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Clustering of Pacinian corpuscle afferent fibres in the human median nerve

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Abstract To further study the functional organisation of human peripheral nerves, the intrafascicular arrangement of afferent fibres supplying Pacinian corpuscles (PCs) was explored by percutaneous microneurography using thin-calibre, concentric needle electrodes. In normal adults, 20 PC afferents were identified in 13 recording sites. Low-amplitude (less than 30 μm) vibratory stimuli to the skin were applied with tuning forks oscillating at 128 Hz or 256 Hz and response patterns of individual PC units were studied. In many recording sites, two, sometimes even three, PC afferents with adjacent or overlapping receptive fields in the hand were clustered in the nerve. The observed incidence in the records containing a certain number of PC units was compared with the expected probability calculated according to the hypothesis that all nerve fibres are randomly organised in peripheral nerves. The results suggested that PC afferents are partially segregated in the nerve. In addition, PC afferents were neighbouring on slowly adapting type II (SAII) units and skin sympathetic activity in individual fascicles. SAII units often innervated the same skin area as PC units, but did not respond to vibration. The data provided additional information regarding the functional organisation of the human peripheral nerve and the mechanisms underlying the sense of vibration in man with special regard to population behaviour of neighbouring PC mechanoreceptors.

Key words Microneurography · Peripheral nerve · Pacinian afferent · Cutaneous mechanoreceptor · Vibrotactile stimuli · Human

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Introduction

Pacinian corpuscles (PCs) are sensory receptors with wide distribution in the body, but mainly found in the skin of the hand and foot. Their structure and function as vibrotactile detectors have been extensively studied (Hyvärinen et al. 1968; Talbot et al. 1968; Mountcastle et al. 1969; Knibestöl and Vallbo 1970; Horch 1991; Birder and Perl 1994). They exhibit extreme sensitivity to high-frequency vibration and a time-locked response, usually 1:1, to the stimulus. There is a narrow range between the mechanical threshold evoking any response at all and the stimulus intensity at which the PCs have their tuning points. The results of psychophysical tests have suggested that sensory information from PCs might be mediated via a distinct sensory channel (Bolanowski et al. 1988, 1994), which could act as a critical band within the somatosensory system (Makous et al. 1995). In agreement with this concept, decreased vibration perception has been detected early in suspected neuropathies with normal skin sensibility (Lindblom 1981). Deficient vibrosensation has also been found during aging (Gescheider et al. 1994). Based on the outcome of psychophysical studies, it has been proposed that groups of PC mechanoreceptors might work together within the Pacinian channel (Verrillo 1963; Bolanowski et al. 1988; Gescheider et al. 1994).

Percutaneous microneurography with a thin-calibre concentric electrode (Hallin and Wiesenfeld 1981) allows simultaneous studies of both functional aspects and anatomical arrangements of nerve fibres within even a part of a fascicle (Hallin 1990; Hallin et al. 1991). Data acquired with this technique has provided evidence

against the idea of a random intraneural nerve fibre organisation (Sunderland 1945; Schady et al. 1983) Instead, there seemed to be a general segregation of human cutaneous afferents by function, but segregation of PC afferents was not conclusively demonstrated in these earlier studies, which in part might have been due to the methods employed (Hallin 1990; Hallin et al. 1991, 1994; Ekedahl et al. 1996a, 1997). However, by using interspike interval analysis of mechanically evoked responses, it has been shown recently that PC units tend to cluster intraneurally (Ekedahl et al. 1996b).

The aim of this study was to further examine the intrafascicular arrangement of human PC afferents. Statistics different from those previously used (Hallin et al. 1991, 1994) were developed. Since PC units are infrequently encountered in microneurographic recordings (Johansson and Vallbo 1979; Hallin 1990), we evaluated the likelihood of encountering one, two or more PC units at any individual recording site under the hypothesis that all nerve fibres are randomly organised intraneurally.

Materials and methods

Subjects

More than 100 experiments were performed on normal volunteers (16 women and 25 men) in this study, which was approved by the local ethics committee. The subjects were 20–42 years old and without any neurological or dermatological disease. According to the Declaration of Helsinki, the detailed experimental procedures were explained to each subject and informed consent to participate in the trials was obtained from all subjects. The subject was reclining with one arm extended during the experiment. Skin nerve activity was recorded from the median nerve at elbow level.

Recording procedure

The experiments were carried out at room temperature (ca. 22°C). Standardised concentric needle electrodes with a single recording surface at the tip, about 20×30 µm, were used (Hallin and Wiesenfeld 1981). The electrode was freshly sterilised by formalin steam or an autoclave before the trial. After sterilisation the electrical property of the electrode was checked and its tip configuration inspected. The electrode was manually inserted through the skin into the nerve. Electrical stimulation was applied through the electrode tip in order to guide the passage of the electrode into the nerve (1–5 V, 0.2 ms, 3 Hz; Grass S8800, USA). Paraesthesia irradiating into the innervation area was reported by the subject when the tip of the electrode impaled the nerve fascicle. After switching the system from stimulation to recording (Neurolog System, Digitimer, UK), single-unit recordings with a high signal-to-noise ratio were encountered following repeated electrode adjustments. The signals were monitored on an oscilloscope and were simultaneously stored on tape (TEAC XR-30H Cassette Data Recorder, Japan) for later off-line analysis. The bandwidth of the whole system, including the tape recorder, was from 200 Hz to 10 kHz. For a detailed description of the procedure, see Hallin and Wu 1998.

Test stimuli

The cutaneous stimuli included constant skin pressure or intermittent light touch stimuli exerted with a cylindrical wooden stick, 1 mm in diameter, or an algometer probe (Somedic, Sweden). Skin vibration was performed with tuning forks oscillating at 128 Hz or 256 Hz. Their shafts were cylindrical and had a contact area with the skin with a diameter of about 1 cm (Förbandsmaterial, Partille, Sweden).

The vibratory displacements of the tuning forks were evaluated (Fig. 1). The instrument to be tested was securely fastened at its branching point between two flexible beams of glass fibre epoxy laminate. This way the clamping of the fork in the test rig did not obstruct or interfere with the free movements of the legs and shaft of the fork. At rest a simple U-shaped metal tool held the legs of the fork tightly together. Excitation of the fork was accomplished by momentarily freeing the legs from the grip of the tool by instantly pulling away the tool from the secured fork. Like the vibratory oscillations applied during the experiments, the vibrations of the tuning fork in the test rig were perceived for a fairly long time, about 20 s. The excursions of the shaft of the unloaded fork in air were measured 3 s and 4 s after excitation (Fig. 1). There was a similar time lag between excitation of the fork and vibratory testing during the microneurography experiments. Thus it was considered that the measured excursions roughly corresponded to the vibratory oscillations the fork delivered at the moment when it, during the trials, was applied to the skin. It was presumed that the decay rate of the oscillations increased when the fork was loaded during experimentation.

A fibre-optic displacement sensor was used to measure the amplitudes of the oscillations at the shaft of the tuning fork. The distance between the fibre-optic probe and the centre of the end of the shaft was 0.6 mm. The probe was kept perpendicular to the circular surface at the end of the shaft where a piece of white tape was attached to obtain uniform optical conditions. The probe, operating according to the light intensity variation principle (Krohn 1992), was manufactured from PMMA (Plexiglas) plastic optic fibre with a core diameter of 1 mm. The easily installed one-fibre probe method was applied (Krohn 1992) after the fibre was cut and bonded in a Y-configuration. Constant light emitted at a wavelength of 660 nm with a spectral bandwidth of 25 nm was fed through one branch of the bonded fibre, whereas the other branch was used for detection of the light intensity variations reflected from the moving tuning fork shaft (Fig. 1). A micrometer translation unit was used for calibration of the sensor before and after the trials. The photo detector output was recorded on a Tektronix TDS 210 digital oscilloscope, stored and transformed by a PC computer and finally analysed using computer software. The different steps of the applied procedures, briefly touched upon above, will be described in detail in a subsequent report.

Classification of single units

The receptive field characteristics of the units were tested with von Frey hairs (Research Media, USA) exerting a force 4 times threshold. Four types of cutaneous mechanoreceptive units with myelinated fibres were classified. PC units were characterised by their rapid adaptation to constant pressure. They had a large innervation area and responded with high fidelity to vibration (Talbot et al. 1968; Mountcastle et al. 1972; Knibestöl 1973; Johansson and Vallbo 1979, 1983; Vallbo et al. 1979; Hämäläinen et al. 1985; Hallin et al. 1991; Horch 1991). Unlike PC units, rapidy adapting (RA) units had a small receptive field with a well-demarcated border (Talbot et al. 1968; Knibestöl 1973; Johansson and Vallbo 1979, 1983; Vallbo et al. 1979; Hallin et al. 1991) and did not reliably follow high-frequency vibration (Talbot et al. 1968). Slowly adapting type I (SAI) and slowly adapting type II (SAII) units discharged continuously during constant skin pressure (Chambers et al. 1972; Johansson and Vallbo 1979, 1983; Vallbo et al. 1979; Hallin et al. 1991; Birder and Perl 1994). SAI units had small and well-defined receptive fields, whereas SAII units had relatively large receptive fields with obscure boundaries. Some SAII units carried a spontaneous discharge in the absence of any specific stimulation (Chambers et al. 1972). On constant skin pressure, SAI units fired irregularly, whereas SAII units discharged regularly. In particular, SAII units were easily excited by skin stretch in a preferred direction (Chambers et al. 1972; Edin 1992).

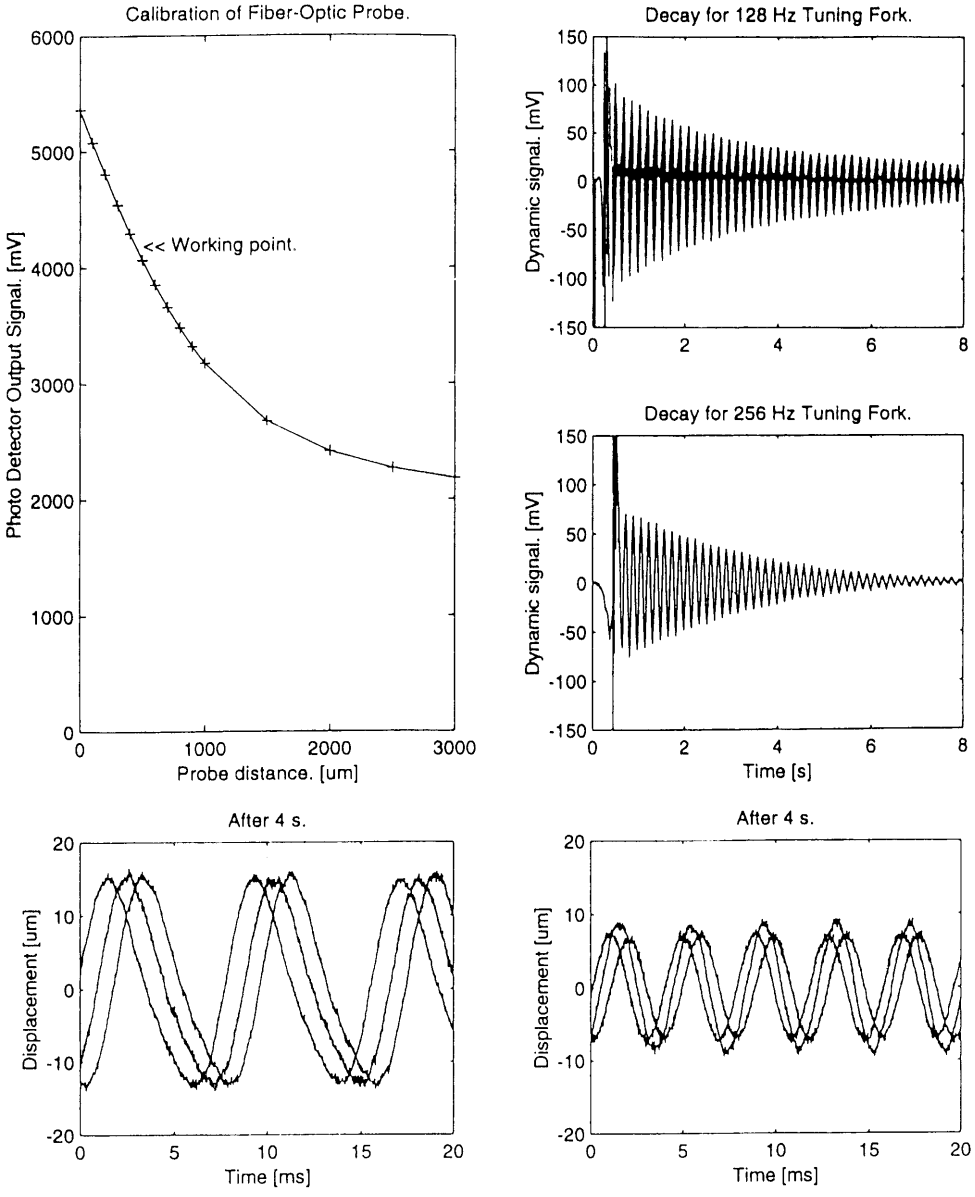

Fig. 1 Photodetector calibration curve demonstrating how the magnitude of the photodetector output signal changes as a function of the distance between the probe and the studied object (*upper left*). The amplitude decreases in vibratory stimuli with time deriving from tuning forks oscillating at 128 Hz and 256 Hz are displaced to the *right*. In the decay plots, the waveforms are scrambled by alias components owing to insufficient sampling speeds. For these particular cases, however, the decay envelopes are still relevant. The large artefacts to the *left* in the records indicate moment of excitation of the tuning forks. The *bottom panels* show the reproducability of the tests and display the displacement amplitudes (micrometres) and shapes of the tuning fork oscillations at 128 Hz (*left*) and 256 Hz (*right*) 4 s after fork activation

Signal analysis

Off-line analysis of the neural data was performed with CED data analysis software (Spike2, 2.0β2; Cambridge Electronic Design; Wu et al. 1996, 1997, 1998; Hallin and Wu 1998). Neural signals were played back from the tape into a computer (Power Macintosh 7100/66) via an interface (CED 1401 plus; Cambridge Electronic Design) with a sampling rate of 100 kHz. Template matching, interspike interval analysis and spike-triggered waveform averaging were performed. All action potentials were identified and classified as numbered events by extracting a small waveform fragment (wavemark) of each spike from the unprocessed neural sequence during template matching processing to build up a wavemark channel. Interspike interval histograms were compiled from the spikes displayed on the wavemark channel. By using selected wavemarks as trigger signals, it was possible to separately average different action potentials in the original neurogram. The various steps of the applied procedures have been described in detail elsewhere (Hallin and Wu 1998).

Table 1 Occurrence of recording sites with *k* Pacinian corpuscle (PC) units among a total of *n* units

k	*n*	Probabilities			Observed numbers
		P_k[a]	P_n	$(P_k \cdot P_n)$	
1	1	0.1500	0.6566	0.0985	4
1	2	0.2550	0.2349	0.0599	2
2	2	0.0225	0.2349	0.0053	2
1	3	0.3250	0.0663	0.0215	1
2	3	0.0574	0.0663	0.0038	1
2	4	0.0975	0.0301	0.0029	2
3	5	0.0244	0.0060	0.0001	1
			Total	0.1920	13

[a] $P_k = nCk \cdot P^k \cdot (1\text{-}p)^{n-k}$. Assumption for calculation of P_k was that all fibres irrespective of functional class are randomly distributed in the nerve fascicle with a proportion of 0.15 for PC units (P=0.15; Johansson and Vallbo 1979). The values of P_n were derived from the data obtained in our previous study (Hallin et al. 1994)

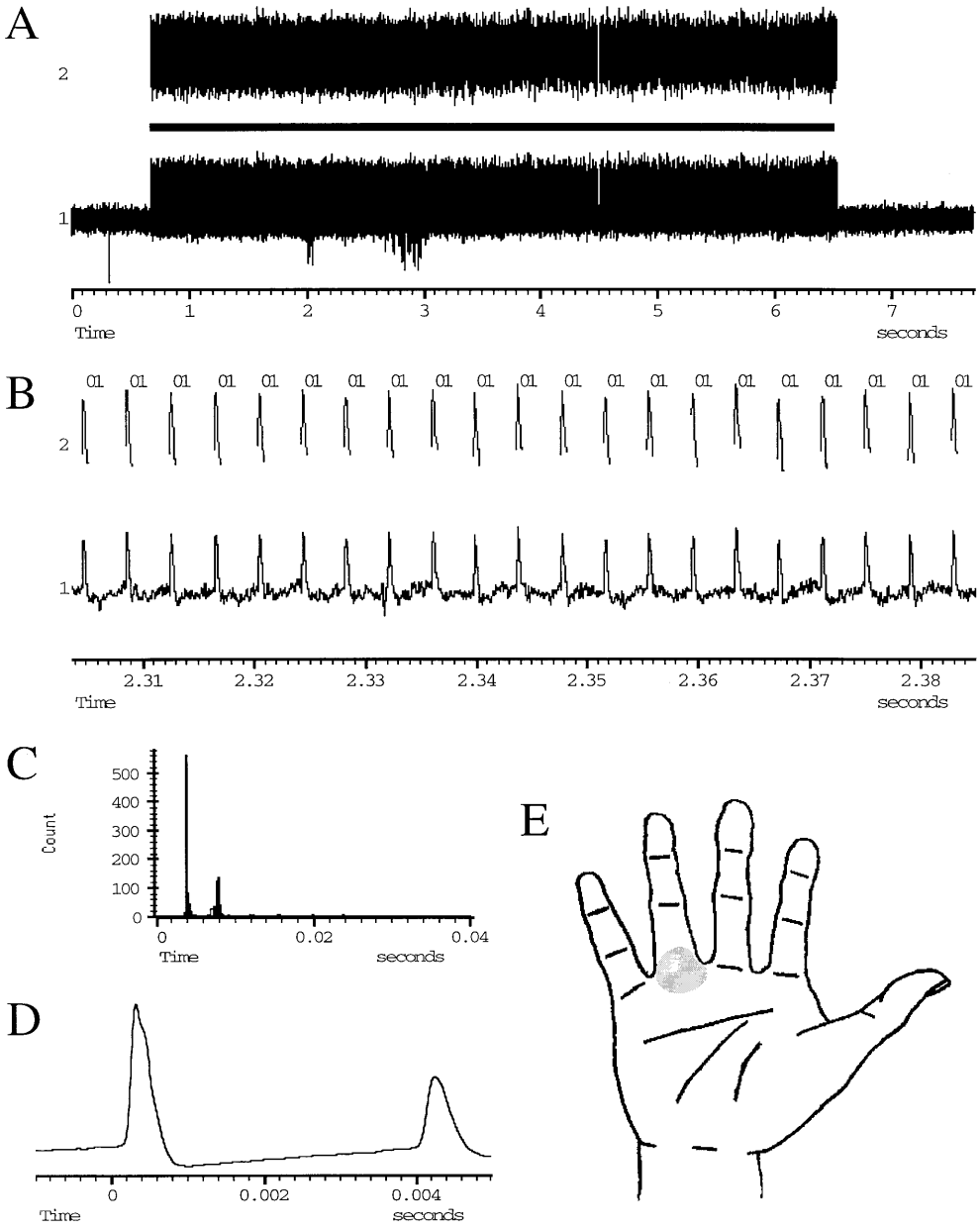

Fig. 2A–E Typical response of a single Pacinian corpuscle (PC) unit to vibration. In **A**, unprocessed signals are shown in *trace 1* (waveform channel), and small fragments of the action potentials extracted from the original signals by template matching are displayed in *trace 2* (wavemark channel). This PC unit did not fire spontaneously at rest, but it responded vigorously to vibrotactile stimulation (tuning fork, 256 Hz, indicated by *bar*). A part of the response is shown at an expanded time scale in **B**. The unit discharges appeared at regular intervals with uniform shapes (same wavemark numbers). In **C**, an interspike interval histogram computed from the wavemark channel is displayed. Two main peaks occurred at 3.9 ms and 7.8 ms, which corresponded to firing at 1:1 or 1:2 (i.e. one spike per 1 or 2 stimulus cycles). In **D**, signals averaged by spike-triggered signal averaging are shown. The first big deflection derived from spikes used as sweep triggers. A flat baseline followed before a small deflection appeared at an interval of ~4 ms. In **E**, the receptive field extension of the unit is schematically shown

Statistics

The relative frequencies of the occurrence of the four main classes of mechanoreceptive units encountered in microneurography have been previously reported (Johansson and Vallbo 1979). In the human median nerve, PC units occurred less frequently than the other three types of units, and the proportion of PC units was about 0.15 among the unit population as a whole. According to the law of binomial distribution, the probability (P_k) of recording one or more (k) PC units at a recording site among a large number of randomly obtained recordings containing a given number (n) of units can be calculated with the assumption that the proportion of PC units was 0.15 (Table 1). Under this assumption, 26% of all sites with a total of two units would be sites with one PC unit and one additional unit of any other modality and only 2% would be sites yielding two PC units. In the rest of the sites with two units (72%, not listed), no PC units would be encountered. To further calculate the general probability of recording k PC units in recordings with various numbers (n) of units among all the experimentally obtained recording sites, the proportion (P_n) was considered as listed in Table 1. P_n constituted the likelihood to encounter n units, irrespective of modality, in any site among all recording sites. The values of P_n listed in Table 1 were derived from a previous study on the median nerve where a large number of randomly encountered recording sites, each yielding a variable number of units,

Table 2 Expected and observed incidence of recording one or more (k) PC units per site (data derived from Table 1)

	Expected	Observed
k=1	0.1799/0.1920=0.94	7/13=0.54
k>1	0.0121/0.1920=0.06	6/13=0.46
Total	1.00	1.00

The 95% confidence interval for the observed proportion (6/13) is 19.22–74.87%. The expected proportion (6%) is outside this interval; thus, P<0.05

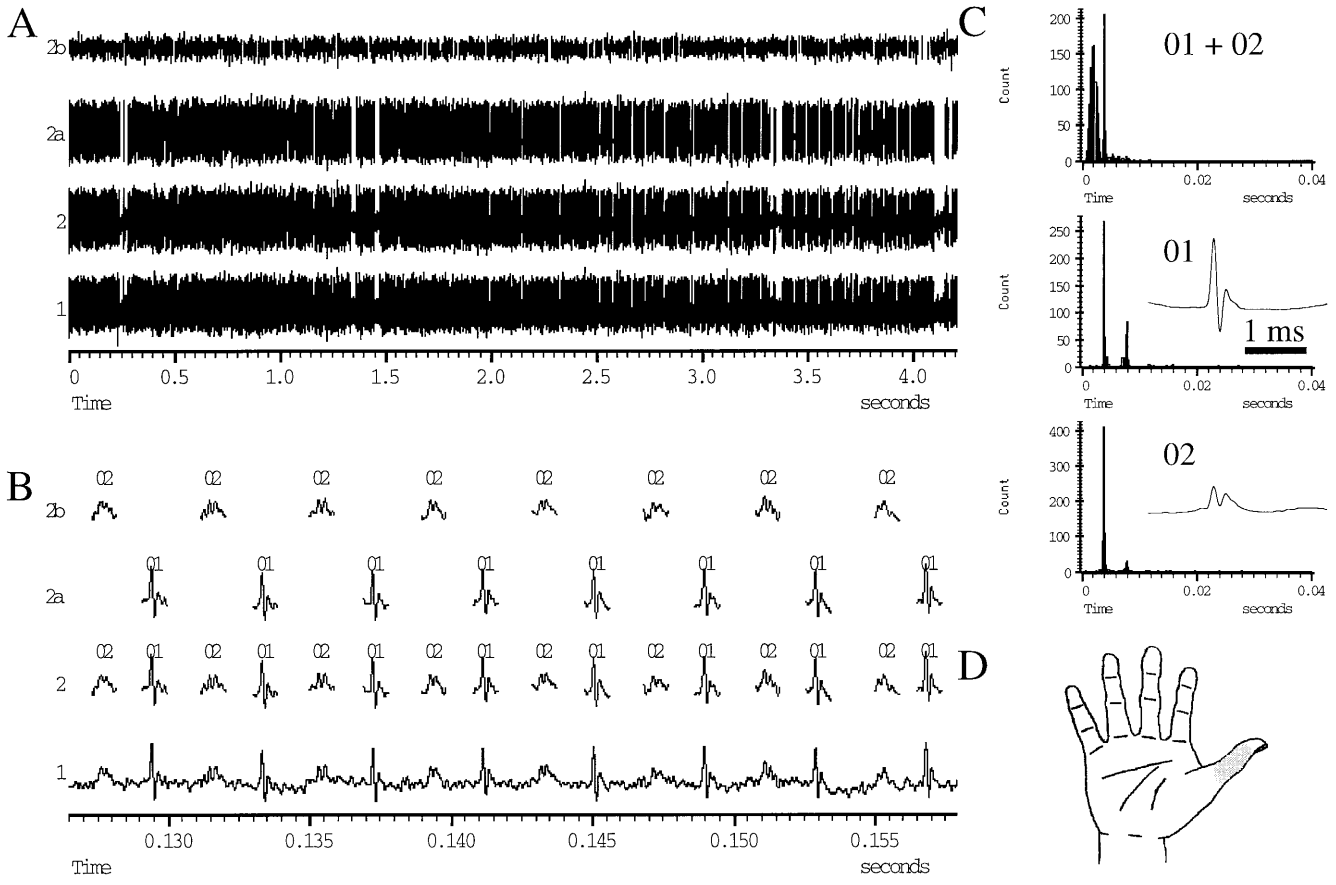

Fig. 3A–D Co-activation of two PC units by vibrotactile stimuli. In **A**, the vibratory induced response is shown: *trace 1* displays unprocessed signals and *trace 2* extracted spikes. Two PC units were identified by differences in action potential waveforms and displayed separately in *traces 2a and 2b*, two traces split from trace 2. The details of the separation are shown in **B**. The action potentials of the big unit were labelled *01* by the template matching and the potentials of the small unit *02*. In **C**, interspike interval histograms computed from each unit separately or the two together indicated that each unit had a 1:1 or 1:2 firing pattern, which confirmed the PC character of the neural activity. Together they produced short interspike intervals easily detected in the histogram, which proved that they were of different neural origin. *Insets* are averaged action potentials of the two units. The extension of their overlapping receptive fields is schematically shown in **D**

were investigated using concentric needle electrodes (Hallin et al. 1994). For instance, in 66% of all recordings only one unit (n=1) was encountered and in 23% two units (n=2) were recorded. By multiplying P_k by P_n, the general probability of predicting how often k PC would occur with n units among all recordings could be calculated (see the column $P_k \cdot P_n$ in Table 1). For instance, the likelihood of encountering one recording site yielding three PC units among a total of five units has a probability of occurring in less than 0.1‰ of the recordings.

Non-parametric statistics were performed with a binomial test to compare the calculated probabilities of encountering k PC units with n units in the recordings with the actually observed incidence frequencies.

Results

PC units were encountered in 13 of 110 experiments (a total of 128 recording sites) performed for this study. In all experiments, cutaneous median nerve fascicles were explored, and in each of the 13 experiments with PC recordings one site was obtained. A total of 20 PC units were identified at the 13 sites. Two PC units had a spontaneous discharge, whereas the rest were silent until activated by tactile stimulation. In many of the recording sites (6/13), skin sympathetic activity (SSA) was also recorded at the same time (e.g. Fig. 2).

Clustering of PC units

Some important features of all the PC recordings are summarised in Table 1. The “observed numbers” to the right list the number of recording sites with different numbers of PC units combined with other units. Many recording sites contained more than one PC unit. A further evaluation of potential PC clustering is shown in Table 2, where the expected incidence of recording one or more PC units in any site was compared with the experimentally observed findings. The outcome of the comparison indicated that recordings containing more than one PC unit occurred more frequently than would be expected if the nerve fibres were randomly organised, suggesting that PC units are segregated in the nerve.

Figure 2 illustrates a typical response of a single PC unit elicited by cutaneous application of a tuning fork oscillating at 256 Hz. Four features of the response were noted: (1) the unit had uniform action potentials of similar amplitudes; (2) a 1:1 (one spike per stimulus cycle)

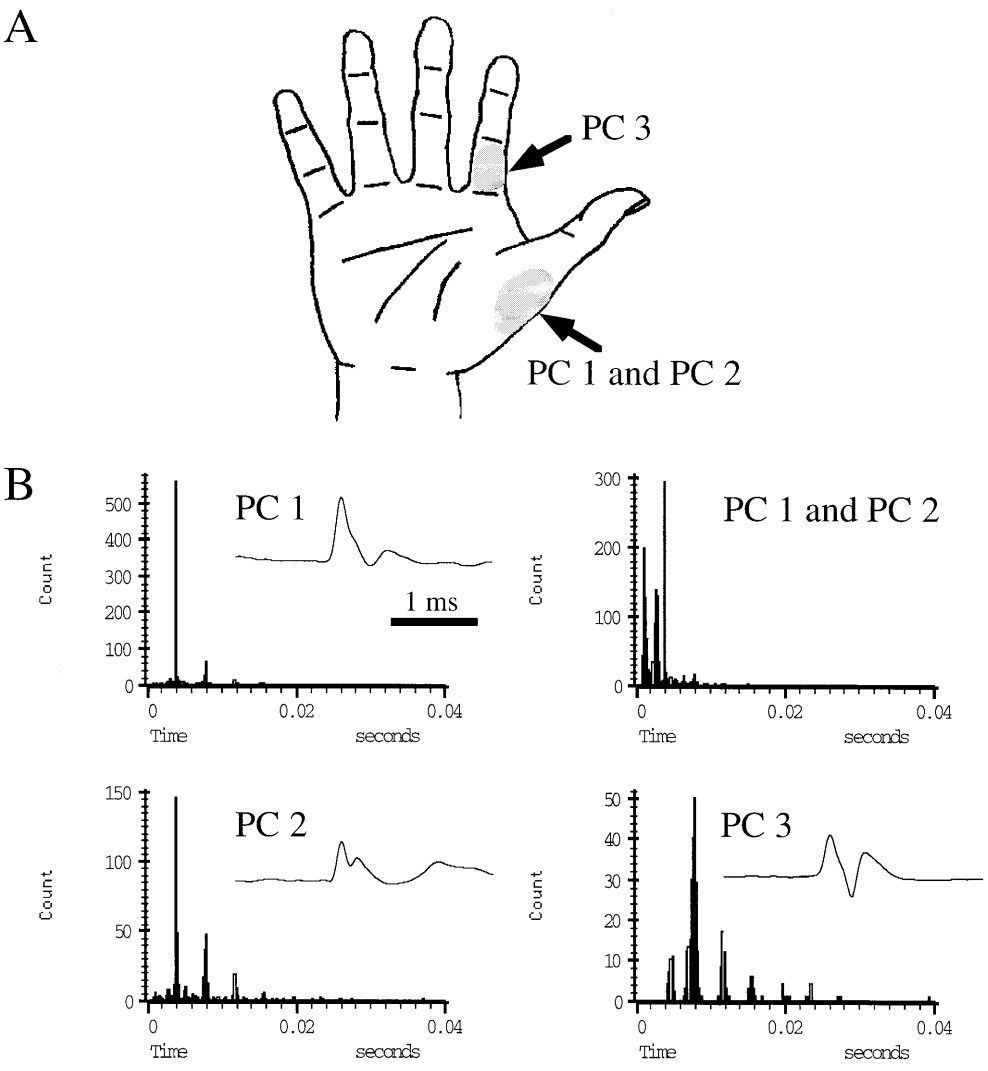

Fig. 4A, B Three PC afferents recorded as neighbours in the median nerve. Location of the three PC units is shown in **A**. PCs 1 and 2 innervated the same skin area and their receptive fields were separated from PC 3. The discharge patterns and action potential waveforms are illustrated in **B**. PCs 1 and 2 discharged simultaneously when vibration (256 Hz) was applied to the thenar eminence of the hand, whereas PC 3 was activated separately when the vibration was applied to the base of the index finger. These three PC units had distinctly different action potential waveforms and slightly different interspike interval distributions, which indicated that they were of different neural origin

or 1:2 (one spike per two stimulus cycles) discharge pattern was apparent from the compiled interspike interval histogram; (3) there were no short intervals in the response; (4) the receptive field of the unit was diffusely outlined at the base of the 4th digit. All these features indicated that a single PC unit was recorded. In addition, Fig. 2, trace 1, demonstrates that negative-going mass discharges of sympathetic outflow also occurred irregularly in this recording.

In many recordings more than one PC unit was discriminated. Some additional characteristics in the obtained records revealed their presence. A pair of PC units responding to vibration at 256 Hz, which was clearly distinguishable by interspike interval analysis, is illustrated in Fig. 3. Two individual units of different amplitudes were observed in the original neurogram. The large-amplitude unit had triphasic action potentials and the small one a double-peaked waveform. Both units were characteristic PCs and were robustly entrained by the vibration. Short interspike intervals only occurred in the histogram when the two populations of spikes were computed together (Fig. 3C), which verified their different neural origin.

In one experiment, three PC units were recorded simultaneously, illustrated in Fig. 4. Two of the PC units responded when a vibrating tuning fork (256 Hz) was applied to the thenar eminence. At the same recording site, another PC unit was recorded when the tuning fork was moved to the base of the 2nd digit. All three PC units had different action potential amplitudes and waveforms. Additionally, unit 3 discharged in a 1:2 manner during most tests, which was different from units 1 and 2, which responded 1:1. It is worth noting that no cross-activation was observed between the two test areas by vibration with the tuning fork, which suggested that the spread of the vibratory oscillations from the stimulus site was limited during the tests. The outcome of the trials also indicated that the receptive field of individual PC units might be more restricted than previously realised.

The clustered PC afferents responded synchronously to the applied vibration, which suggested that they had at least partly overlapping innervation areas in the hand. Testing with non-vibratory stimuli for detailed localisation of their receptive fields also indicated that the clustered PC units regularly were located in the same digit or

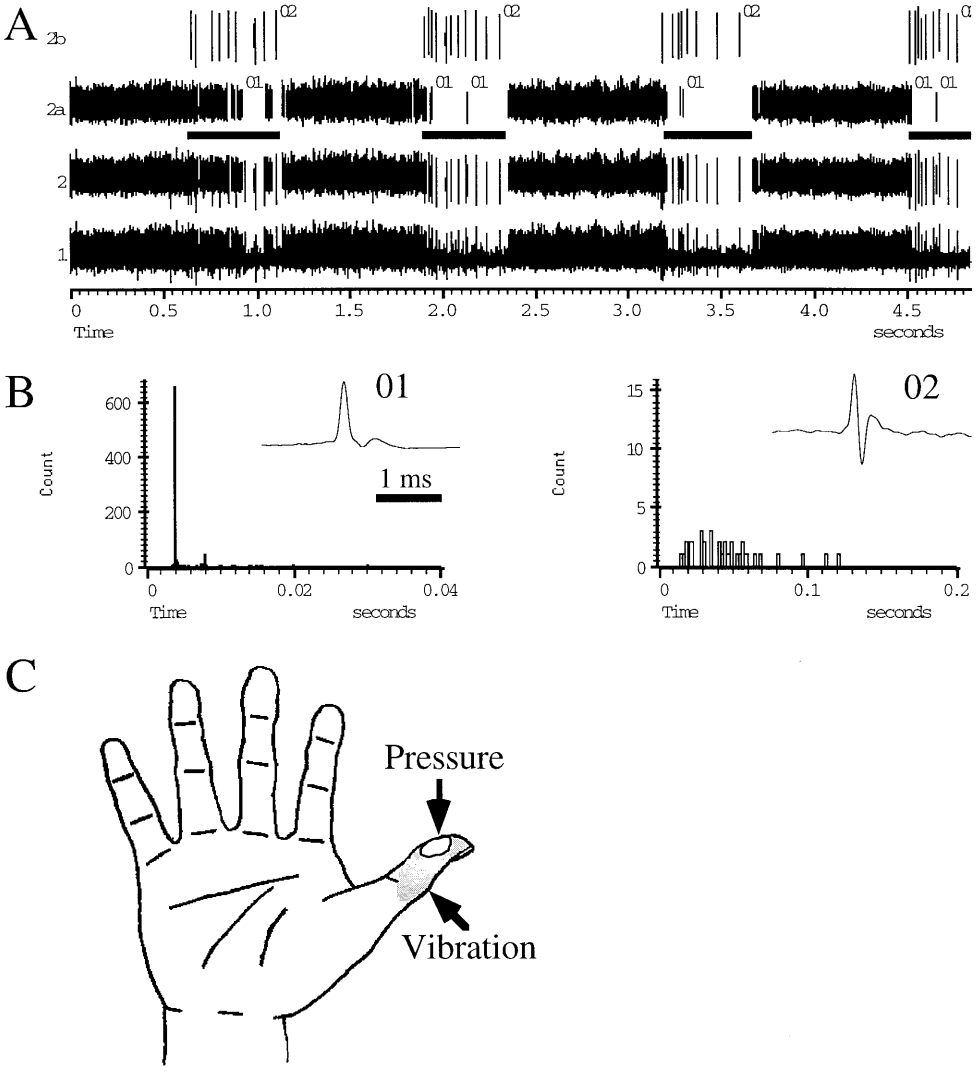

Fig. 5A–C Alternative firing of a PC and an SAII unit in responses to continuous skin vibration and intermittent local pressure in the pulp of the thumb. In **A**, the original neurogram is shown in *trace 1* and processed signals in *trace 2* as well as *traces 2a and b*. The PC unit with action potentials 01 (*trace 2a*) discharged vigorously during vibration but its firing was perturbed by local pressure (*bars*). The SAII unit with action potentials 02 (*trace 2b*), however, only discharged during local pressure. Interspike interval distributions and action potential waveforms of the identified big amplitude PC and the SAII unit are shown in **B**. The receptive fields are illustrated in **C**, where the sites for the applied vibration respectively pressure are indicated

even the same phalanx of that digit and sometimes they were even found in the same region of either the palm or the thenar eminence (not illustrated). Thus, PC afferents that clustered in the nerve appeared to have neighbouring, sometimes even overlapping, receptive fields in the hand.

PC afferents are linked with SAII units

PC units were often recorded together with other types of mechanoreceptive skin afferents. The sites in Table 1 where *n* was larger than *k* represented such recordings. These data are summarised in Table 3. In six of seven recording sites, SAII units occurred and a total of 11 non-PC units were identified. The unit distribution among these 11 units by modality was evaluated under the assumption of a random intraneural organisation of nerve fibres (Table 4). The outcome of the test indicated that SAII units occurred more frequently than would be expected in these PC recordings, which suggested that PC units tend to be coupled with SAII units in the human median nerve.

Table 3 Coupling of PC units with other modalities of mechanoreceptive afferents: results of individual recordings (*SA* slowly adapting units, *RA* rapidly adapting units)

Recording site	PC units (*k*)	Other units (*n*–*k*)	Total (*n*)
1	2	2 SAI	4
2	1	1 SAII	2
3	1	1 SAII	2
4	2	1 SAII	3
5	1	2 SAII	3
6	3	2 SAII	5
7	2	1 SAII+1 RA	4
Total	12	11(1 RA, 2 SAI, 8 SAII)	23

The relative frequencies of occurrence of the four main types of mechanoreceptive units were previously established to about 0.40 (RA), 0.15 (PC), 0.25 (SAI) and 0.2 (SAII); (Johansson and Vallbo 1979; Hallin et al. 1991). It was hypothesised that all non-PC units irrespective of category are randomly distributed in the nerve with the above frequencies. The 95% confidence interval corresponding to the observed proportion of RA and SAI units in the records (3/11) is 6.02–60.97%. The expected proportion (76%) is outside this interval; thus, $P<0.05$

Table 4 Coupling of PC units with other modalities of mechanoreceptive afferents: proportion of certain types of units

	Expected	Observed
SAII	0.20/0.85=0.24	8/11=0.73
RA+SAI	0.65/0.85=0.76	3/11=0.27
Total	1.00	1.00

As for Table 3, $P<0.05$

This link between PC and SAII mechanoreceptors is illustrated in Fig. 5, where continuous vibration at 256 Hz was applied to the thumb with a tuning fork. At the same time, localised pressure was intermittently delivered to the pulp region of the thumb. Two single units were excited by these stimuli. The PC unit that responded 1:1 to vibration did not discharge during local pressure of the tested area, whereas an SAII unit reacted to local pressure but not vibration. The manner by which these two units alternatively responded to the test stimuli demonstrated that PC and SAII mechanoreceptors may be encountered as neighbours both in the nerve and at the receptor level in the skin. In such situations, however, only the PC units responded to the applied vibratory stimuli. This SAII unit also fired robustly when tested with skin stretch (not shown).

Discussion

Some technical considerations

The search procedure for units was the same as described previously (Hallin 1990; Hallin et al. 1991, 1994; Ekedahl et al. 1997; Wu et al. 1998). Four types of low-threshold mechanoreceptive afferents were identified (cf. Johansson and Vallbo 1979; Vallbo et al. 1979; Hallin et al. 1991). In prior studies with tungsten electrodes, the highest proportion of the infrequently occurring PC units has been reported to be 13% (Johansson and Vallbo 1979). Also, in monkeys, PCs are seldom found (Talbot et al. 1968). Similarly, there are a scarcity of PCs in other human nerves and/or other species (Järvilehto et al. 1976, 1981; Konietzny and Hensel 1977; Roberts and Elardo 1986; Edin and Abbs 1991; Leem et al. 1993; Vallbo et al. 1995). By analogy, in the present study we found only 13 of 110 sites with PC units. Thus, it seemed unlikely that any bias in the recording and/or sampling procedures were present in these studies.

RA and SA units in monkey glabrous skin are entrained 1:1 at 100–200 Hz by vibratory stimuli exceeding 100 µm (Talbot et al. 1968). A few sensitive human RA units in the hand respond about 1:1 to skin displacements of approx. 60 µm at 128 Hz or 256 Hz, but much higher amplitudes are needed at these frequencies to entrain SAI and SAII units (Johansson et al. 1982). According to other workers, both RA and SA units in human foot or leg respond 1:1 to vibration at 100–200 Hz with amplitudes of 200–500 µm (Vedel and Roll 1982; Ribot-Ciscar et al. 1989). Interestingly, SAI and SAII units in cat follow vibratory stimuli at amplitudes of less than 100 µm at these frequencies when the probes are of small (e.g. 250 µm) rather than large (≥1–2 mm) diameter (Gynther et al. 1992; Vickery et al. 1992). By contrast, PCs in both animals and humans always exhibit reproducible and reliable entrainment at 1:1 during extended periods, even at stimulus amplitudes of less than 20 µm, irrrespective of stimulus parameters used (Lindblom and Lund 1966; Johansson et al. 1982; Hämäläinen et al. 1985; Leem et al. 1993). During testing of the PCs the oscillations of our tuning forks had similar low magnitudes and none of the RA, SAI and SAII units studied responded faithfully in a reliable phase-locked manner to prolonged vibration. Therefore, most likely only PCs were excited by these tests.

Distribution of PC afferents in the human median nerve and palm

Despite the infrequent ocurrence of PCs in the recordings, more than one PC unit was often identified at each site, which statstically should occur very rarely. More importantly, the degree of PC clustering might even be underestimated: the estimated proportion of PCs in the population of myelinated afferents, 0.15, used here and elsewhere (Hallin et al. 1991, 1994) to avoid any statistical bias, was derived from a study with tungsten electrodes (Johansson and Vallbo 1979). In all unit samples acquired with concentric electrodes, the proportion of PCs was always lower than 0.15 (Hallin 1990; Hallin et al. 1991; 1994; Ekedahl et al. 1997; Wu et al. 1997). This might in part be due to unfavourable topographical arrangement of the nodes of Ranvier of clustering PC afferents so that a negative bias for sampling pairs of PC units could be present. In the recordings there were almost always differences in the amplitude of the action potentials between pairs of PC units, which was generally not the case with pairs of studied units of other modalities. Such significant amplitude differences might indicate a certain distance between the fibres either cross-directionally over the fibres and/or only between the nodes of the two fibres in the longitudinal direction. Thus, we may not always discover neighbouring PC afferents in the nerve. In addition, low-amplitude vibration with tuning forks evoked the responses of the PCs in this study. Possibly, more PC afferents might have been activated if vibrotactile stimuli of higher intensities had been applied (Johnson 1974; LaMotte and Mountcastle 1975).

A grouped distribution of PCs has been found in hairy skin of the cat forelimb (Lynn 1969; Kumamoto et al. 1993b) and in glabrous skin in the monkey hand (Kumamoto et al. 1993a). Also PCs in the human palm were clustered anatomically, especially close to nerves and vessels (Stark et al. 1998). In this study, clustered PC afferents in the nerve were typically activated together by restricted skin vibration, which suggested that they often

had adjacent or even overlapping receptive fields. By contrast, previous anatomical (Cauna and Mannan 1959) as well as electrophysiological studies with tungsten electrodes in man have shown a random distribution of PC receptors in the palm (Johansson and Vallbo 1979). The receptive areas of these PC units often covered a large part of the whole hand (Knibestöl 1973; Johansson and Vallbo 1979), which was not the case with the units described in this study.

Many more fibres abut to the recording area of an intraneural tungsten electrode than that of a concentric electrode positioned in the same site (Hallin 1990). Thus, it cannot be excluded that PCs with a large receptive field as described previously (Knibestöl 1973; Johansson and Vallbo 1979) might have reflected activity in more than one PC afferent with adjacent receptive fields, which were not possible to classify as a group of fibres due to the recording technique used (Ekedahl et al. 1997). Thus, our data suggested clustering of PC afferents in human nerves and also at least partial clustering with anatomical overlap of their innervation areas in palmar skin.

Population behaviour of PC mechanoreceptors

All our PC units responded robustly 1:1, 1:2, etc. to the vibratory oscillations. They never discharged twice or with triplets at each vibratory cycle as previously described (Talbot et al. 1968; Johansson et al. 1982). This latter phenomenon was attributed to strong stimulation and termed "disorganisation" of the response (Talbot et al. 1968). Instead we observed that several PC units were tuned to the stimulation without interaction, which facilitated discrimination of individual units. Synchronous discharges of several PC afferents to each vibratory stimulus cycle, as described here, might represent a new type of discharge pattern for coding of vibrotactile stimuli. It may be speculated that the previously described repetitive firing of single PCs (Talbot et al. 1968; Johansson et al. 1982) perhaps instead reflected synchronous firing in a small group of PC units supplying the same skin area.

Based on previous work in animals and humans, it was claimed that SA mechanoreceptors might signal vibrotactile stimuli at high frequencies (Vedel and Roll 1982; Ribot-Ciscar et al. 1989; Gynther et al. 1992; Vickery et al. 1992). Also results from psychophysical experiments suggested that SAII mechanoreceptors might respond in the high-frequency range similar to PCs (Bolanowski et al. 1988). Interestingly, palmar PC afferents and nearby SAII fibres in the median nerve often supplied the same glabrous skin area (Fig. 5). Moreover, inputs from these two types of mechanoreceptors projected to mixed areas in the brain (Dykes et al. 1982). However, we never observed frequency locked 1:1 responses of SAII fibres to high-frequency, low-amplitude skin vibration that activated PC afferents. Previously acquired evidence has suggested that SA mechanoreceptors only may detect low-frequency vibration (Talbot et al. 1968; Järvilehto et al. 1976; Konietzny and Hensel 1977; Freeman and Johnson 1982; Hämäläinen et al. 1985). In agreement with these data, simultaneous monitoring of evoked SAII and PC unit activity did not demonstrate that SAII mechanoreceptors transmit high-frequency vibrotactile stimuli in man. Instead, our data favoured the concept that the Pacinian channel may work as a critical band for the perception of touch (Makous et al. 1995).

Some miscellaneous aspects

The existence of a functional neural organisation by both modality and somatotopy is well founded and commonly accepted in the central nervous system, especially at cortical levels of the somatosensory system, both in subhuman species (e.g. Mountcastle 1957) and in man (Penfield and Boldrey 1937). It might therefore be argued that clustering of afferents of the same class would be likely to occur also in peripheral nerves, particularly in consideration of evolutionary aspects. Yet prevailing orthodoxy has rather emphasized the lack of such an organisation in the peripheral part of the neuraxis, in particular within individual nerve fascicles (Sunderland 1945; Schady et al. 1983). In the light of the present and previous data (Ekedahl et al. 1996a, 1996b, 1997; Wu et al. 1998), it is tempting to suggest that the peripheral systemization of neural elements might be of profound significance under normal conditions and in disease. Some possible clinical implications of a peripheral nerve fibre systemization with grouping of different types of cutaneous afferents have been elaborated upon in a previous study (Ekedahl et al. 1997).

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