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REVIEW

Translational nociceptor research as guide to human pain perceptions and pathophysiology

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Abstract Microneurography is a method for recording single unit action potentials with microelectrodes from the nerves of awake cooperating humans. Although this method is now in use since almost 40 years, its potency has been strengthened by the recent technical developments. A great progress was the discovery that different functional groups of nociceptors are characterized by a distinctly different post-excitatory slowing of their conduction velocities. Microneurography is now powerful enough to analyze the nerve activity pattern of enigmatic sensations such as pruritus. Furthermore, it is the only method providing direct insight in the changes which human nerves undergo with aging. Recently, reliable recordings from patients suffering from painful neuropathies came into reach. It has been shown that different types of neuropathies are characterized by different patterns of abnormal nociceptor functions. Although some of them are characterized by abnormal spontaneous activity in C-nociceptors, others show mainly signs of denervation. Microneurography is, therefore, a tool for translational studies on human nociceptor functions by linking direct animal studies on experimental neuropathies with human diseases.

Keywords Microneurography · Neuropathy · Itch · Aging · C-fiber

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Introduction: evolution of microneurography

Shortly before the Second World War, the development of electronic amplifiers and oscilloscopes had reached a level that enabled recordings of single nerve fibers. In 1932, Edgar Adrian got the Nobel Prize for the discovery of the encoding of nerve signals in streams of action potentials. Shortly afterwards his co-worker, Y. Zotterman published recordings from presumably unmyelinated nociceptors in cat nerves (Zotterman 1939). In the late 1950s, Ainsley Iggo and later Ed Pearl established our knowledge of the conduction velocities and functional properties of this class of nerve fibers in various tissues (Iggo 1958, 1959; Bessou and Perl 1969, 1973).

Animal experiments are limited, however, regarding the contribution of nervous elements to sensory experiences, and in particular as to their contributions to pathological pain states of human beings. This gap was closed by the technique of microneurography, i.e. recordings from single nerve fibers in peripheral nerves of cooperating human volunteers. The method was developed by Hagbarth and Vallbo in the late 1960s (Vallbo and Hagbarth 1968) and it was adapted to recordings of C-fibers by their students Torebjörk and Hallin (1970). In the following decades, microneurography was employed by several groups for studying pain mechanisms (for a review see Torebjörk et al. 1996), but also mechanisms of tactile sensations, motor control (for a review see Vallbo et al. 1979; Macefield 2005), and sympathetic nerve functions (for a review see Mano et al. 2006; Macefield and Wallin 1999; Macefield et al. 2002; Vallbo et al. 2004). Microneurography has been carried out in awake and cooperating human subjects who are able to report sensory experiences that can be matched to the nerve fiber discharges.

When comparing properties of human nociceptors with the respective findings in laboratory animals, most often the so-called polymodal nociceptors are in the focus. This class of C-fibers was originally described in cat experiments by Bessou and Perl (1969). They respond to mechanical-, heat-, and different forms of irritant chemical stimulation. This nociceptor type is the most frequent kind of nerve fiber in skin nerves of rat (Lynn and Carpenter 1982), other rodents, and also primates, in particular man (Schmidt et al. 1995), In elaborate and careful experiments, it has been shown that the heat responses of polymodal nociceptors are in the same range as human pain thresholds and their suprathreshold responses match quantitatively the magnitude of heat pain experiences under experimental conditions (Robinson et al. 1983). Furthermore, sensitization of the nociceptive nerve endings by burn injuries or chemicals is accompanied by hyperalgesia (LaMotte et al. 1982). Even the fatigue of spike responses following repetitive heat stimulation has a parallel in smaller pain responses (Adriaensen et al. 1984). This obvious concordance of nerve fiber discharges and pain experiences supported the hypothesis that polymodal C-nociceptors are instrumental for mediating heat pain and hyperalgesia. However, such a nice concordance was not found for mechanical stimulation. Typically, polymodal nociceptor units can be excited by probing with von Frey hairs of different stiffness. In several studies, it was found that the average threshold of these units to von Frey-hair probings was in the range of 50 mN and rarely exceeded 120 mN (Van Hees and Gybels 1981). Similar thresholds were found for "polymodal" Aδ-fibers, the other class of nociceptive nerve endings in human skin (Adriaensen et al. 1983). However, probing the skin with von Frey hairs of double this strength is rarely regarded as painful.

Therefore, the pains induced by mechanical stimuli such as pin pricks or by tonic pressure with a small probe are probably conveyed by another class of nociceptors that was not found in the early microneurography studies, probably for technical reasons. To avoid a sampling bias, our group developed the method of "marking" by which individual C-fibers can be characterized without the recourse to mechanical search stimuli. This method is based on the slowing of C-fiber conduction following activation. We were able to prove that marking occurs even if only one additional spike is interpolated at a repetition rate of 0.25 Hz of the conditioned response (Schmelz et al. 1995). The method provides the additional advantage, that traces of individual C-fibers can be easily discovered by their coherent conduction latencies in multiunit recordings even when the signal to noise ratio is small. This led to a much higher yield of individual experiments, a prerequisite of successful clinical studies.

Figure 1a shows an experiment proving the sensitivity of the marking technique by showing the latency shifts due to interpolated conditioning spike responses. The marking method led to the discovery of the mechano-insensitive afferent C-fibers in human skin which were called CMi units by our group (Schmidt et al. 1995). These units cannot be excited by acute mechanical stimulation, even when needles penetrate the skin in the receptive territory. They become responsive, however, to noxious tonic pressure stimuli when the pressure is applied for longer than a few seconds and to von Frey hair stimulation when the skin is inflamed (Schmidt et al. 2000). Owing to this plasticity they have been called "sleeping nociceptors", a term that was originally introduced by RF Schmidt for C-fibers found in the rat′s knee joint which responded to joint movements only after inflammation (Schmidt 2007).

Figure 1b shows that CMi units which make up about 20% of the afferent C-fibers in human skin have another interesting and unexpected feature: they show more pronounced slowing during frequent electrical stimulation. Slowing upon repetitive stimulation is obviously dependent on post-excitatory hypo-excitability as the "marking" phenomenon to interpolated spikes. CMi units show significantly more slowing than the mechano-responsive polymodal nociceptors (Weidner et al. 1999, 2000). The third group of C-afferents studied in the skin of the lower leg and foot, the cold-sensitive fibers, show the least slowing (Campero et al. 2001).

"Sleeping nociceptors" in humans and in animals?

In the last decade, it turned out that the "sleeping nociceptors" are constitutive for many physiological reactions and pain phenomena. They are assertive for the pain to tonic pressure (Schmidt et al. 2000) and the sustained pain induced by intracutaneous capsaicin injections together with the subsequent mechanical hyperalgesia (Schmelz et al. 1997b). Generally, they seem to have a great impact on hyperalgesia. Neuropeptide release from their terminals leads to the large flare reactions in human skin following noxious stimuli (Schmelz et al. 2000). A subgroup of them mediates the histamine-dependent itch sensations (Schmelz et al. 1997). Recently, we have demonstrated different cerebral projections of both fiber classes (Ruehle et al. 2006).

It is still unclear in which other mammalian species this type of nociceptors exists and whether it plays there the same role. Employing electrical stimulation of single axons, Lynn and Pierau have shown, that in the domestic pig the flare responses, which are in size similar to those of humans, are mediated by unmyelinated mechano-insensitive "heat fibers" (Lynn et al. 1995, 1996a, b; Gee et al. 1997). Recently, the group of Schmelz co-workers have extended these results. Apparently, CMi units in the pig skin show the same type of pronounced slowing as CMi

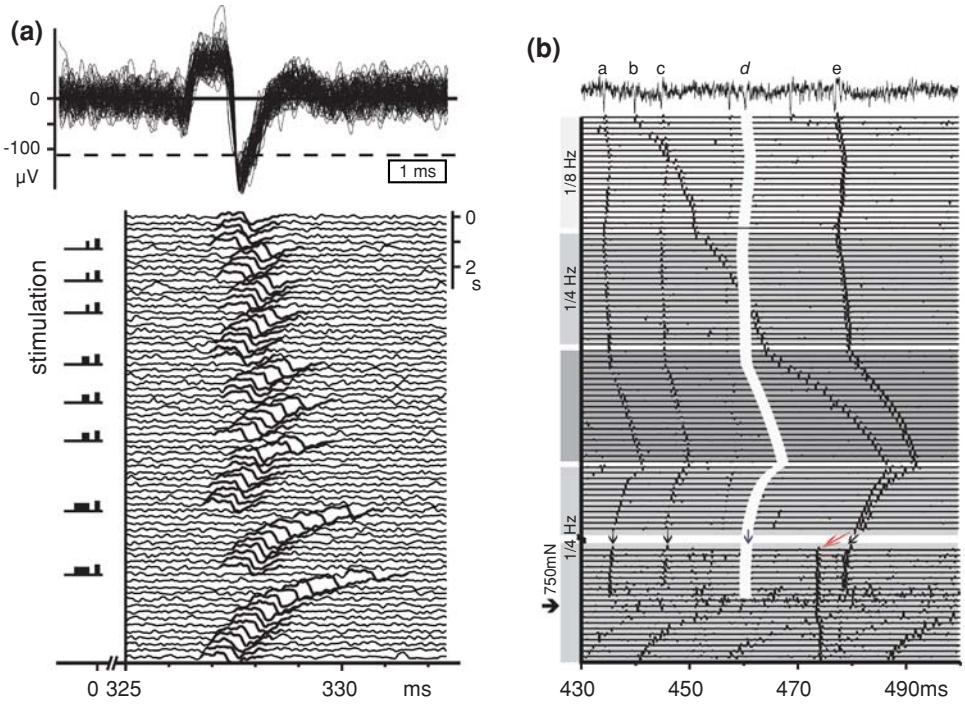

Fig. 1 **a** The response of a C-unit to intracutaneous electrical stimulation. From *top* to *bottom* successive recordings of the unit during electrical stimulation at 4 s intervals are depicted. One to four additional electrical pulses lead to increasing delays of the response. The stimulus pattern is shown at the left of the recordings. The figure shows the sensitivity of the marking method. One interpolated spike is sufficient for inducing a notable marking (Data from Schmelz et al. 1995). **b** Slowing of conduction velocity during repetitive stimulation. The figure shows a multiunit recording of C-fibers in which five units could be discriminated. From top to bottom, the changes in conduction velocity of the units due to different stimulus repetition rates are shown (indicated to the left of the traces). In the bottom traces responses of the units to mechanical stimulation of their receptive fields with a stiff von Frey bristle are shown. Four of the units are mechano-responsive (CM, probably polymodal nociceptors), the unit (**b**) mechano-insensitive (CMi unit). This unit, marked in *red* shows the most pronounced slowing characteristic. The spike responses of unit *d* are overlaid by a *white band* for better visibility of the trace (modified from Weidner et al. 1999)

units in humans (Obreja et al. 2008). These findings allow for using the pig as an experimental model of "sleeping nociceptor" functions. Bostock et al. have studied the slowing behavior of C-fibers in rat skin and found different groups of units with different slowing. The units with the most pronounced slowing may be equivalents of the human CMi units. Unfortunately, mechanical thresholds and responses were not systematically assessed in this study (George et al. 2007). At present no publications exist on the respective nociceptor properties in mice a desideratum for studying the membrane mechanisms of the slowing in transgenic animals.

Perception-guided nociceptor research: the enigma of pruritus

Itch is one of the most enigmatic sensations: apparently, it belongs to the realm of nociception, but it is distinguished from pain by several characteristic features: it originates only from the upper layers of the skin and transitional mucosa (e.g. lips and anal mucosa) and it is characterized by the urge to scratch (Rothman 1941). It has been difficult to study itch in rodents, because the distinction between itch and pain behavior is not always obvious and only recently it has been explored systematically (Shimada and LaMotte 2008).

In man, itch has been related to the release of histamine from mast cells in the skin. Histamine applied into the superficial skin layers by microinjection or iontophoresis lead invariably to itch (Magerl et al. 1990). It has been puzzling that polymodal nociceptors turned out to be insensitive, or at best weakly sensitive to histamine (Schmelz et al. 1997a), although experiments with differential nerve blocks have shown that histamine-induced itch is predominantly mediated by C-fibers (Koltzenburg et al. 1993). Characterization of the mechano-insensitive C-nociceptors led to the solution of this puzzle: a subpopulation of these fibers have been characterized which respond strongly to histamine iontophoresis with a time-course matching the itch sensations of the subjects (Schmelz et al. 1997). Histamine-sensitive CMi units have a characteristic profile of chemical excitability which is distinct from other CMis and from polymodal nociceptors. They are, e.g. more sensitive to

prostaglandin E2 and this matches the potency of this inflammatory mediator to produce itch (Schmelz et al. 2003a, b). On the other hand, histamine-sensitive CMi units often respond to heating and to capsaicin application. Because both stimuli are related more to pain than to itch, one has to adopt "selectivity", rather than "specificity" hypothesis to explain the peripheral mechanisms of itch induction: when the larger group of capsaicin and heat-sensitive C-nociceptors is excited by either of the two stimuli the ensuing pain suppresses the itch sensation and pain prevails.

In microneurography experiments on patients with prurigo nodularis suffering from severe itching, spontaneously active histamine-sensitive CMi units were found. These findings indicate that this fiber class plays also a role in pathological pruritus (Schmelz et al. 2003a).

Histamine release in the skin is followed by weal and flare reactions apart from itch. However, there are many forms of pathological pruritus that are not accompanied by these reactions which are probably histamine-independent. A conspicuous example is the often severe pruritus in atopic dermatitis, a very common affection in the Western World and in Japan, which is rather resistant to anti-histaminic therapies.

An experimental model of non-histaminergic pruritus is the itch induced by the spicules of cowhage (*Mucuna pruriens*) which induce strong itching when brought into the superficial layers of the skin, but do not induce weal and flare reactions (Arthur and Shelley 1955; Shelley and Arthur 1955, 1957; Johanek et al. 2007, Kosteletzky et al. 2008). The active ingredient of these plant spicules is mucunain, a peptidase acting at the PAR4—receptor (Reddy et al. 2008). Interestingly, it has been shown that mucunain acts on a quite different population of C-fibers compared with histamine (Namer et al. 2008b). Cowhage spicules excite polymodal nociceptors and not the histamine-sensitive CMi units. A similar dichotomy of the peripheral pathway for itch has been found in the monkey (Johanek et al. 2008), where in addition A-delta units strongly responsive to cowhage have been encountered (Schepers et al. 2008) which have not yet been studied in man. In monkey, the dichotomy into at least two different pathways for pruritus seems to extend up to the thalamic level, since in the thalamic projection nuclei separate populations of units have been encountered for histamine and for cowhage stimulation (Davidson et al. 2007). Figure 2 shows microneurography recordings from a healthy human subject which clearly demonstrate the two types of itch mediating C-fibers in one multi-unit record.

Little is known at present about the neuronal mechanisms of pruritus in patients suffering from kidney and liver failure. In both cases, anti-histaminic drugs are largely inefficient and exploration of these mechanisms is of great importance for developing effective therapeutic options.

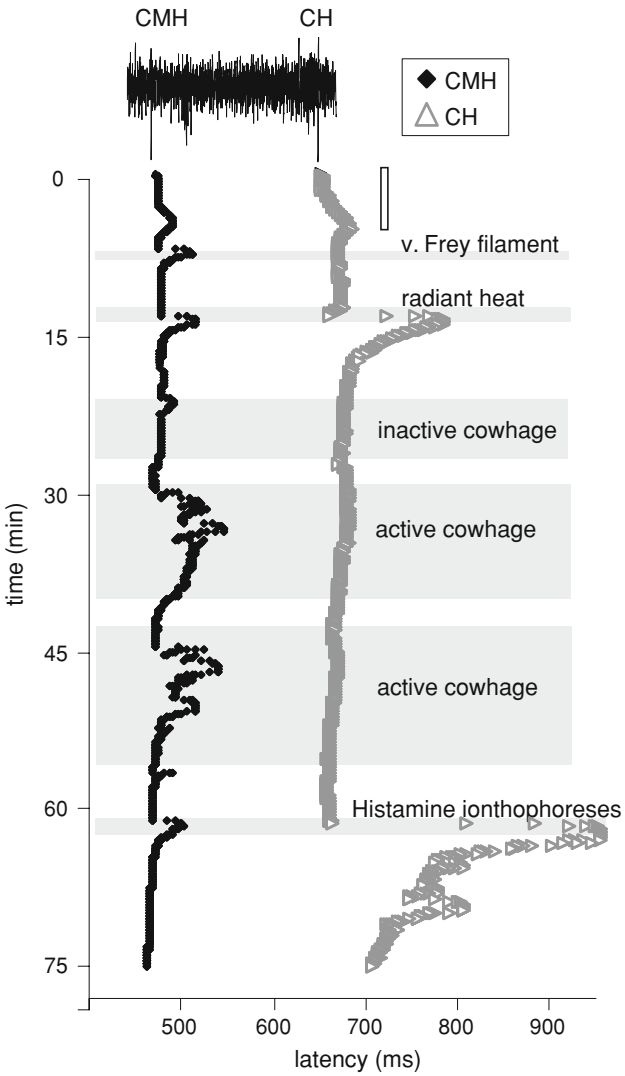

Fig. 2 Specimen of a multifiber recording with one mechano-responsive (CM) and one mechano-insensitive nociceptor (CMi) (see *trace* at *top*). The protocol shows the reaction of the two units to different kinds of stimulation in their slowing responses. From top to bottom: differential slowing during frequent electrical stimulation (*open bar*). Mechanical stimulation with a von Frey filament (only the CMH unit reacts). Radiant heat stimulation. Stimulation with inactive and active cowhage spicules. Histamine ionthophoresis. The CMH unit shows a vigorous reaction to cowhage, but not to histamine. The mechano-insensitive CH unit reacts vigorously to histamine, but not to cowhage (Namer et al. 2008a, b)

Aging nociceptors

The lifespan of humans comprises many decades, and life expectancy is still increasing. In this long time-course, changes occur in the peripheral nervous system that may lead to sensory deficits, and changes in pain perception. Obviously, the life span of rodents is much shorter. Rats are regarded as "old" at an age of 2–3 years, and have been

experimentally used as comparison group for age influences. Most data on aging of pain perception are derived from behavioral studies, e.g. the tail flick test to heat which obviously depends not only on the nociceptor input, but also on the nervous control of the motor reaction. In some studies on aged animals, the tail flick latency was increased in old rats (Hess et al. 1981), in others it was decreased (Crisp et al. 1994), and finally, in some studies it was unchanged (Gagliese and Melzack 2000). Mechanical sensitivity to paw pressure increased, while sensitivity to von Frey stimulation decreased in 29-month-old rats (Jourdan et al. 2000).

Unfortunately, no systematic electrophysiological studies on nociceptive primary afferents in old rats have been published to date. In recordings from secondary neurones in the spinal dorsal horn signs of sensitization to noxious heat were found in elderly rats which could not be enhanced by inflammation (Iwata et al. 2002; Kitagawa et al. 2005). The authors assumed that these findings were due to changes in spinal synaptic transmission or to deficits in descending pain inhibiting pathways, rather than in changes of nociceptor excitability. Morphological studies show a degeneration of predominantly unmyelinated fibers in mice older than 24 months (Ceballos et al. 1999).

In humans, indirect functional studies of the peripheral nervous system have predominantly shown decreased C-fiber functions in aged people, e.g., increased temperature thresholds may indicate functional deficits in afferent C-fibers (Claus et al. 1987; Rolke et al. 2006). Diminished axon-reflex flare sizes point in the same direction (Helme and McKernan 1986; Munce and Kenney 2003; Namer et al. 2004). However, in other studies no clear differences between younger and older subjects were found regarding temperature thresholds (Lautenbacher et al. 2005).

Efferent sympathetic C-fibers also seem to have decreased functions in aged persons as shown by reduced quantitative sudomotor axon reflexes (QSART) (Low et al. 1990, 1997; Foster et al. 1976). Again, changes obtained with these indirect methods may also depend on extrasensory changes, e.g., reaction times in the case of motor reactions, or vascular changes in the case of sudomotor and flare reactions.

Some studies have also shown morphological changes such as a degeneration of C-fiber endings resulting in lower intraepidermal nerve fiber counts or fiber counts in sural nerve biopsies of aged subjects (McArthur et al. 1998; Kanda et al. 1991; Jacobs and Love 1985). In other studies, however, morphological changes were either absent or detected only in people over an age of 65 (Kanda et al. 1991; Lauria et al. 1999).

In a recent microneurography study, subjects with a mean age of 25 years were compared with subjects of more than double this age (mean age 56 years) (Namer et al. 2008a). In aged subjects, changes were found in the composition of the C-fiber population and in sensory and axonal properties. Although the relative incidence of afferent to efferent C-fibers remained almost constant, the ratio of mechano-responsive to mechano-insensitive nociceptors was 7:3 in aged subjects, while it was 8:2 in the young controls. In aged subjects, 13% of the fibers showed atypical discharge characteristics, while those "atypical" fibers were extremely rare in young subjects. Figure 3 shows the distribution of C-afferent fiber types in young and in old subjects and in patients suffering from diabetic neuropathy (data from Orstavik et al. 2003). The average age of the patients was in the same range as that of the group of the older subjects. The figure indicates that the aging has mainly the effect to increase the number of two types of "abnormal" afferent C-fibers, the "Xi" units which were of the "polymodal type" according to their use dependent slowing but probably have lost their mechanical- and heat sensitivity in the course of a degenerative process, and the "Xsens" units which most often had the characteristic slowing of the mechano-insensitive units, but were excitable by von Frey hair stimulation and/or spontaneously active. Xsens fibers had also significantly lower heat thresholds compared with CMi units of young subjects supporting the notion that they were sensitized. For comparison, neuropathy patients had a much higher incidence of desensitized fibers compared with age-matched healthy controls (Fig. 3).

The majority of the afferent C-units could still be classified as "polymodal nociceptors" and "mechano-insensitive units" as in the younger controls. The receptive properties of the "normal" polymodal C-units were not significantly altered, in particular their average heat threshold (42–43°C). In contrast, the heat thresholds of the mechano-insensitive C-units were significantly lowered from 48°C in the young to 44°C in the old group (Namer et al. 2008).

In summary, discrete signs of "loss of function" and of "sensitization" were observed in the nerves of older subjects. The former, manifested in the "Xi" units did by far not reach the extent found in neuropathic nerves (c.f. Figs. 3c, d). Nevertheless, the functional deficit in part of the C-nociceptors could lead to slight degrees of hyposensitivity to painful stimuli. However, the surprising signs of increased sensibility and sensitization in the other group of C-units, the "sleeping" nociceptors might compensate for this loss. One may speculate that this phenomenon might be due to a stronger action by a surplus of NGF released, e.g. by keratinocytes related to a loss of targets at the degenerated fibers.

The combination of degeneration and sensitization due to aging may render the aging peripheral nervous system more susceptible for intervening events, e.g. neuropathies and toxic effects, e.g. by chemotherapy.

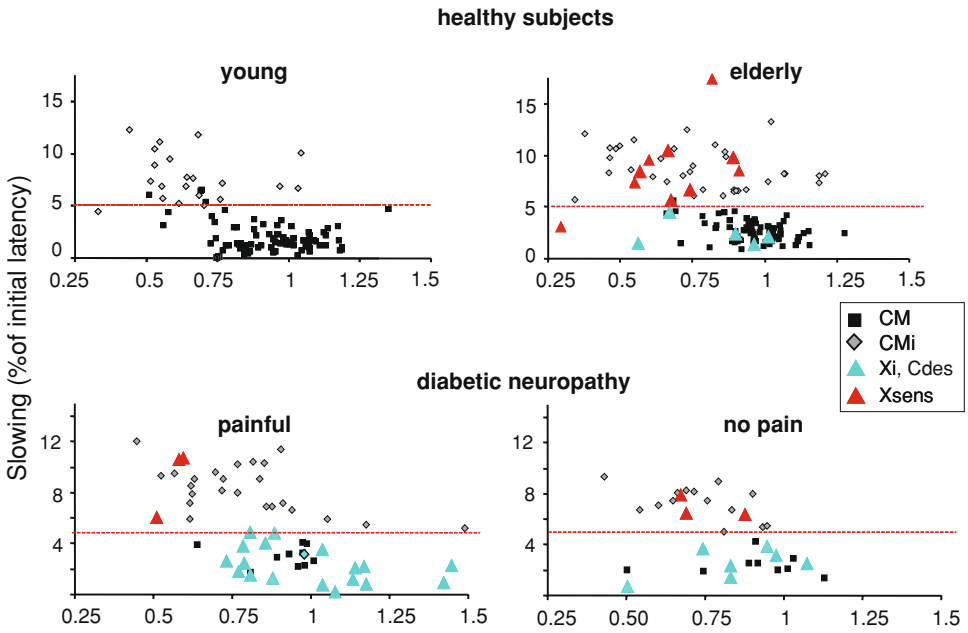

Fig. 3 Populations of C-fibers in young and elderly healthy subjects (*upper diagrams*) and in painful and non-painful neuropathies (*lower diagrams*). Singe units are characterized by their initial conduction velocity (abscissa) and total slowing during a standardized protocol (*ordinate*). *CM* mechano-sensitive units, polymodal nociceptors, *CMi* mechano-insensitive units, *Xi and Cdes* mechano-and thermoinsensitive units with slowing characteristics of CM—units, Xsens: spontaneously active and/or mechano-sensitive units with slowing characteristics of CMi. *Upper diagrams* data from Namer et al. 2008a, *lower diagrams* data from Orstavik et al. 2006

The older population in this study (41–67 years) was not "old" as by nowadays standards. However, already middle-aged patients seem to experience more impairment (Cutler et al. 1994a, b) and psychological distress (Naliboff et al. 1985) from painful events. The majority of patients admitted to pain clinics are between 40 and 60 years (Gagliese and Melzack 1997). The development of chronic pain states might be most likely in a stage when both degeneration and regeneration combined with sensitization processes are in a kind of balance. This hypothesis needs to be proven in future studies.

Neuropathies

The pathomechanisms and symptoms of human neuropathic pain are in many respects different from animal models of neuropathies (Rice et al. 2008), in the light of the different pathomechanisms, time span and life expectancy. It is not surprising that drugs which have been found to abridge pain behavior in animal models of neuropathy turned out to be ineffective in human neuropathic diseases. Recent examples are the drugs targeting NK1 receptors developed for the treatment of neuropathic pain (Boyce and Hill 2008).

Animal models of neuropathic pain are mostly based on the nerve injury, such as chronic constriction injury (Bennett et al. 2003). In this and other animal neuropathy models most emphasis was placed on testing mechanical and thermal thresholds in search of hyperalgesias, whereas less attention was directed to spontaneous ongoing pain, perhaps because of the lack of reliable and easily practicable assessment methods (Mogil and Crager 2004). When looking into studies on patients with traumatic nerve injury, it is obvious that humans suffer most from spontaneous ongoing pain and much less from mechanical or heat hyperalgesia (Leffler and Hansson 2008; Jaaskelainen et al. 2005). In patients suffering from polyneuropathies (see below!) spontaneous pain is even more often the most disturbing symptom (Backonja and Stacey 2004).

Electrophysiology of peripheral nerve fibers provides a more analytic access to changes in neuropathies than behavioral studies. One feature, generally described in animal neuropathy models used to be spontaneous activity of nociceptors, which is thought to induce spontaneous ongoing pain. Spontaneous activity occurred in both, injured (Blumberg and Janig 1984; Devor 2006), and in neighboring uninjured nerve fibers (Wu et al. 2001, 2002; Ringkamp and Meyer 2005). It may be generated at different sites of the axon from the peripheral nerve terminal to the dorsal root (Amir et al. 2005). Spontaneous activity and sensitization of primary afferent C-fibers were also found in animal models of neuropathic pain because of diabetes (Burchiel et al. 1985). An interesting finding in non-human primates was abnormal sensitivity of afferent nerve fibers to catecholamines in uninjured C-fibers neighboring injured axons (Ali et al. 1999).

Not many microneurography studies exist on humans with neuropathic pain caused by nerve injury. In a first such study on amputees Hagbarth et al. obtained recordings from injured nerve fibers and found ectopic activity in C-fibers probably related to stump pain (Nystrom and Hagbarth 1981). In A-fibers spontaneous discharges were observed in microneurographic experiments on patients with paresthesias (Campero et al. 1998). Another microneurographic study on a patient with sympathetically maintained pain

due to a nerve injury related to a compartment syndrome in the legs, showed evidence for catecholamine induced activation of nociceptive nerve endings (Jorum et al. 2007). This result fits with the findings on non-human primates mentioned above. The patient benefited from sympathetic blocks, so that in this case a direct line could be drawn from basic research to clinical treatment (Jorum et al. 2007).

Many patients with neuropathic pain had not experienced traumatic nerve injury, but suffer from small fiber neuropathies caused by systemic diseases, e.g., diabetes, or are of unknown origin. In several microneurography studies on patients suffering from painful small fiber neuropathies abnormalities of C-fiber nociceptor functions have been found, which may be a source of the ongoing spontaneous pain and hyperalgesia. In patients with erythromelalgia, a disease which is accompanied by hyperalgesia and attacks of spontaneous pain, abnormal C-fibers were frequently found which have been described above as "Xsens" (see also Fig. 3, here only a few of these units are shown (red symbols) since these units are not typical for diabetic polyneuropathy). They were mechanically excitable while having the slowing characteristics of CMi units. Some CMi fibers were found to be spontaneously active and may cause the spontaneous ongoing pain of these patients (Orstavik et al. 2003).

In patients with chronic painful feet many C-fibers with a great amount of spontaneous activity were found by Serra et al. (Ochoa et al. 2005) (see Fig. 4) which probably originated in the peripheral terminals of the nociceptors because nerve conduction block by a local anesthetic proximal to the recording site did not abolish the spontaneous activity. Also, sensitization of C-nociceptors with pronounced postexcitatory slowing to mechanical stimuli was reported in this study, although the fibers were interpreted as polymodal nociceptors with altered axonal properties and lowered mechanical thresholds (Ochoa et al. 2005).

In the same patient population, a phenomenon called unidirectional block was described, which could lead to amplification of spike numbers arriving at the spinal cord level (Bostock et al. 2005). Usually an action potential evoked in one axonal branch invades the axonal tree retrogradely and abolishes all other action potentials, which were simultaneously evoked in other branches but conducted more slowly. An unidirectional block at the branching points prevents the action potential from invading this branch antidromically. Therefore, action potentials generated in this slower conducting branch by the same stimulus are not abolished by collision with an antidromic spike and can than reach the stem axon and finally the central nervous system. Consequently, this mechanism leads to an amplification of the input in the CNS. This phenomenon has been described first in healthy volunteers in about 3.2% of the recordings (Weidner et al. 2003), but occurred much more

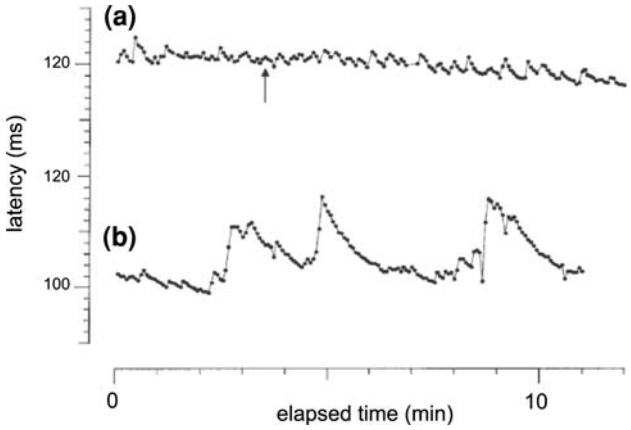

Fig. 4 Different patterns of spontaneous discharge among units in a patient with painful neuropathy of unknown etiology. **a** Unit showing regular low frequency spontaneous discharges which is uninfluenced by local anesthetic application proximal to the recording site (indicated by *arrow*). **b** Another unit from the same recording as in 4a exhibits more sporadic bursts, with greater activity-dependent latency increases and longer recovery time than the unit in 4a. The marking method, as explained in the text was used for characterizing the spontaneous activity of the units. Note that the display is rotated by 90° compared to Figs. 1 and 2. By courtesy of J. Serra, modified from Ochoa et al. 2005

frequently in about 48% of the fibers of patients suffering from neuropathic pain. This could account in part for the hyperalgesia under which all patients of this group suffered. There was clear temperature dependence. During warming, the occurrence of unidirectional block increased, which correlates well with the worsening of hyperalgesia of the patients during warming their feet (Bostock et al. 2005).

In a patient group suffering from diabetic neuropathy, we encountered a different pattern of nerve pathology (Orstavik et al. 2006). A prominent finding in these patients were C-fibers which would have been classified as CMH fibers according to their conduction slowing properties, but were entirely unexcitable by mechanical or heat stimuli applied to their terminals. They were labeled "Xi" and "Xdes" (see Fig. 3c, d). It has been suggested that these unresponsive fibers may represent degenerated CMH fibers, in which only the conductile membrane of the terminal axon was left intact. Correspondingly, fewer normal CMH fibers were observed in these patients (see Fig. 3). In young healthy subjects, a ratio of 8/2 CMH to CMi fibers has been found in microneurographic studies and in older subjects a ratio of 7/3. In the diabetic patients, this ratio was nearly reversed to 3/7. This result indicates that polymodal nociceptors are more prone to degeneration in this form of "dying back" neuropathy than CMi units. In contrast, the number of sensitized Xsens units so typical for erythromelalgia and other forms of neuropathy, are not significantly increased in these patients.

Apparently, there is a discrepancy between the microneurography findings on different patient groups suffering

from neuropathies. The patients studied by Ochoa et al. (2005), Bostock et al. (2005) and the erythromelalgia patients studied by our group (Orstavik et al. 2003) show predominantly spontaneous activity and signs of hyperexcitability. On the other side, the patients suffering from chronic diabetic neuropathies exhibit mostly "negative" symptoms indicating neurodegeneration (Orstavik et al. 2006). It has often been assumed on the basis of animal models that neuropathic pain is directly proportional to spontaneous activity either in damaged nerve fibers, or alternatively in "spared" fibers of neuropathic nerves. This may fit with the findings in the first mentioned patient groups. However, in the diabetic neuropathy patients there was little evidence for that. Several possible reasons are conceivable: (1) the patients in this group did not suffer from such a degree of spontaneous pain at the time of recording as the patients in the other two groups, or (2) the pain had its origin in a region outside the innervations territory of the studied nerve, e.g., in the sole of the foot in case of the burning feet syndrome from which most of our diabetic patients suffered. However, these explanations are not striking in diabetic neuropathy patients who suffered from extended pain in the lower extremities. In these cases, one has to assume that the massive loss of input of polymodal nociceptors has led to central synaptic denervation plasticity that may be able to sustain the chronic pain state even in the absence of ongoing input from the periphery. To test this hypothesis, future microneurography experiments on neuropathy patients should be aimed at recordings specifically from nerve fibers with innervation territories in acutely painful skin areas.

Thus, microneurography can contribute substantially to the solution of the old question whether neuropathic pain is mainly of peripheral or central origin in different neuropathic diseases.

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