most common outcome was that C nociceptor units were identified within the intraneural recording focus (Table 1). In nineteen instances, the area encompassing the receptive field of these C nociceptor units overlapped or was within 10 mm (centre to centre) of the skin area to which pain had been projected, whereas in ten instances, C nociceptor units were found at greater distances (Table 1, column 5). The spatial correspondence was particularly good for the glabrous skin of the hand where close association (within 10 mm) between receptive and projected fields was found in fifteen of nineteen instances. Thus, the subjective projection and the dull or burning quality of pain evoked by intraneural microstimulation had a predictive value for both type and innervation territory of recordable C nociceptor units. By contrast, low threshold mechanoreceptor units but no C nociceptor units were typically recorded in intraneural sites where stimulation gave rise to non-painful tactile sensations (Torebjörk & Ochoa, 1989*a*).

Clustering of nociceptor units

Figure 1 shows location of the cutaneous receptive fields (●) of fifty-two C nociceptor units recorded microneurographically in twenty-three experimental

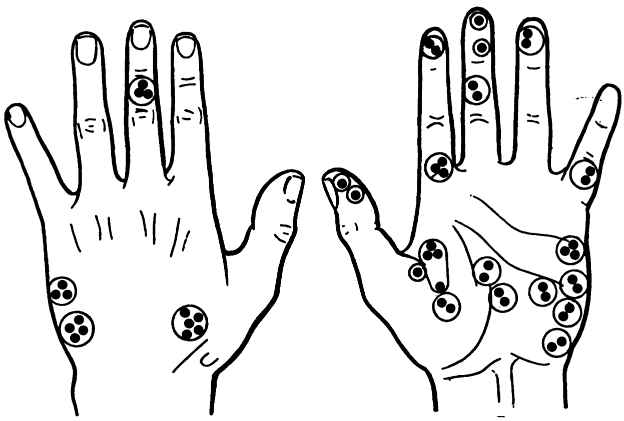

Fig. 1. Location of receptive fields (●) of fifty-two C polymodal nociceptors in the hand. Units recorded together in any one recording site are encircled.

sessions in which previous intraneural microstimulation had evoked threshold sensations of dull or burning pain. The receptive fields of different C units that triggered afferent impulse activity recordable from any one intraneural recording site are enclosed together in a circle. It may be seen that in most instances two or three C nociceptor units which had receptive fields closely clustered in the glabrous skin of the hand were recorded from the same intraneural site. Single C nociceptor units were sometimes recorded from fingertips, whereas clusters of five to ten C nociceptors were recorded in fascicles supplying hairy skin in the dorsum of the hand or in the leg.

Polymodal receptor properties of C nociceptors

C nociceptors in glabrous skin of the hand were polymodal in the sense that they all responded to noxious mechanical and heat stimuli (Fig. 3*C* and *D*). A few units tested also responded to histamine injection and became spontaneously active thereafter. The receptive fields were small, usually 2×2 to 3×3 mm. Conduction velocities ranged from 0·5 to 1·5 m s^{-1}. A detailed description of the receptor properties of human C nociceptors in the glabrous skin of the hand will be reported separately.

'Marking' C nociceptor units by intraneural microstimulation

The application of the experimental strategy introduced by Torebjörk & Ochoa (1989*a*) allowed the conclusion that C nociceptor units identified by recording had been effectively activated by liminal intraneural microstimulation during psychophysical testing. As shown in Fig. 2*B*, a single C unit in focus for recording displayed a fixed latency when excited by electric shocks (0·3 Hz) delivered through intradermal needles. The unit proved to be a nociceptor, responding to noxious heat, and as a consequence of repetitive afferent firing the latency of the electrically

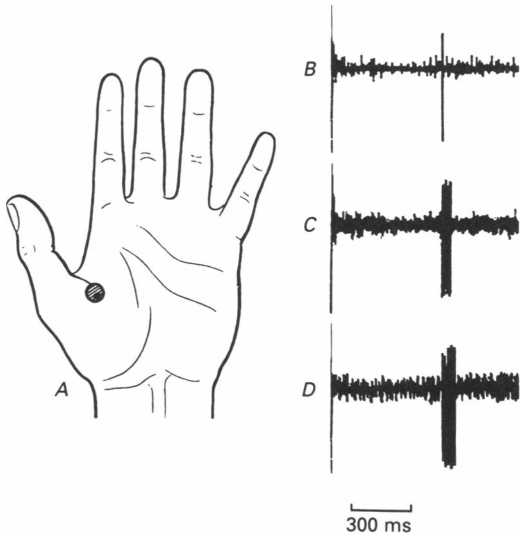

Fig. 2. 'Marking' of C nociceptor unit. *A*, intraneural stimulation at threshold intensity for detection of evoked dull pain projected in the thenar region (hatched area). Inside that area, the receptive field of a C nociceptor was found (●). *B*, constant latency, in the C range, of unitary response elicited by intradermal electrical stimulation (0·3 Hz) in receptive field, recorded at elbow level (conduction velocity: 0·9 m s^{-1}) (five superimposed sweeps). *C*, sudden increase in latency of unitary response elicited by intradermal electrical stimulation (0·3 Hz), caused by activation of the unit by noxious heat stimulus applied to receptive field. Progressive recovery follows (twelve superimposed sweeps). This strategy verified the afferent nature of the recorded C unit (Hallin & Torebjörk, 1974). *D*, increase in latency of unitary response to electrical stimulation of the receptive field (0·3 Hz), caused by intercurrent INMS delivered at intraneural recording site, followed by progressive recovery (twelve superimposed sweeps). This verifies that the unit identified by recording was actually stimulated during INMS and psychophysical testing (Torebjörk & Ochoa, 1989*a*).

evoked response transiently increased (Fig. 2*C*). When the latency recovered, the intraneural microelectrode was used for stimulation at the very same recording site. Delivery of intraneural stimuli at 10 Hz elicited at 0·21 V a threshold sensation of dull pain projected to the receptive field of the unit (Fig. 2*A*). After 40 s, intraneural stimulation was discontinued, and the microelectrode was instantly switched to recording mode and intradermal electrical stimulation to the receptive field was resumed at 0·3 Hz. As a consequence of intraneural activation at the frequency higher than the steady 0·3 Hz, a sharp delay in latency was detected which recovered exponentially over several minutes; the C unit was 'marked' (Fig. 2*D*).

Mismatch between pain projection and innervation territory of C nociceptors

Even though matching was typically found for quality and projection of pain evoked by intraneural microstimulation relative to type and innervation territory of recordable C nociceptor units, mismatching also occurred (Fig. 3), particularly in areas other than the hand. For instance, if the stimulus amplitude necessary to evoke a pain sensation was higher than 0·22 V, then the localized sensation did not predict recordable C nociceptor fibres. The reason for this was probably that the

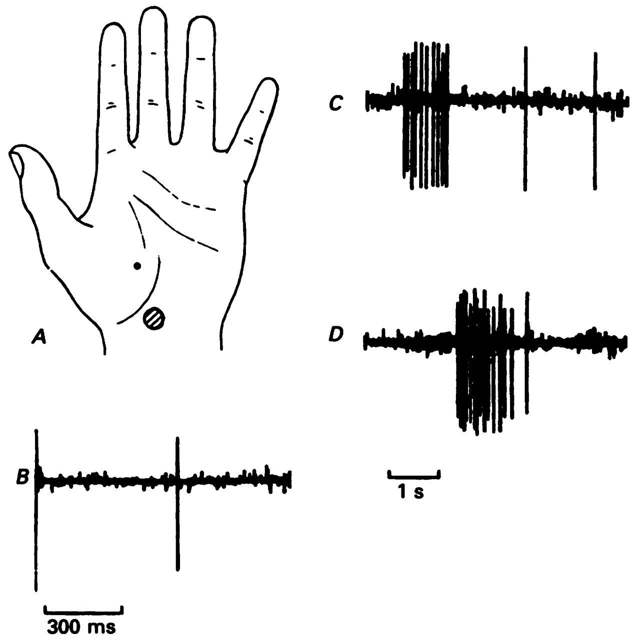

Fig. 3. *A*, projected field of dull pain (hatched area) and mismatching receptive field (●) of recordable C polymodal nociceptor unit. *B*, long latency to unitary response, conduction distance 45·5 cm. Calculated conduction velocity = 0·83 m s^{-1}. *C*, intermediately adapting response to stroking unitary receptive field with blunt stick. Note two after-discharges. *D*, unitary receptor response to heat applied to receptive field.

unmyelinated fibres of these units lay at some distance from the electrode tip and their small signals were not recordable, even though the fibres could be stimulated by large enough currents. Also, for a few single C nociceptor units recorded, intraneural stimulation at 5 and 30 Hz evoked not a sensation of pain, but, rather, non-painful tactile sensations projected outside the receptive field of the recorded unit. Finally, even when pain was perceived as a threshold sensation during stimulation, it was occasionally projected several centimetres outside the receptive field of the C nociceptor(s) present within the intraneural recording focus.

C locognosia

The remarkable matching between the sensory projections of dull or burning pain evoked by INMS, and the innervation territories of C nociceptor fibres in the skin, indicate that subjects can locate a painful event in the glabrous skin of the hand with a substantial degree of accuracy, based on the input from C nociceptive fibres alone. To test this hypothesis further, C locognosia experiments were performed. It was found that the mean error in localizing a hot stimulus during A fibre block was 7·5 mm in the fingers ($n = 52$), and 10·5 mm in the palm of the hand ($n = 44$). This may be compared with the results obtained without nerve block, when the mean error in localizing a tactile stimulus was 4·2 mm in fingers ($n = 13$) and 7 mm in the palm ($n = 11$).

DISCUSSION

Others have also taken advantage of microneurographic recordings of human cutaneous C nociceptors to study somatic sensation. In those studies, the afferent messages have been induced by natural or electrical stimulation of cutaneous receptors, which unavoidably co-activate unknown numbers of units of imperfectly established physiological identity. The latter inconvenience was largely obviated through dissociated A fibre blocks to show that when only C fibres conduct nerve impulses, the residual sensations are warmth and pain (Torebjörk & Hallin, 1973; MacKenzie, Burke, Skuse & Lethlean, 1975). Subsequent studies correlated natural stimulus with C nociceptor frequency response and with subjective experience. Indeed, upon analysing subjective correlates of excitation of single C units activated by natural mechanical, heat or chemical stimuli, Van Hees & Gybels (1981) concluded that, while C nociceptor input correlates well with pain from heat or chemical stimuli, similar C input triggered by mechanical stimulation does not necessarily evoke pain. Comparable findings were reported in the same year by Hallin, Torebjörk & Wiesenfeld (1981). Both studies agreed in concluding that co-activation of other afferents might inhibit nociceptor input at central levels.

The uniquely vast material of C nociceptors collected for the present report, particularly from glabrous skin, can be credited to the application of a new technique for identification of human nociceptors, whereby projected pain evoked by INMS guides the examiner to their receptive fields (Torebjörk & Ochoa, 1989*a*). Issues emanating from the observations that deserve discussion include: (*a*) whether human C nociceptor fibres can be stimulated selectively through intraneural microelectrodes, (*b*) the general meaning of subjective quality and localization of elementary sensations evoked by INMS at C recording sites, and (*c*) clinical implications.

Can human C nociceptor fibres be excited selectively by INMS?

It is possible to document firmly excitation and conduction of impulse activity in human C fibres through supraliminal intraneural stimulation and separate intraneural recording when using two pairs of Vallbo–Hagbarth microelectrodes inserted into the same fascicle, as illustrated in Fig. 1 from Schady *et al.* (1983*a*). It is also clear from similar preparations that block of conduction in the C population, achieved through excitation failure induced by high stimulus frequency, reduces the painful component of the complex 'electrical' evoked sensation, as illustrated in Fig. 2 from Torebjörk, Schady & Ochoa (1984*b*). Thus, it can be accepted that the method used in the present study can effectively induce generation of impulses in mid-axon of C fibre populations, which propagate centrally, eventually to evoke a painful sensation.

Furthermore, the present study shows that intraneural stimulation at liminal intensity, to evoke a purely dull painful sensation projected to the glabrous skin of the hand, may indeed activate the particular V nociceptor unit(s) present within the intraneural recording focus. Thus, it can be accepted that even very low amplitudes of stimulation are sufficient to activate unmyelinated nociceptive fibres, provided that the electrode tip is very close to the fibres. In that sense, the microstimulation technique differs from the conventional technique of stimulating strands of nerve fibres via hook electrodes, which typically activates the largest fibres first at lowest stimulus intensities.

The fact that one particular C nociceptive fibre has been proven to be stimulated does not mean that the resulting painful sensation is necessarily mediated from that fibre alone. On the contrary, several lines of evidence support the view that in most experiments several C nociceptive fibres were co-activated by intraneural stimulation. For instance, it was the rule rather than the exception that several C nociceptive fibres were recordable in any one intraneural electrode site where stimulation gave rise to dull or burning pain, and it is likely that several C fibres in such close proximity to the electrode tip were co-activated during intraneural stimulation. Furthermore, the observations that projected areas of dull or burning pain were usually larger than the receptive fields of any individual C nociceptor unit, and that the projected fields grew in area and the pain sensation increased in magnitude when the stimulus amplitude was gently increased, all speak in favour of co-activation of several C nociceptive fibres. This notion is further strengthened by the fact that several C nociceptor fibres recordable at one site in the nerve had their receptive fields clustered closely together in the skin of the hand. Such intraneural clustering and implied parallel course of C human nociceptor axons within the multiaxonal Schwann cell columns would result in monofocal projection of pain even when several fibres are co-activated. Thus it must be accepted that in most instances several C nociceptor units were stimulated together when a purely dull or burning pain sensation was perceived.

The next question is whether sensory units other than C nociceptors were also co-activated intraneurally. This must have happened on many occasions when subjects announced tactile or pricking pain sensations in addition to dull or burning pain. It has been shown in previous work that non-painful tactile sensations projected to the glabrous skin of the hand are evoked by intraneural stimulation of certain types of low-threshold mechanoreceptor units (Torebjörk & Ochoa, 1980; Vallbo, 1981; Ochoa & Torebjörk, 1983; Schady *et al.* 1983*a*, *b*; Vallbo *et al.* 1984), while pricking pain sensations projected to the glabrous skin are associated with stimulation of A mechano-nociceptor units (Torebjörk & Ochoa, 1989*b*). Since preparations contaminated with tactile or pricking pain sensations were not included in the present material, there is no reason to suspect co-activation of units other than C units when purely dull or burning pain was the threshold evoked sensation. It is conceivable that co-activation of units which require considerable spatial or temporal summation to elicit a conscious sensation may have occurred without reaching consciousness. It is, in fact, known that when 'silent' units, like single SA II mechanoreceptors are activated, no sensation is evoked (Torebjörk & Ochoa, 1980; Ochoa & Torebjörk, 1983).

Other features that speak in favour of fairly selective stimulation of C nociceptive axons in the present material are the temporal summation of dull or burning pain reported here, which is consistent with temporal summation of second pain (Price, Hu, Dubner & Gracely, 1977), and the identification of burning pain evoked by INMS with second burning pain evoked by noxious heat during A fibre block. The ability to induce purely dull or burning pain and the occasional contamination with pricking pain finds a satisfactory anatomical correlate since, in the endoneurium of human cutaneous nerve fascicles, unmyelinated fibres tend to segregate from large myelinated fibres while sometimes associating with small myelinated fibres (Ochoa, 1976; Thomas & Ochoa, 1984).

Qualities of pain from C nociceptor activation

The present observation that C polymodal nociceptors were recorded in intraneural sites where stimulation gave rise to painful sensations, but were usually not recordable in intrafascicular sites yielding non-painful tactile sensations upon stimulation, constitutes by itself a strong argument towards the concept that dull or burning pain is a natural subjective response to impulses initiated in primary C nociceptor units. This is not to say that excitation of primary sensory units with electrophysiological characteristics of C nociceptors *must* evoke dull pain when excited, since a minority of them evoked itch (Torebjörk & Ochoa, 1981), as will be reported separately. The same observation serves as indirect evidence for our claim that excitation of a particular identified C nociceptor preparation, either pre-recorded or predicted by sensation evoked by INMS, was the primary basis for the pure dull or burning pain sensation evoked at threshold.

It was an interesting finding that pain evoked from intraneural stimulation of C nociceptor fibres was often described as dull when projected to glabrous skin, while a burning quality was usually reported when pain was projected to hairy skin, and clearly the qualitative difference was real and not a semantic artifact. However, this does not mean that burning pain from C fibre stimulation cannot be felt in glabrous skin. Unsurprisingly, in control experiments using a large Marstock probe (Fruhstorfer *et al.* 1976) for stimulation, naïve subjects reported burning pain from glabrous as well as from hairy skin of the hand in response to noxious heat stimuli during block of impulse conduction in A fibres. Thus, burning pain emerges as a normal sensory quality of C fibre pain from hairy and glabrous skin, provided that the area of stimulation is fairly large. By contrast, a pointed noxious stimulus from a needle applied on the glabrous skin of the hand was reported as either dull or burning pain during A fibre block. Thus, it seems that the spatial characteristics of the stimulus are important as determinants of whether C fibre pain from glabrous skin is felt as dull or burning. As reported elsewhere (Torebjörk & Ochoa, 1989*a*), fewer C nociceptor units were grouped together in recording from cutaneous nerve fascicles supplying glabrous rather than hairy skin. It is reasonable to assume that fewer C nociceptor units were also microstimulated in cutaneous nerve fascicles destined for glabrous as opposed to hairy skin. Thus the spatial content of the afferent C fibre message may differ when stimulating nerves supplying glabrous *vs.* hairy skin, and this may perhaps explain the observed regional differences in quality of evoked C fibre pain. This interpretation would be in harmony with the suggestion of Chery-Croze & Duclaux (1980) that 'a burning sensation would arise only when a sufficient number of polymodal nociceptors were activated'. Alternatively, co-activation of different types of sensory units, for example, warmth-specific units, might perhaps occur more frequently when stimulating nerve fascicles supplying hairy rather than glabrous skin, thereby contributing to the burning quality of pain.

Localized projection

Experimental evidence put forward by Nathan & Rice (1966) indicated that the sensations of warmth and heat from thermal stimulation of the skin are localized with a degree of accuracy which is almost as good as the localization of tactile stimuli. Their observations are supported by the C locognosia tests presented here, showing that the mean error in locating a hot stimulus to glabrous skin in the hand, from a pure C fibre input, is of the order of 7–10 mm. This is to be compared with a mean error of the order of 4–7 mm for localization of tactile stimuli, when using all available inputs provided by the unblocked nerves, as shown both in this and in previous studies (Hamburger, 1980; Schady, Torebjörk & Ochoa, 1983*c*). Thus, it appears that the C fibre system can provide useful input for fairly accurate localization of noxious events, at least in the glabrous skin of the hand. Furthermore, this capacity for localization persists even if the amount of nociceptive C fibre input is reduced to a minimum for conscious detection, as observed in our microstimulation experiments. In fifteen of twenty instances, the threshold sensation of pain from intraneural microstimulation was projected within 10 mm of the receptive field areas of recordable C units in the glabrous skin of the hand, as detailed in Table 1. Thus, it appears that for the C nociceptor system, as for the A fibre tactile systems, little is gained in terms of stimulus localization by co-activating numerous cutaneous units with overlapping receptive fields (Schady *et al.* 1983*c*). This is surprising in view of the traditional notion that C pain is poorly localized. Such an obviously erroneous generalization probably originates from clinical experiences of diffusely localized dull pain from visceral irritation. The same applies to pain experimentally evoked by INMS of muscle afferents, which we have shown, even at liminal stimulus amplitude, to project to a rather large, deep, vague locus and often to refer remotely (Torebjörk *et al.* 1984*a*). It seems obvious that accurate cerebral localization function requires very refined circuitry, and thus is not available for pain as an internal alarm. Such function appears reserved for pain as a signal of noxious challenge to the body surface which needs to be well localized for the purposes of removal of the agent, projection of the injured site or inhibition by gentle rubbing or scratching.

Clinical implications

The experimental data support the view that the delayed cutaneous pain sensation, often described as dull or burning, is normally mediated by activation of C polymodal nociceptors, and that such pain is projected to the innervation territory of the stimulated nociceptors in the skin with fairly good accuracy, at least in the hand. It is therefore not surprising that spontaneous pain or the hyperalgesic response emanating from pathologically hyperexcitable human C polymodal nociceptors has a dull, often burning, subjective quality. These subjective response characteristics have now been reported not only for experimentally sensitized human C polymodal nociceptors (Torebjörk & Hallin, 1977; Torebjörk, LaMotte & Robinson, 1984*c*; Culp, Ochoa, Cline & Dotson, 1989) but also for C polymodal nociceptors sensitized by disease (Cline, Ochoa & Torebjörk, 1989). It must be pointed out, however, that the dependence of pain upon nociceptor input and the relatively good accuracy in localizing pain from cutaneous C nociceptor stimulation, as reported here for weak pain sensations induced in normal subjects, may not hold true for severe experimental or pathological pain, where changes in the central processing of nerve signals might upset both specificity in afferent input for pain, and accuracy in projection of pain (H. E. Torebjörk, L. E. R. Lundberg & R. H. LaMotte, unpublished observations).

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