Microneurography in relation to intraneural topography: somatotopic organisation of median nerve fascicles in humans

Rolf G Hallin

Abstract
Microneurography was performed in median nerve sensory fascicles with concentric needle electrodes and with conventional tungsten microneedles. The latter electrodes preferentially recorded activity from the myelinated fibres in the whole fascicle. By contrast, due to its special design, a concentric needle can record activity selectively from even a small part of a fascicle. High amplitude signals in C fibres can be discriminated close to Schwann cells that envelope unmyelinated axons. Apart from being biased for activity in thin fibres, the concentric needles can also record signals from nearby myelinated fibres. The palmar receptive fields of such fibre groups were not congruent with the areas traditionally attributed to multiunit skin afferents in humans, namely the innervation zone(s) of one or two adjacent digital nerve(s). Instead, the multiunit fields often comprised small parts of a digital nerve innervation area, frequently only the pulp of a finger. Single units were always localised within previously screened multiunit areas.

By using surface electrodes, Eichler succeeded in recording synchronous mass activity through the skin from a mixed nerve in situ.1 The subsequent use of improved macroelectrodes placed in the vicinity of a peripheral nerve,2-4 later combined with averaging procedures, refined the technique of recording activity from intact limb nerves in humans.5 The procedures have been used for both experimental and clinical purposes.4,5,7

Single unit activity in humans was first recorded experimentally from excised and/or exposed nerves in situ, an approach which obviously is precluded from wide adoption in humans.6 Valbo and Hagbarth described a percutaneous technique to explore human limb nerves with intraneurally positioned solid tungsten needle microelectrodes. This technique records multiunit and single unit activity in myelinated fibres.8 Multiunit activity in C fibres innervating muscle10 and skin,11,12 as well as single unit activity in both afferent and sympathetic C fibres,13-15 can also be discerned.

Hallin and Wiesenfeld introduced a standardised, thin diameter concentric needle electrode for percutaneous recording of A and C fibre units in humans.16 In this investigation the recording properties of the concentric needle electrode were compared with those of the tungsten needle. Further, individual median nerve fascicles were screened for their relative contents of myelinated and unmyelinated fibres and to obtain information about the cutaneous palmar distribution of these fibres. Preliminary data16-17 and our results indicate that the concentric electrode is biased towards recording C fibre activity. The data obtained also suggest the presence of a hitherto not described somatotopic organisation in human sensory nerve fascicles.

Material and methods
Our findings were obtained from 67 experiments performed on 15 healthy subjects of both sexes aged between 20-42 years. They all gave their informed consent to participate in the trials. In eight experimental sessions skin nerve activity was recorded from the median nerve at the wrist and in 49 sessions from the nerve just proximal to the elbow. For comparison ten experiments with tungsten electrodes were carried out under equivalent conditions.

A Recording electrodes
In most experiments concentric needle electrodes were used.18 They consisted of commercially available hypodermic needles with outer shaft diameters of 200-250 μm and inner diameters of 90-100 μm (fig 1). A thin insulated tungsten wire, diameter 10-30 μm or a thin platinum-iridium wire of diameter 20-30 μm was positioned inside the needle, which was then filled with Araldite. The leads from the central core and the shaft (ground lead) were thin copper wires. The impedance of electrodes with small diameter recording wires (10 μm) was 500-700 kΩ, and of those with the largest threads (30 μm) 200-300 kΩ at 1000 Hz.18 Solid tungsten electrodes (bare tip length 30-100 μm, impedance 50-75 kΩ) were used in a few experiments.9,19,20
Microneurography in relation to intraneural topography: somatotopic organisation of median nerve fascicles in humans

The flat oval recording areas of the concentric electrodes were much smaller than the conical recording surfaces of the tungsten needles. The recording area of the concentric electrode in fig 1C was calculated to 785 µm². With a core diameter of 30 µm the maximal recording areas of these electrodes were about 2100 µm². The somewhat asymmetrical mantle area of the tungsten electrode shown for comparison in fig 1A was calculated to about 14 200 µm². If the tip of such a tungsten electrode had extended only 70 respectively 100 µm, the base of the cone would have been about 28 µm respectively 38 µm and the recording areas would have been about 3100 µm² respectively 6000 µm².

B Recording and display system
The recording electrodes were connected to commercially available preamplifiers (Neurolog System, Digitimer Ltd.). For maximal recording safety the ground was close to the recording point/intraneural stimulating site. The safety measures taken corresponded to those of class I, group B. The bandwidth of the whole system was generally 200 Hz–10 KHz (−40 dB/decade), but it could be modified when needed by applying other filter settings. An improvement to the signal-to-noise ratio was obtainable by an amplitude discriminator which could eliminate about 50% of the noise. The noise of the recording system peak-to-peak was about 10–15 µV. The best units had amplitudes of 40–60 µV. During the experiments the signals were displayed on an oscilloscope, fed into a loudspeaker and were simultaneously stored on tape.

C Mechanical and thermal stimuli
The receptive field location and extent of low threshold mechanoreceptors was mapped by a number of stimuli, such as application of skin taps or pressure with various small objects. The thresholds of individual units and boundaries of the unitary receptive fields were examined by using von Frey’s hairs. Afferent C fibre activity was also evoked by squeezing or applying pressure to the skin, needle pricks in the receptive area or by applying warm or hot stimuli derived from a radiant heat source (Quartslampen GmbH, Original Hanaan, West Germany) or from a commercially available temperature stimulator (Somedic AB, Stockholm, Sweden) according to the Peltier principle.

D Electrical stimulation
Electrical stimulation (square wave pulses of 0.1–1 ms duration) was performed using a DISA stimulator unit (Type 14E D1). Surface or needle electrodes were used for skin stimulation. Intraneural electrical stimulation was performed with the recording area of the electrode as cathode and the shaft of the needle as anode.

E Nerve exploration procedure
The experiments were carried out as described previously. To minimise the risks of inducing nerve fibre damage the bevelled surface of the concentric needle was directed through the skin in parallel with and into the nerve to be explored. Weak electrical pulses (1–6 V, 0.2 ms duration) intraneurally eliciting paraesthesias in the fascicle territory, were delivered through the electrode during the search procedure to guide the electrode tip into the nerve. A number of criteria were used to decide whether the electrode picked up activity from fascicles destined for skin or muscle. Occasionally it was tedious to reach and keep a recording position but sometimes the nerve activity had stable amplitudes allowing studies for up to several hours.

PRECAUTIONS
The electrodes were sterilised in 35% formaline vapour at 80°C at a variable under-pressure. The skin at the electrode site was carefully cleaned with pure alcohol and then with sterile injection swabs. The same recording site was not explored again until 4–6 weeks after an examination. With these measures no enduring neurological symptoms were encountered in any of the subjects.

Results
NERVE TOPOGRAPHY IN RELATION TO RECORDING SITES
Extrafascicular sites
The concentric electrode could be gently pushed stepwise through the nerve without causing too much discomfort to the subject. Electrical silence on a number of tests suggested that the recording surface often was located in extrafascicular sites. Judging from the frequency of occurrence of such situations a substantial portion of human peripheral nerve is made up of non neural tissue elements.
Intramuscular sites dominated by A fibre activity

I Single unit activity

When the needle was in a stable intramuscular site single unit activity in myelinated fibres was regularly identified either directly or after repeated needle adjustments. The occurrence of single unit activity in the recording was sometimes preceded by the appearance of injury discharges. Injury activity often occurs before unit activity is reached with tungsten microelectrodes.9 The characteristics of stable single A fibre units agreed well with those previously described in microneurography.10-12 Out of a total of 92 units 42 RA (rapidly adapting) units, 5 PC (Pacinian afferents), 27 SAI (slowly adapting type I) and 4 SAII (slowly adapting type II) units were reliably identified (figs 2, 3) on the basis of action potential amplitude and shape, distribution and size of receptive fields, firing characteristics etc. Four units could not be classified satisfactorily. The localisations of the units' receptive fields (fig 2) were essentially the same as previously reported when using tungsten microelectrodes for sampling26 with a high concentration of the RA and SAI units on the tip of the digits and a lower density in the rest of the hand. The sizes of their receptive fields were also as previously reported. Furthermore, the extent and sensitivity of an individual unit's receptive area remained the same during an experiment and no tendency to "blocking" of even a part of a unitary receptive field was seen.25 Representative recording sequences illustrating the firing characteristics of these units are shown in fig 3. Various details in the firing characteristics of single units were sometimes possible to test over several hours.

Generally only one (core diameter 10 μm) or a few units (1-3) (core diameter 20-30 μm) were recorded at each site even at elbow level. By contrast, tungsten electrodes may in a single site discriminate several units in the median nerve, especially at the elbow. The RA and SAI units were most commonly identified in recording sites where the corresponding multunit receptive field(s) involved the pulp and an adjacent small skin region at the
Microneurography in relation to intraneural topography: somatotopic organisation of median nerve fascicles in humans

Figure 4  Relationship between identified single unit receptive sites and corresponding multiunit receptive fields. RA and PC receptor sites are shown in A, SAI and SAII sites in B. The units were either identified when the multiunit area was initially mapped or encountered in the course of a few needle insertions when abandoning the original recording site. The hatched areas display the established extent of the receptive fields for PC and SAII receptors and the black dot the point of maximal sensitivity of these receptors.

A: The RA units (circles) were always localised within the previously established multiunit field (grey area) irrespective of field size and complexity. The outcome of five different experiments are shown to the left. To the right above an example of a split multiunit receptive field comprising a relatively large part of the pulp of the thumb (maximal extension of not visible area indicated by broken lines) and part of the index finger is shown. Below to the right is displayed the field of one PC receptor.

B: Seven SAI receptor sites (dots) and one SAII site (below right) obtained from different experiments and their relation to the corresponding multiunit receptive fields are shown. Moderate skin tension in the directions indicated by the arrows enhanced the firing of the SAII unit.

finger tip (see below). When sampling tactile afferents in such a situation by repositioning the electrode intraneurally, the units were always encountered within or sometimes on the border of the receptive field for the previously recorded multiunit activity (fig 4) which was reduced in amplitude when a large amplitude single unit was discriminated. These findings were consistent irrespective of recording site and irrespective of whether the previously identified multiunit field was small, large or split (figs 4, 6, 7). Sometimes successive units were of the same modality and had adjacent receptive fields. In one recording at the elbow four units, three RA units and one SAI unit were found in a cluster in the pulp of the thumb (fig 4A, bottom row left).

At the end of the experiments the electrode was sometimes successively withdrawn from the recording site in the nerve. During this procedure previously identified units tended to reappear in the neurogram in the reverse order to that in which they had been identified.

II Multiunit activity

The multiunit activity evoked by weak mechanical stimuli appeared as low amplitude, mainly negative directional mass discharges. The most intense activity was evoked by brief repeated stimulation with, for example, rough emery cloth or vibratory stimuli (fig 5A). Responses occurred at the onset and offset of local pressure whereas often only relatively sparse discharges were recorded during maintained stimulation. The multiunit discharges were often lower amplitudes than such activity recorded with tungsten needles (5C, D). In some cases, however, and particularly when the activity was derived from pulp afferents, well discriminated responses to both local touch stimuli and maintained pressure were discerned, especially in the integrated neurogram (fig 5A, B).

The receptive field locations and distribution in the hand were as described earlier. However, the multiunit areas recorded with the concentric needle electrodes were usually smaller (50–70°, less) than those normally found with the tungsten needles (figs 4, 6). When exploring the median nerve at the wrist the receptive area generally included an elongated, slightly asymmetrical part of a finger tip and a coherent small region on the phalanx next to the pulp. Sometimes the innervation area was more proximal and then involved glabrous skin of the two proximal phalanges on the ulnar or radial aspect of the finger (fig 6A). Similar size multiunit receptive fields were found at the elbow level, although larger receptive areas were also found there, corresponding to the innervation area of one or two or sometimes parts of two adjacent digital nerves (fig 6B). In almost all experiments the position, extension and complexity of the multiunit receptive fields of low threshold skin afferents changed slightly when repositioning the electrode surface in the nerve fascicle in small steps. This finding was consistent irrespective of whether the recording site was at the wrist or at the elbow level (fig 7).
Intratascicular sites dominated by C fibre activity
In many experiments the initial recording was that of C fibre activity, generally as sympathetic multiunit discharges. This was less common with tungsten than concentric needles, especially when exploring the median nerve.

The general occurrence of the sympathetic bursts during resting conditions and under a variety of manoeuvres, such as cooling or stressing the subject, fully agreed with previously described characteristics of cutaneous sympathetic outflow in humans. The sympathetic multiunit discharges often had higher amplitudes than concomitantly recorded A fibre multiunit activity (fig 5) and the corresponding receptive area for myelinated fibres on many occasions was positioned proximally in the fingers and palm. Slight needle adjustments occasionally dramatically improved the signal-to-noise-ratio in such a recording so that single unit activity in sympathetic fibres could be recorded.

Afferent C fibre activity was recorded on several occasions, but was not, like sympathetic activity, encountered at every recording site. The activity was in many instances of a unitary character already when first identified and the signal-to-noise-ratio of the discharges could sometimes be improved on needle adjustment. In most recording sites about two or three C afferents were identified. Only exceptionally was an afferent C unit recorded in isolation. Additional data on C fibre activity will be published separately.

Figure 5 Integrated neurograms (above) and multiunit responses (below) to repeated strokings of the skin with an emery cloth (left, bars) and to a continuously applied constant pressure with a metal springholder (right, bar). Time constant of integrator 500 ms. Time calibration 1s. A, B: The afferent multiunit discharges were sometimes well discriminated. The phasic component of these responses appeared most distinctly. C, D: Different recording site than A–B. The multiunit receptive field was split and comprised the pulp region and the skin covering the ulnar part of the first phalanx of the thumb and the basal radial part of the index finger on the left hand. This recording was dominated by sympathetic activity. The afferent multiunit discharges to skin strokings and constant pressure in the thumb were discrete but sometimes the amplitudes of the responses were as high as that of spontaneous outbursts of sympathetic skin nerve activity (arrows).

Figure 6 Typical examples of multiunit receptive fields of low threshold mechanoreceptive afferents (shaded areas) as encountered when exploring the median nerve with concentric needle electrodes at the wrist (A) and at the elbow (B).
Microneurography in relation to intraneural topography: somatotopic organisation of median nerve fascicles in humans

Figure 7  Transition of localisation and extent of multiunit receptive fields (shaded areas) in successive intraneural sites (1–3) when mapping the median nerve at the wrist (A–C) and at the elbow (D–F). Both at the wrist (three experiments) and at the elbow (three experiments) changes in the multiunit receptive field were noted upon minute adjustments of the concentric recording needle (1–3) irrespective of whether the needle was advanced into (A, D, E) or withdrawn from the nerve (B, C, F).

Discussion
Possible hazards involved in the exploring procedures which might influence the interpretation of the results
Electrodes inserted into human nerve fascicles might mechanically or otherwise interfere with and distort the intraneural environment so that neurophysiological events become misrepresented in the recorded activity. Wall and McMahon31 paid particular attention to this problem and suggested that single unit recordings from human peripheral nerves only are possible because almost all of the nerve fibres near the electrode tip are pressure blocked during the exploring procedure and therefore unable to conduct action potentials. Only one or a few fibres near the tip would stay active, making action potentials recordable as unitary discharges.31

In a study utilising concentric needle electrodes with two recording surfaces we established the probability of recording neural activity at both surfaces at the same time.31 In 80% of the experiments nerve signals were discerned at both recording areas simultaneously. Furthermore, the properties of the identified units to a diversity of stimuli were similar to characteristics ascribed to normal, previously established receptor types in sub-human species.32–36 Therefore, gross functional abnormalities engaging the explored nerve fascicle do not seem to be prevailing during microneurographic investigations.

Electrophysiology related to intraneural topography. Impulse generators for single and multiunit A and C fibre activity
As pointed out in a critical review unitary discharges in microneurography are not likely to be recorded intra-axonally.37 Furthermore, unit activity in myelinated fibres recorded with the concentric needle can hardly originate from an intra-axonally positioned electrode surface. On the other hand, the suggestion of a pressure block for most nerve fibres at the electrode site, thereby making unit identification possible, could not be verified.31

Another unconsidered possibility to account for how unit activity can be recorded in these studies would be that the unitary signals in the case of myelinated fibres represent activity at the nodes of Ranvier. This does not exclude the possibility that the tip of a tungsten electrode recording unit activity may occasionally be partially penetrating the axon.35–39 As will be discussed, the present data would be easily accounted for if the proposed idea of unit origin is accepted. Furthermore, some major discrepancies between the present results and previous microneurographic data acquired with the tungsten electrode could then be explained.

Concentric electrodes with small recording surfaces seldom recorded single unit activity in myelinated fibres whereas electrodes with somewhat larger surfaces did so regularly, sometimes from several units. These data fitted well with our experience that sampling of units with myelinated fibres tended to be more rewarding when using tungsten electrodes (unpublished observations). Obviously, the big three dimensional tip area of such an electrode will record from a larger number of nodes of Ranvier than the concentric needles used (fig 8).

The unitary characteristics, for example, shape and duration of the recorded potentials and firing pattern to various stimuli were virtually the same irrespective of recording technique. However, injury discharges were more rare with the concentric needle and the identified units (fig 3) in many cases showed no overt signs of nerve fibre damage.31 In this context it should be recalled that previously identified but abandoned unit(s) tended to reappear in focus for the electrode when it was moved out of the nerve. The likelihood for such events to occur would be much higher if the unit recordings originated near some anatomical structure(s) than if relocation of the recording surface intra-axonally in one or more previously penetrated axons would be neces-
likely at the internodes. Also, the shape of the unitary impulses were of the types to be expected when recording the electrical activity from a fibre very close to a node of Ranvier with the kind of electrodes used in this investigation (Frankenhaeuser, personal communication).

Multunit A (discussed under next heading) and C fibre signals probably represented extracellular activity recorded close to a number of fibres, but distant from a node. Since the intervals between the nodes may amount to several hundred microns, which is long in relation to the length of the electrode surface, and the nodes are not always randomly distributed within the fascicle (Hallin and Ekedahl, unpublished observations), the location of the active surface of the needle will often be at the internodes. Solely multunit activity therefore should (and does) occur in the recordings quite frequently during microneurography. The receptive fields were smaller than those found using solid tungsten electrodes for nerve exploration and the evoked multunit mechanoreceptor activity from these fields was often of very low amplitudes. Reasons were given above to explain why the concentric needles used in this investigation often sampled fewer units in a recording site than tungsten needles. The same line of argument could explain why the multunit mechanoreceptor activity often had lower amplitudes and regularly derived from smaller receptive fields when screened with the concentric needle than when the tungsten electrode was used for similar nerve explorations (fig 8).

Multunit C activity was often better discriminated than multunit A fibre discharges with the present technique (fig 5). This finding would also relate to the design of the concentric needle. Since it often records activity from only a small part of the myelinated fibres of the fascicle, multunit discharges in A fibres were less prominent than they would have been if recorded by a tungsten electrode in the same site (fig 8). Thus simultaneously recorded C fibre activity tended to be favourably enhanced, especially close to the electrode when exploring nerve fascicles with the concentric needle.

**Somatotopy in human median nerve fascicles**

A number of mapping experiments were performed to study intraneural fascicular topography with the more selective concentric needle. In many trials the multunit fields of myelinated afferents comprised a part or the entire pulp of a finger (figs 4, 6, 7). In about 30% of the cases the fields were split in at least two distinct areas, suggesting simultaneous recordings from more than one nerve fibre population. Following adjustments of the electrode position there were minor changes in the extent and localisation of the multunit innervation area (fig 7). There were also striking transitions from fields composed by separate parts to fields which were coherent and vice versa. The single units, however, which were found in the course of the changes in recording site were all situated within the boundaries of the previously screened multunit receptive fields (fig 4) and in one experiment several single A fibre elements

---

**Figure 8** Schematic illustration of the proposed recording site when using the concentric needle (A) and the tungsten electrode (B) for microneurography. The small illustration in the middle indicates that a recording electrode inserted in a nerve has entered into a fascicle. In A is shown how 10 fibres lie near to the recording surface (shaded area measuring about 30 × 85 μm) of the concentric electrode (hatched oval indicates the Araldite filled lumen of the needle). Sometimes a node of Ranvier may lie close to the recording area resulting in a unit recording. Many more fibres (20) lie close to the recording area of a tungsten electrode (length of free tip ‘shaded area’ in this case 100 μm) in the same recording site (B). Apart from a few more fibres lying in front of the needle, most of the additional fibres (indicated by black ellipses to the right) are located “behind” the needle. (Their course behind the needle is not indicated.) Additional nodes of Ranvier may get close to such a needle with its conical recording area resulting in additional unit recordings. It is also apparent that the concentric needle records multunit activity preferentially from fibres close to and in front of its recording surface. The tungsten electrode records multunit activity from fibres abutting on any part of its bare conical tip and will therefore reflect the overall activity from a large part or the entirety of the explored fascicle. Bar 100 μm.
(three RA and one SAI unit) with separate but nearby receptive areas were successively identified in a cluster in the pulp of the thumb. These findings provide strong evidence for the presence of intrafascicularly localised microbundles of myelinated fibres in human nerves. Some microbundles seem to be destined to the finger pulp, others to more proximal parts on the side of a finger. Furthermore, cutaneous median nerve fascicles at the elbow level, which typically contain fibres supplying the innervation areas of two adjacent digital nerves, probably contain at least four (fig 7), probably more, bundles of myelinated fibres which innervate adjacent sides and pulp regions of two neighbouring fingers.

In contrast to our results, Schady et al found that the myelinated fibres in human peripheral nerve fascicles are randomly distributed intrafascicularly. The issue then is whether or not the interpretation of our data can also account for the discrepancies in the results between the two studies. Due to its design, the concentric electrode is quite useful in analysing the neural activity from small groups of fibres near to the recording surface (fig 8A). Since the tungsten microelectrode discriminates activity in many more fibres around its entire conical tip (fig 8B) it is simply not possible by physiological recordings to study microanatomy relating to small portions of the fascicle by using this technique. Hence, the now discovered fascicular organisation was not detected in previous microneurographic recordings with tungsten electrodes. Neither microstimulation through the intrafascicularly inserted tungsten electrode, used by Schady et al to study intrafascicular fibre organisation in detail, seems accurate enough in the present context. In the trials described it was assumed that individual nodes of Ranvier within small groups of fibres close to the electrode tip were stimulated. However, since the recording/stimulating tungsten electrode sometimes had a free tip extending as much as 180 μm, which presumably was totally enclosed intrafascicularly, and there was no way to judge the distribution of the stimulating currents used, the nodes of Ranvier of the stimulated fibres near such a big tip might well have been spread out within a comparatively large intrafascicular volume. Intraneural stimulation of several dispersed nodes of Ranvier near the whole free tip would evoke randomly scattered projected fields, indicative of unit activation, within a large cutaneous area within the explored fascicular territory, thus accounting for the reported results. However, in view of the present data it is apparent that the microstimulation technique is not sufficiently precise to use as a tool for studying the intrafascicular fibre organisation in detail.

Thus we conclude that an orderly micromosaicotopy is present in human median nerve fascicles. The results adhere to a common principle of a somatotopical organisation in the somatosensory system which starts in the peripheral nerves and relays via the spinal cord to end in a well organised manner at more central levels.  

Some clinical implications
The fingers in primates have a dense mechanical innervation. The intrafascicular grouping of the myelinated fibres supplying palmar skin must also have bearing on tactile gnosia in humans. Thus the relatively common early complaint of finger tip numbness occurring in patients with carpal tunnel syndrome might be accounted for as a compression involving microbundles destined to the finger tips. If neighbouring median nerve fibres destined to the whole hand and the finger tips were randomly distributed in each individual fascicle as suggested in another study the distal numbness occurring in the carpal tunnel syndrome would be much less likely to develop especially if the massive central representation of the fingers is considered. Clinically, reality points in the opposite direction.

Our results may also be of relevance for corrective micro-surgery aiming to re-establish innervation after nerve trauma. It is well known that the manual discriminatory capacity restored after peripheral nerve suture to a substantial part depends on the extent of the nerve injury. Patients with partial nerve lesions generally regain a functionally more complete tactile gnosia than those with total severance of the nerve which is easily understood in the light of the present findings.

Judging from the number of recording sites where sympathetic activity occurred, sympathetic fibres are quite numerous in the median nerve. This is not surprising as the unmyelinated fibres far outnumber myelinated fibres in human nerves but quite the contrary to the experience with the tungsten microelectrode which records preferentially from the myelinated fibre population of the whole fascicle. The fact that sympathetic fibres are numerous in the median nerve might be one necessary prerequisite for the occurrence of pain syndromes involving the median nerve area.

I am grateful to Professor Bernhard Frankenhuesser for valuable discussion of the manuscript. The expert secretarial aid of Maj-Len Holm and Eli Fryen-Stenberg is gratefully acknowledged. This research was supported by funds at the Karolinska Institute and the Swedish Society of Medicine.


