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## Microneurography as a tool to study the function of individual C-fiber afferents in humans: responses from nociceptors, thermoreceptors, and mechanoreceptors

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**The technique of microneurography – recording neural traffic from nerves in awake humans – has provided us with unrivalled insights into afferent and efferent processes in the peripheral nervous system for over 50 years. We review the use of microneurography to study single C-fiber afferents and provide an overview of the knowledge gained, with views to future investigations. C-fibers have slowly-conducting, thin-diameter, unmyelinated axons, and make up the majority of the fibers in peripheral nerves (~80%). Using microneurography in humans, C-fiber afferents have been differentiated into discrete sub-classes that encode specific qualities of stimuli on the skin and their functional roles have been investigated. Afferent somatosensory information provided by C-fibers underpins various positive and negative affective sensations from the periphery, including: mechanical, thermal and chemical pain (C-nociceptors); temperature (C-thermoreceptors); and positive affective aspects of touch (C-tactile afferents). Insights from microneurographic investigations have revealed the complexity of the C-fiber system, methods for delineating fundamental C-fiber populations in a translational manner, how C-fiber firing can be used to identify nerve deficits in pathological states, and how the responses from C-fibers may be modified to change sensory percepts, including decreasing pain. Understanding these processes may lead to future medical interventions to diagnose and treat C-fiber dysfunction.**

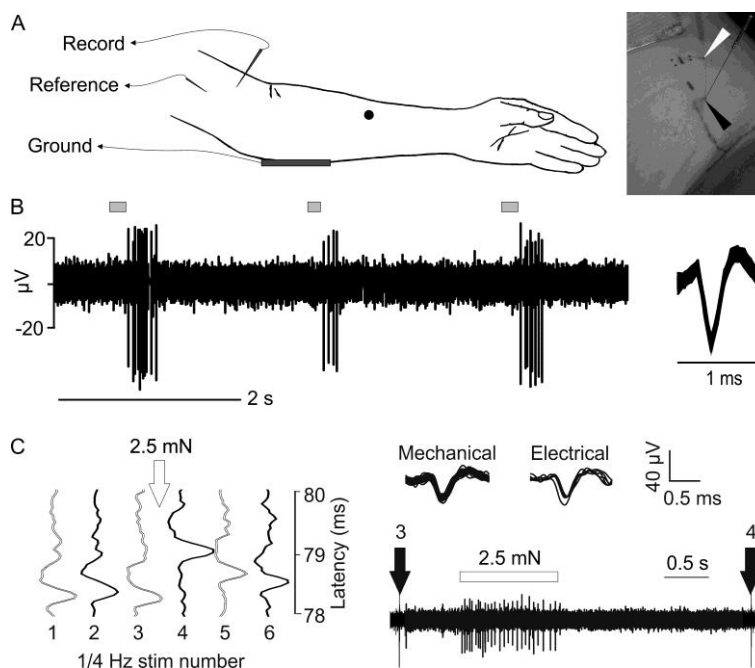
Microneurography is a technique involving the insertion of a microelectrode into a peripheral nerve, in order to register axonal electrical activity, where it is possible to record unitary activity from individual neurons in awake, relaxed humans. This approach offers unique insights into the peripheral bases for somatosensation and the activity of first-order, afferent neurons can be measured in response to varied stimuli, and correlated with subjective sensations. Responses can be recorded from slowly-conducting ( $<2 \text{ m.s}^{-1}$ ), unmyelinated, C-fibers, where combinations of peripheral stimuli (e.g. touch, temperature, electrical stimulation) can be used to group C-fibers into well-defined, discrete, populations subserving different sensory functions. Several classes of C-afferent have been identified, including: C-nociceptors (both C-mechanosensitive (CM) and C-mechanoinsensitive (CMi) nociceptors), C-cold afferents, C-warm afferents, and C-tactile (CT) afferents (Table 1). The aims of the present review are to (i) provide a background into how microneurography can be used to study C-fibers, (ii) outline the different peripheral afferent C-fiber populations in humans, including their physiological response properties, differences in their biophysical axonal properties, and their putative perceptual correlates, and (iii) highlight their significance in pathologies.

### **A brief history of single unit C-fiber microneurography**

The technique of microneurography was developed over 50 years ago in Uppsala, Sweden, by Karl-Erik Hagbarth and Åke Vallbo, in order to record from human peripheral nerves in a minimally invasive manner (Vallbo and Hagbarth 1968; see also the historical perspective by Vallbo 2018). The approach offered an alternative to traditional teased fiber techniques, typically conducted in animals, which involves surgical nerve exposure and partial transection. Although the teased fiber approach can maximize data generation, in terms of the number of high amplitude single unit recordings that can be obtained (Zimmermann et al. 2009), surgery on human participants is invasive and complicated, where the risk of nerve damage is high (Hensel and Boman 1960). Conversely, microneurography involves the percutaneous insertion of a needle electrode into a peripheral nerve, in order to register electrical potentials from axons (see Fig. 1A, B for a typical set-up). This is far less invasive than surgical techniques, as the electrode is gently pushed through the skin and underlying tissue, adjusting the electrode position until an intra-neural position is reached. When the procedure is conducted by an experienced microneurographer, it can be almost painless, although transient sensations can be generated from the movement of the electrode in the external skin layers, pressure on subcutaneous structures (e.g. blood vessels, tendons), the electrode activating passing nerve fibers by pressure, or pressure exerted on the epineurium (Vallbo and Hagbarth 1968; Vallbo et al. 1979).

The original microneurography recordings demonstrated the feasibility of the approach and that unitary potentials originating from single, presumably myelinated, cutaneous and muscle afferents could be registered (Hagbarth and Vallbo 1967, 1969; Vallbo and Hagbarth 1968). A further paper measured what appeared to be bursting of sympathetic C-fiber efferents, but unitary potentials were not discriminable in these responses (Hagbarth and Vallbo 1968), in part due to the relatively low signal amplitudes of C-fibers. Subsequent work showed that it was possible to register unitary potentials from afferent unmyelinated C-fibers using microneurography (Torebjörk and Hallin 1974a). This was surprising, as it was initially believed that single C-fibers could not be recorded from using microneurography, due to the ratio between the small diameter C-fiber axons ( $<2\ \mu\text{m}$ ) and the larger needle electrode (tip  $\sim 5\ \mu\text{m}$ ), and that C fibers are grouped together in Remak bundles in the nerve. This was especially relevant at the time, as single unit C-fiber recordings in animals had only been identified 10 years previously using teased fiber techniques (Douglas and Ritchie 1957), and this was itself contentious among the science community (Vallbo et al. 2004).

The vast majority of C-fiber microneurography studies have investigated responses in human hairy skin, i.e. the non-glabrous skin that covers the majority of the body. Most studies have recorded from afferents in the hairy arm (e.g. responses in Fig. 1B, C) or leg skin, with a few recordings from the face (e.g. Nordin 1990). Conversely, the majority of studies into A $\beta$  mechanoreceptive afferents have been conducted on the glabrous skin of the hands (e.g. Vallbo and Johansson 1984), but only a limited number have investigated C-fiber function here (e.g. Ochoa and Torebjörk 1989; Torebjörk and Ochoa 1990). Methodological considerations that make C-fibers in glabrous skin more difficult to record from include: higher mechanical forces that must be applied to the skin in order to localize recordings from C-fibers as compared to hairy skin, potentially differing profiles of responses, with more easily identifiable prolonged responses in hairy skin, and potentially sparser afferent C-fiber innervation of glabrous skin (Torebjörk and Ochoa 1990). Since differences exist between the thinner hairy skin and thicker glabrous skin, both in terms of receptor type and function, it is possible that the work described below may not be fully applicable to C-fibers in the glabrous skin.



**Figure 1: An example recording from a C-tactile (CT) fiber.**

(A) A typical experimental set-up for microneurography recordings is presented diagrammatically, showing a recording in the lateral antebrachial nerve, from a single C-tactile (CT) unit, represented as a black dot on the arm. The photograph to the right shows an electrode inserted into this nerve. (B) Overlaid CT spikes ( $n = 40$ ) from (C). (C) An example recording from the CT, positioned at (A). Negative-going spikes are shown to four indentations of the skin (shown by the gray boxes above). (D) Marking responses in a (different) CT afferent. Responses to mechanical marking are shown between pulses of electrical stimulation at 0.25 Hz. The physiological stimulation increased the latency of the CT to electrical stimulation. (E) The relative timings of electrical stimuli and mechanical stimuli (delivered between electrical stimuli 3 and 4) are shown on the original recording. (F) Overlaid CT spikes from the mechanical and electrical stimuli.

### Methods for studying C-fibers using microneurography

In order to study C-fibers using microneurography, a target nerve must first be identified, along with an optimal peripheral location for accessing the nerve, based on anatomical considerations (e.g. see Fig. 1A (left) for a typical set-up for recording from the left lateral antebrachial nerve in the arm and a photograph of the electrode in the nerve (right)). Participants must be relaxed in a position that permits experimenters to access the nerve, as even small participant movements may cause instability in a recording. Several techniques may be used to help implant a high impedance recording electrode into the nerve, including knowledge and experience in finding a specific nerve (e.g. depth, angle of the electrode), transcutaneous electrical stimulation to identify the trajectory of the nerve, a guide electrical search electrode, direct electrical stimulation through the

recording electrode, or the more recently used ultrasound imaging of the recording electrode placement (Curry and Charkoudian 2011; Granata et al. 2016; Dunham et al. 2018).

Once the nerve has been penetrated by the recording electrode, it is often accompanied by a neural discharge that can be heard audibly and visualized, sometimes with reports of paresthesia from the participant. When a stable intraneural recording position has been achieved, the skin innervation territory of the impaled nerve fascicle can be identified. This is commonly assessed using feedback from near-instantaneous myelinated, A $\beta$  mechanoreceptive afferent discharges, which typically respond to the stroking of a wide area of skin (Vallbo and Hagbarth 1968; Schmidt et al. 1995; Vallbo et al. 1999; Serra et al. 2012; Watkins et al. 2017). Alternatively, the peripheral innervation of a fascicle may be defined by the use of intraneural microstimulation (detailed below) to define the region of projected sensation for further study (Serra et al. 1999, 2004; Simone et al., 1994). Micro-adjustments of the electrode can then be performed, in order to identify and register unitary potentials.

#### *Identifying C-fibers in microneurography recordings*

C-fibers may be initially identified based on the appreciable comparative conduction delay after mechanical or electrical stimulation at the receptive field. Such conduction delays can be distinguished clearly by an experienced microneurographer if the receptive field-to-recording electrode distance exceeds ~50 mm, with a comparative delay in the responses of greater than ~50 ms as compared to myelinated fibers (Vallbo et al. 1999). The spike shape can also be used to identify single C-fiber units, which is predominantly triphasic, with a major negative deflection (Fig. 1B, C), compared to a bi/triphasic spike with a major positive deflection in A-fibers. A combination of these auditory and visual signals is ideal for the identification of single unit C-fibers, as a minority of A-fiber recordings will involve a triphasic spike with a major negative deflection, which is thought to indicate that the electrode is recording from near a node (Vallbo et al. 1979). Below, we give an overview of the main methods used to identify C-fibers.

#### *Separation of C-fiber spikes*

Amplitude and/or spike shape separation in recordings has been used to directly define the receptor encoding properties of several afferent C-fiber populations (Konietzny and Hensel 1977; Van Hees and Gybels 1981; Serra et al. 1999; Vallbo et al. 1999; Campero et al. 2009; Watkins et al. 2017). Sufficient amplitude and/or spike shape differences are required to allow the unambiguous identification and separation of unitary potentials by software. This approach is usually used during the application of natural stimulation to the receptive field. It becomes problematic when studying afferents with high thresholds, as the repeated application of intense mechanical or thermal stimulation may be painful and/or alter receptor responses, entailing the sensitization or desensitization of C-fiber responses (Bove and Dilley 2010). Using an electrical search procedure that stimulates the whole nerve is best to identify single unit recordings in an unbiased manner, and is commonly carried out in animal studies (Leem and Bove 2002), although this is technically difficult in microneurography recordings and not well-tolerated in humans. A related procedure can be employed in humans, using a combination of mechanical and electrical stimulation of the skin to identify single unit recordings (Schmidt et al. 1995), although this may bias recordings toward neurons with lower electrical thresholds.

#### *Electrical intraneural microstimulation (INMS) of C-fibers*

A method that may be used in the identification C-fiber receptive fields, particularly to identify C-fibers with high mechanical thresholds, is that of intraneural microstimulation (INMS; Konietzny et al. 1981; Ochoa and Torebjörk 1989; Torebjörk and Ochoa 1990), which stimulates small bundles of grouped C-fibers. Depending upon the intraneural site of the electrode, isolated tactile, painful, or mixed-sensation percepts may be generated during trains of electrical stimuli, generally experienced as unpleasant by subjects. These percepts are perceived as projecting to a well-defined peripheral area. At intraneural positions where projected painful dull or burning percepts are generated during INMS, single unit recordings from C-nociceptors with amplitude discrimination sufficient for single unit recordings are commonly identified, with receptive fields near the site of projected painful precepts on the skin (Konietzny et al. 1981; Ochoa and Torebjörk 1989). This has permitted the characterization of units with high thresholds in the glabrous skin of the hand (Torebjörk and Ochoa 1990) and in muscles (Simone et al. 1994).

Performing INMS to identify C-fibers for recording cannot be used to identify the perceptual correlates of an individual fiber in the same way as for myelinated afferents (i.e. *single unit* INMS, Torebjörk and Ochoa 1980; Vallbo 1981; Torebjörk et al. 1987), as several lines of evidence suggest multiple C-fibers may be activated even at liminal INMS stimulation intensities (Jørum et al. 1989; Ochoa and Torebjörk 1989). However, the sensations generated by INMS can tell us about general differences in isolated C-fiber mediated sensations at different peripheral innervation sites, for example, sensations projecting to the glabrous skin feel dull, whereas sensations projected to hairy skin feel sharp (Ochoa and Torebjörk 1989). This can help in investigating integrative aspects of sensation, such as the analgesic effects produced by tactile stimulation upon C-fiber mediated pain (Bini et al. 1984), without exploring the contribution of specific C-fiber receptor classes to perceived sensations.

#### *Latency separation, marking, and activity dependent slowing of C-fibers*

Investigations of C-fiber response properties can be performed using electrical stimulation of the skin with the latency separation of evoked spikes (Torebjörk and Hallin 1974a; Serra et al. 1999). This method can be used to classify most C-fiber populations based on their distinct response profile (Serra et al. 1999; Obreja et al. 2010) and may be used in comparative translational studies to identify similar C-fiber populations in animals, but responses to natural stimulation cannot be monitored directly, except when exceptional separation happens to be present for an individual fiber (Campero et al. 2001, 2011). The identification of C-fibers via their response latency can be performed in situations in which amplitude separation is not possible or desirable, and alternatively can be used to study multiple fibers simultaneously, which increases the yield of data as compared to single unit studies (Serra et al. 2012).

The *latency separation* method is performed by the insertion of two stimulating electrodes into the skin, at the peripheral innervation territory identified by multiunit C-fiber (and also A-fiber) activity (Torebjörk and Ochoa 1990; Serra et al. 1999), at the location of a single unit identified with natural stimulation (Schmidt et al. 1995; Serra et al. 2012; Watkins et al. 2017), or at the location of projected sensations from intraneural microstimulation (Serra et al. 1999; Bostock et al. 2003). This method can be used in order to infer activity in C-fibers based on latency changes, as repeated activity in C-fibers acts to increase their conduction latency (Hallin and Torebjörk 1974; Torebjörk and Hallin 1974b; Schmeltz et al. 1995; Schmidt et al. 1995). This increase in conduction latency is the basis for the marking technique, described below.

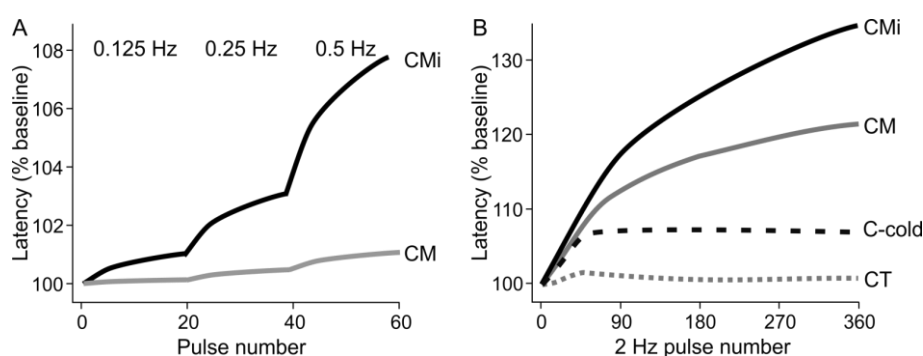
The *marking technique* is a way of monitoring C-fiber activation to a combination of electrical and natural stimulation of the receptive field, based upon response latency changes (Hallin and Torebjörk 1974). First, electrical stimulation is delivered to the skin at a low rate (e.g. 0.25 Hz), where the latencies of evoked spikes are relatively constant and can be tracked in individual fibers (Fig. 1C). Additional natural stimuli are then applied to the receptive field in the skin (e.g. mechanical stimuli between the electrical pulses; Fig. 1C), where conditioning of the response can be observed if there is activity evoked by the test stimulus, and the unit is considered 'marked' as responsive to the test stimulus. Conditioning of the response is identifiable by an abrupt latency shift that is produced during the ongoing low frequency electrical stimulation (Fig. 1C), the magnitude of which is dependent on the intensity of evoked activity (Hallin and Torebjörk 1974; Schmidt et al. 1995; Watkins et al. 2017).

Using the marking method, the functional response characteristics of individual C-fibers can be monitored in multiunit recordings, by virtue of latency deflections caused by conditioning activity from a natural stimulus. This allows the relative magnitude of suprathreshold responses to stimuli (based on the extent of latency shifts) to be studied, the approximate receptor thresholds (Schmeltz et al. 1995; Schmidt et al. 1995), the receptive field structure (Schmeltz et al. 1996; Schmidt et al. 1997, 2002), and pathological/ongoing activity (Kleggetveit et al. 2012; Serra et al. 2012). Two situations in which this technique is problematic to use are: when studying cooling responses, as latency shifts caused by thermodynamic effects of spike propagation cannot be reliably separated from latency shifts induced by cooling evoked activity (Campero et al. 2001), and when studying gentle touch encoding CT afferents, as these show particularly small latency shifts for small numbers of spikes (Watkins et al. 2017). The marking method cannot be used to resolve the timings of spikes, where the classification of units depends on the timing of evoked activity (as with the classification of subpopulations of C-mechano-heat nociceptors in non-human primate studies (Wooten et al. 2014)). However, this method has proved useful in identifying responses in afferents with particularly high mechanical thresholds, and in patient

studies, where signs of pathological spontaneous and evoked activity can be identified without the need to resolve individual spontaneous spikes in units (Kleggetveit et al. 2012; Serra et al. 2012).

*Activity dependent slowing* is a method related to the marking technique that involves the delivery of repetitive peripheral electrical stimulation whilst monitoring latency changes in one or more single unit responses over time (Schmelz et al. 1995; Serra et al. 1999). Latency changes produced by different frequencies of stimulation are dependent upon both the stimulation frequency and the type of C-fiber. The original mechanism proposed for the activity dependent slowing was that of activity dependent hyperpolarization, similar to that documented in myelinated fibers (Thalhammer et al. 1994). Subsequent pharmacological investigations suggested that sodium channel inactivation was likely to be the major mechanism behind this in C-fibers (De Col et al. 2008; Kankel et al. 2012; Obreja et al. 2012), further supported by alterations in activity dependent slowing seen in patients with sodium channel mutations (Namer et al. 2015). This approach can be used to determine action potential generation/propagation (spontaneous or evoked) in experimental and/or pathological conditions. Typically, three different protocols are used for examining activity dependent slowing: low frequency (below 0.5 Hz), intermediate frequency ( $\sim 2$  Hz) and high frequency (up to 200 Hz) stimulation.

Latency changes during low frequency stimulation can differentiate C-nociceptor populations, which show discrete patterns of latency changes (Fig. 2A; Serra et al. 1999; Weidner et al. 1999; Obreja et al. 2010). C-mechanosensitive nociceptors show low latency increases during low frequency stimulation, whereas C-mechanoinsensitive nociceptors show higher latency increases (Weidner et al. 1999; Serra et al. 2004). Low frequency stimulation provides unequivocal separation of C-nociceptive afferents as compared to intermediate frequency stimulation, which produces more modest differences between CM and CMi nociceptors (Weidner et al. 1999; Serra et al. 2004; Obreja et al. 2010). Latency changes during intermediate frequency stimulation are monitored during 2 Hz stimulation (usually 3 minutes in duration; Fig. 2B), followed by recovery pulses delivered at 0.25 Hz (Serra et al. 1999; Campero et al. 2001; Obreja et al. 2010; Watkins et al. 2017). This protocol can be used to differentiate between a wider range of C-fiber populations (e.g. C-tactile, C-nociceptor, C-cold afferents), where there are characteristic slowing profiles that define each type (Serra et al. 1999, 2004; Weidner et al. 1999; Campero et al. 2001; Obreja et al. 2010; Watkins et al. 2017), and can be additionally used to separate C-fiber afferents from sympathetic efferents (Serra et al. 2004; Obreja et al. 2010). High frequency stimulation protocols can complement the classification of different fiber types, as defined by the previous stimulation protocols (Weidner et al. 2000; Bostock et al. 2003) and may provide insights into the excitability and adaptation of C-fibers at firing frequencies observed during natural stimulation (Weidner et al. 2002; Watkins et al. 2017).



**Figure 2: Schematic of activity dependent slowing profiles.** Activity dependent slowing protocols induce latency changes in afferent responses over time. Latency changes are dependent upon both the stimulation frequency and the type of C-fiber, where characteristic profiles are found.

(A) Activity dependent slowing in C-mechanosensitive (CM) and C-mechanoinsensitive (CMi) nociceptors to low frequency electrical stimulation of 0.125, 0.25, and 0.5 Hz. (B) Activity dependent slowing to intermediate frequency 2 Hz electrical stimulation in C-tactile (CT), C-cold, CM, and CMi afferents.

### **Populations of C-fibers found with microneurography and their perceptual correlates**

C-fiber afferents are typically classed by the natural stimulus that they primarily respond to i.e. C-nociceptors for noxious stimuli (including C-pruritic afferents encoding itch-producing substances), C-thermoreceptors for temperature, and C-mechanoreceptors for gentle tactile stimuli (see Table 1). However, it is clear from the literature that C-fibers can respond to heterogeneous stimulation, where an individual C-fiber may respond to multiple stimulus modalities e.g. C-nociceptors that are activated by both noxious cooling and heating; (Campero et al. 1996), or a combined stimulus (e.g. touch and temperature) may modulate firing (e.g. CT afferents are mechanoreceptors, but are subject to thermo-modulatory effects; Ackerley et al. 2014). Below,

we describe the main classes of C-fiber afferents as defined in microneurography recordings from human subjects, their classification by natural stimulation, their activity dependent slowing profiles, and their putative somatosensory perceptual correlate.

### C-nociceptors

C-nociceptors respond to mechanical, thermal, and/or chemical stimulation of the skin, and their responses encode stimuli into the noxious range. C-nociceptors are a heterogeneous population of afferents, consisting of several subpopulations (Table 1). The major subdivision within the C-nociceptors is based upon their sensitivity to mechanical stimulation, and a separation can be made into C-mechanosensitive (CM) and C-mechanoinsensitive (CMi) nociceptors. The differentiation between these groups of afferents is made upon their responsiveness to a forceful mechanical stimulus (~750 mN; Weidner et al. 1999; Serra et al. 2004) where CM fibers will show a strong response, but CMi fibers will not respond. Other functional and anatomical differences between these two groups include their receptive field sizes, heat activation thresholds, chemical sensitivity, electrophysiological properties (e.g. conduction velocity, electrical activation threshold), and activity dependent slowing profiles. Additionally, these two groups of C-nociceptors have been shown to be differentially affected by pathological conditions, with predominantly CMi fibers displaying sensitization and pathological activity (Ørstavik et al. 2003; Serra et al. 2004, 2012, 2014; Kleggetveit et al. 2012).

Classification	Afferent type, nomenclature	Sub-populations	Preferred stimulus
Nociceptor	C-mechanosensitive (CM) nociceptor, type 1A, polymodal	C-mechano-heat nociceptors (CMH)	Noxious touch (CM); the sub-classes also respond to noxious temperature
		C-mechano-heat-cold nociceptors (CMHC)	
Nociceptor	C-mechanoinsensitive nociceptors (CMi or C-MIA), type 1B	C-mechanoinsensitive-heat-insensitive nociceptors (CMiHi)	Noxious heat, little mechanical sensitivity within measurable limits. CMiHi have little thermal sensitivity either. CMi(His+) are pruriceptors and sensitive to histamine
		C-mechanoinsensitive histamine-positive (CMi(His+)), C-pruritic, C-itch	
Thermoreceptor	C-cold afferent, type 2 (C2)	No defined sub-populations	Cooling, with no sensitivity to touch; can show activity at typical skin temperature and fire down to 0°C. May show paradoxical responses to heating.
Thermoreceptor	C-warm afferent	Low threshold warm receptors (LTWRs)	Warming, with no sensitivity to touch.
		High threshold warm receptors (HTWRs)	
Mechanoreceptor	C-tactile afferent (CT; C-low threshold mechanoreceptor, CLTM), type 3	No defined sub-populations	Responds preferentially to slow, gentle, stroking touch delivered at skin temperature.

Table 1: The main classifications of C-fiber afferents.

C-fibers compose three main subclasses of nociceptors, thermoreceptors, and mechanoreceptors, although they often respond to more than one type of stimulus (mechanical/thermal/chemical). Note that C-sympathetic efferents (not included) are described as 'type 4'.

### C-mechanosensitive (CM) nociceptors

The most commonly encountered C-fibers are CM nociceptors, which are activated by mechanical stimulation of the skin, and may additionally be activated by thermal and/or chemical stimulation. The receptive field of CMs are ~1 cm<sup>2</sup> (ranging from 0.1 cm<sup>2</sup> up to 3 cm<sup>2</sup>) and are relatively uniform in shape, although some CMs display more complex multi-spot receptive fields (Van Hees and Gybels 1972; Torebjörk 1974; Torebjörk and Hallin 1974a; Nordin 1990; Schmeltz et al. 1994; Schmidt et al. 1997). Their mechanical thresholds, as assessed using monofilaments, range from 10-300 mN, with a median ~30 mN (Gybels et al. 1979; Schmidt et al. 1995), although occasional units may be encountered with thresholds either above or below this range (Schmidt et al. 1997; Watkins et al. 2017). A sharp stimulus, such as a pin-prick, may evoke a maximal instantaneous firing frequency of ~60 spikes.s<sup>-1</sup> (Torebjörk and Hallin 1974a; Nordin, 1990). CM neurons have conduction velocities of ~1 m.s<sup>-1</sup>, and electrical activation thresholds at the skin of <10 mA, when studied with a monopolar skin surface stimulation (Weidner et al. 1999; Watkins et al. 2017). Marking in CMs suggests that their responses increase in magnitude with increasing force of mechanical stimulation (Schmidt et al., 1995), which implies the encoding of mechanical information into the noxious range.

CMs are a large and varied class of C-afferent and are often referred to as 'polymodal nociceptors' or 'type 1A C-fibers'. Afferents that only respond to mechanical stimuli have been found (CM/C-high threshold

mechanoreceptors, CHTMs; Schmidt et al. 1995) and subclasses exist that include C-mechano-heat nociceptors (CMH) and C-mechano-heat-cold nociceptors (CMHC) (Schmelz et al. 1995; Campero et al. 1996) (Table 1). Heat stimulation activates 60-80% of CMs (Schmidt et al. 1995), with thresholds for firing having a mean temperature of  $\sim 40^{\circ}\text{C}$  (CMHs; Yarnitsky et al. 1992; Schmidt et al. 1997; Weidner et al. 1999). Temperatures are encoded up into the noxious range, where a linear relationship is found between the stimulus temperature and frequency of firing, at temperatures exceeding  $45^{\circ}\text{C}$  (Van Hees and Gybels 1972; Hallin et al. 1981; Yarnitsky et al. 1992). A subdivision of non-human primate CMHs has been revealed by their responses to a stepped heat stimulus, where there is a population of quick-responding and slowly-responding CMH afferents (Wooten et al., 2014). No reports of comparable populations have yet been made in humans, but it is of interest to investigate this possibility.

A little under half of CMs exhibit additional responses to prolonged stimuli at colder temperatures (Campero et al. 1996). These CMHCs do not differ much from CMHs in terms of their receptive field size, mechanical sensitivity, and heat sensitivity, apart from their activation to cold ( $<20^{\circ}\text{C}$ ; Campero et al. 1996). The CMHCs likely encode extremes of temperature, where activation by very hot or very cold stimuli are perceived as a burning pain sensation. The maximal response frequency of CMHCs at cold temperatures is much lower ( $<0.5$  spikes. $\text{s}^{-1}$ ), as compared to mechanical or heat stimulation ( $>15$  spikes. $\text{s}^{-1}$ ) (Yarnitsky et al. 1992; Campero et al. 1996). Thus, mechanisms of signaling cold pain via this population of afferents may be via a separate mechanism to mechanical or heat pain, involving either population coding or bivariate central processing based on spiking frequency.

Substances that activate CMs include pain-inducing chemicals: capsaicin (Schmelz et al. 2000, 2003; Serra et al. 2004), mustard oil (Handwerker et al. 1991; Olausson 1998; Elam et al. 1999), bradykinin (Schmelz et al. 2003), and adenosine triphosphate (Hilliges et al. 2002); itch-inducing chemicals: cowhage (Namer et al. 2008a), and to some extent histamine (Schmelz et al. 1997, 2003; Namer et al. 2008a); and chemicals inducing mixed pain and itch sensations: endothelin-1 (Namer et al. 2008b), prostaglandin E2, acetylcholine, and serotonin, which all induce activity in a proportion of CM neurons (Schmelz et al. 2003; Namer et al. 2015b). Table 2 shows these sensitivities and an overview of the sensations elicited by these chemicals.

Chemical	Predominant sensation	C-mechanosensitive (CM) response	C-mechanoinsensitive (CMi) response
Capsaicin	Pain	Yes, but short-lasting	Yes - sustained
Mustard oil	Pain	Yes	Yes
Bradykinin	Predominantly pain, some itch	Some (weak)	Some
Acetylcholine	Mixed	Some	Some (stronger in His+)
Serotonin	Mixed	Some (very weak)	Some (mostly His+)
Cowhage	Itch	Yes	No
Histamine	Itch	Yes – transient	Yes (sustained )
Prostaglandin E2	Mixed	Yes (weak)	Yes (His+) No (His-)
Endothelin 1	Mixed, mostly pain	Yes (sustained)	None

Table 2: The responsiveness of different C-nociceptor afferents to applied chemicals.

An overview of the responsiveness C-mechanosensitive (CM) nociceptors and C-mechanoinsensitive (CMi) nociceptors and the sensations produced to a variety of different chemicals applied to the afferent's receptive field. Note that His+ refers to afferents that respond to histamine.

When studied with activity dependent slowing, CMs show moderately low latency changes during low frequency stimulation ( $<2\%$  during 5 minutes of 0.125, 0.25, and 0.5 Hz stimulation; Fig. 2A) (Weidner et al. 1999; Obreja et al. 2010) or  $<1\%$  during 6 minutes of 0.25 Hz stimulation (Serra et al. 2004) and high ( $>10\%$ ) latency changes during 2 Hz stimulation (Fig. 2B; Serra et al. 1999, 2004; Ørstavik et al. 2006; Namer et al. 2009; Obreja et al. 2010; Watkins et al. 2017). They show progressive latency increases at higher instantaneous stimulation rates, with a positive exponential relationship between the latency shift and the instantaneous frequency of stimulation (Weidner et al. 2000; Bostock et al. 2003). These patterns of latency changes are distinctive of CM nociceptors and can be used to identify these in the absence of peripheral natural stimulation of the skin (Serra et al. 1999, 2004, Weidner et al. 1999, 2000), and even under pathological conditions (Ørstavik et al. 2003; Kleggetveit et al. 2012; Schmidt et al. 2012; Serra et al. 2012, 2014). No reports have been made of differences in activity dependent slowing between the CM nociceptor subpopulations.



Regarding the perceptual correlates of CM nociceptor activity, they primarily signal mechanically-induced pain from non-injured skin. The activation of CMs by transient mechanical stimuli scales with increasing force into the noxious range (Schmidt et al. 1995), and shows a correlation with the intensity of perceived mechanical pain (Koltzenburg and Handwerker 1994). However, mechanical stimuli below the intensity for evoking painful sensations nevertheless evoke sizable responses (Van Hees and Gybels 1972; Watkins et al. 2017), where the activation threshold for CMs does not match pain thresholds. These findings suggest that coding of mechanically evoked pain by CM afferents is intensity dependent, needing >10 spikes to be evoked for a stimulus to become painful, showing a closer association between pain intensity and number of spikes than pain intensity and mean discharge frequency (Koltzenburg and Handwerker 1994). Further, CMs also appear to play a role in only transient painful sensations, where the application of pain- and itch-inducing chemicals provokes weak or phasic responses (Namer et al. 2015b).

CMH nociceptors are thought to encode heat pain, although similar to mechanical pain, heat-evoked activity occurs at stimulation intensities below those producing pain (Gybels et al. 1979), so suprathreshold encoding in CMHs also appears to be key in signaling heat pain. The intensity of heat pain induced by transient stimuli in non-injured skin shows a linear relationship with the intensity of CMH activation, in terms of the number of spikes when varying the peak temperature of the stimulation (Gybels et al. 1979) or the frequency of firing when varying the rate of temperature rise (Yarnitsky et al. 1992). Radiant heat stimuli rated as painful by subjects can evoke firing rates as low as 0.4 spikes.s<sup>-1</sup> in CMH nociceptors (Van Hees and Gybels 1972), although dynamic firing rates evoked by temperature ramps and contact heat stimuli may evoke firing rates of >5 spikes.s<sup>-1</sup> (Yarnitsky et al., 1992). The rate of C-nociceptor firing relates to the intensity of heat pain (Yarnitsky et al., 1992; Van Hees and Gybels, 1972) and the latency of response to heat stimuli suggests that this is predominantly signaled by C-fibers (Yarnitsky et al., 1992). However, precisely what firing rate in a CMH nociceptor corresponds to the signaling of pain may depend on aspects of temporal and spatial summation in the population C-fiber activity, since such disparate rates of firing can be related to heat pain. Upon repeated stimulation, the intensity of activity in CMH afferents decreases, and this is accompanied by a simultaneous reduction in the perceived pain intensity (Adriaensen et al. 1984), providing another link between peripheral activity in CMH neurons and the intensity of perceived heat pain. Further, CMHs are likely involved in the detection of heat pain (i.e. signaling heat pain threshold) and in signaling non-histaminergic itch, as determined by selective nerve blocking experiments (Weinckauf et al. 2016).

#### C-mechanoinsensitive (CMi) nociceptors

The first studies using electrical stimulation and latency marking to identify receptive properties of C-fiber afferents identified a group of lacking responses to even strong mechanical stimulation (Schmelz et al. 1995; Schmidt et al. 1995). These neurons were easily differentiated from CM nociceptors based on their responses to mechanical stimulation, where CMi nociceptors (also referred to as 'type 1B C-fibers') require at least nine times more mechanical force than the highest activation threshold for CMHs (Weidner et al. 1999). The majority (80%) of CMi nociceptors are responsive to heating and the threshold for CMi nociceptor activation by heat is ~48°C, which is significantly higher than in CMH nociceptors (Weidner et al. 1999). Thus, some CMi nociceptors can be activated by intense mechanical stimulation (e.g. forces greater than 750 mN or the insertion of a needle) and by very strong heating, although a minority may be completely insensitive to both modalities (i.e. C-mechanoinsensitive-heat-insensitive, CMiHi) (Schmidt et al. 1995), hence they are often called 'silent' nociceptors (Table 1). Receptive fields in CMi nociceptors are much more expansive than in CMs, with a median receptive field area of 5 cm<sup>2</sup> (Weidner et al. 1999; Schmidt et al. 2002). CMi receptive field structure is often composed of discontinuous patches, irregular in shape, and the physiological response properties may be heterogeneous across different sites within the receptive field (Schmidt et al. 2002). CMi neurons have conduction velocities around 0.8 m.s<sup>-1</sup>, and electrical activation thresholds at the skin of >30mA, when studied with monopolar skin surface stimulation (Weidner et al. 1999).

The chemical activators of CMi neurons include capsaicin (pain-inducing) (Schmelz et al. 2000), histamine (itch-inducing) (Schmelz et al. 1997, 2003; Namer et al. 2008b), and chemicals with mixed pain and itch sensations: prostaglandin E<sub>2</sub>, acetylcholine, serotonin, and bradykinin, which induce activity in a proportion of CMi neurons (Schmelz et al. 2003; Namer et al. 2015b). However, unlike CMs, they do not respond to cowhage (Namer et al. 2008a), which produces the sensation of itch via non-histaminergic mechanisms (Table 2). CMi neurons displaying histamine responsiveness form a population of CMi neurons with the lowest conduction velocities, being significantly slower conducting than histamine unresponsive CMi neurons (Schmelz et al.

1997). When intense heating or mechanical stimulation do not activate CMi afferents, these neurons can begin to respond to such stimuli after chemical sensitization (Schmelz et al. 1996; Serra et al. 2004) or in pathological states (Ørstavik et al. 2003; Kleggetveit et al. 2012; Serra et al. 2014).

When studied with activity dependent slowing protocols, CMi neurons show high latency changes during low frequency stimulation (>5% during 0.125, 0.25, and 0.5 Hz stimulation; Fig. 2A) (Weidner et al. 1999; Obreja et al. 2010), or >2% during 0.25Hz stimulation (Serra et al. 2004) and high (>10 %) latency changes during 2 Hz stimulation protocols (Fig. 2A; Serra et al. 2004; Ørstavik et al. 2006; Namer et al. 2009; Obreja et al. 2010). CMi nociceptors show progressive latency increases at higher instantaneous stimulation rates, up until an instantaneous stimulation frequency of ~20 Hz, where a relative latency decrease/acceleration of subsequent spikes is seen (Weidner et al. 2000; Bostock et al. 2003). These patterns of latency changes are distinctive of CMi nociceptors and can be used to identify these in the absence of peripheral natural stimulation of the skin (Weidner et al. 1999, 2000; Bostock et al. 2003; Serra et al. 2004), and even under pathological conditions (Kleggetveit et al. 2012; Serra et al. 2012, 2014).

A distinct subpopulation of CMi afferents (~30%) exists that is generally classed as nociceptive, yet more specifically, these afferents are pruriceptors signaling itch (pruritus). These have been named CMi(His+) afferents (Table 1), where they are exquisitely sensitive to the application of histamine (Handwerker et al. 1991; Schmelz et al. 1997, 2003), a chemical known to produce the sensation of itch (Handwerker 2010). During histamine application, these afferents are spontaneously active at ~1 spike.s<sup>-1</sup> (Schmelz et al. 1997). Although the sensation of itch may not necessarily be painful, it is generally regarded as unpleasant and a deviation from comfort. Itch is a multidimensional sensation and different subclasses of pruriceptors may signal specific qualities of itch (Ikoma et al. 2006). To scratch an itch may be rewarding, but the entailing effects are often painful and damaging.

The discharges in CMi afferents relate to sensations of pain evoked by tonic pressure (Schmidt et al. 2000) and to chemically induced sensations (Schmelz et al. 1997, 2000), CMi neurons play a clear role in signaling ongoing painful sensations, as well as itch. The sustained and strong responses of CMi afferents to pain- and itch-inducing chemicals (Table 2) correlates with prolonged and increased pain sensations (Namer et al. 2015b). Schmelz et al. (1997) showed that the time course of itch magnitude ratings matched the activity induced in CMi(His+) afferents from the application of histamine. CMi afferents have expansive innervation territories, and when a local nerve is blocked by anesthetic, CMi innervation from other nearby nerves remains in the numbed skin. When this skin is tested for its sensitivity, heat pain threshold is significantly elevated (>50°C), there is an absence of cowhage-induced itch (both signaled by CMs), yet a maintenance of histamine-induced itch (Schley et al. 2013; Weinkauff et al. 2016), further implicating CMi afferents in conveying the sensation of itch.

Due to their sensitivity to irritant chemicals and inflammation, it is likely that CMi nociceptors play a central role in signaling ongoing pain in injured skin, where they become sensitized by some aspect of the damage and/or inflammation, which has clinical implications. In pathological conditions of various etiologies, ongoing activity in CMi neurons appears causal in generating the spontaneous pain felt by patients (Kleggetveit et al. 2012). CMi neurons thus seem to respond only during intense painful stimulation under normal conditions, but may act as inflammation or damage detectors during pathological situations, perhaps serving a protective role against damaging tissue further. In some conditions, for example fibromyalgia, CMi neurons may become pathologically active even in the absence of obvious damage or inflammation (Serra et al. 2014). The investigation of mechanisms by which CMi fibers become sensitized in pathological states, and methods or pharmacological interventions for reducing their activity, may allow for the treatment of the ongoing pathological pain that this group of neurons plays an important role in signaling (Kleggetveit et al. 2012). Since spontaneous pain is the most problematic aspect of pathological pain states for patients (Baron et al. 2010), identifying ways in which pathological CMi activity can be reduced is an important clinical goal.

### *C-thermoreceptors*

Few studies have investigated thermoreceptors in humans, even though our sense of temperature provides a wealth of information about the state of the skin, from feeling the sun on a hot day to sensing wetness (Filingeri and Ackerley 2017). The majority of these have studied thermoreception in the hairy skin, and virtually no direct evidence of thermoreceptors in glabrous skin exists, although it is clear that we feel temperature on the glabrous skin. A psychophysical study by Stevens and Choo (1998) demonstrated the

differences between body sites and temperature sensing, where overall, humans readily sense cooling more than warming, and that the face is the most sensitive area to thermal deviations, with the extremities less so. Thermoreceptors are fewer in number and the main difficulty is finding them during microneurography, as it is much easier to stimulate and identify recordings from afferents using touch, than temperature, and confounding effects of temperature stimulation on evoked spike latency complicates the interpretation of responses identified using the marking technique (Campero et al. 2001). The typical method for finding thermoreceptors during microneurography has been to use an electrical search stimulation approach and/or using thermal stimuli. C-thermoreceptors respond to radiant thermal changes, although skin contact (and thus better temperature exchange) is a more effective method of producing responses (Konietzny 1984).

#### C-cold afferents

C-cold afferents are tonically active ( $\sim 1 \text{ spike} \cdot \text{s}^{-1}$ ) around typical skin temperature of  $\sim 30^\circ\text{C}$  and are very sensitive to changes in skin temperature, where higher discharges are seen at  $<29^\circ\text{C}$  and the tonic activity is suppressed on warming (Campero et al. 2001). They are not activated by touch and encode a range of temperatures to  $0^\circ\text{C}$ , with peak sensitivity  $\sim 20^\circ\text{C}$ , thus underpin cutaneous cold sensations. The firing rates of C-cold afferents are variable, but in general, they all respond with a phasic 2-3 s increase in firing during cold stimulation that decays to an adapted tonic response. They have punctate receptive fields, conduct at  $\sim 1 \text{ m} \cdot \text{s}^{-1}$ , and some C-cold units also respond paradoxically to heating from  $\sim 40^\circ\text{C}$  (Konietzny 1984; Campero et al. 2001, 2009). C-cold fibers slow around 5% with repetitive 2 Hz electrical stimulation, tend to plateau in their response, and recover quickly (Fig. 2B; Serra et al. 1999; Campero et al. 2001). C-cold fibers can be separated from sympathetic efferents, which show a similar extent of slowing, by their lack of reversal in the slowing pattern, and based on latency changes after 5 s of 2 Hz stimulation (Campero et al. 2004).

C-cold afferents do not necessarily encode absolute temperature; rather their response is determined by the change of temperature. For example, on cooling the receptive field to  $20^\circ\text{C}$ , a unit may fire consistently around  $15 \text{ spikes} \cdot \text{s}^{-1}$ ; however, on re-warming, it quickly ceases responding (Campero et al. 2009). This function implicates that they respond preferentially to dynamic changes in temperature. The role of C-cold fibers may provide conscious and/or unconscious information about skin cooling, the perception of paradoxical or illusory thermal sensations, thermoregulatory functions, and signal deviations from thermoneutrality and/or thermal comfort (Filingeri and Ackerley 2017; Filingeri et al. 2017). Green and Pope (2003) suggested the conflicting term 'innocuous cold nociception' for the role of C-cold fibers in somatosensation, where these fibers may sense non-noxious cooling well, but that it is not a particularly pleasant perception. This fits with a possible role in sensing dynamic thermoneutrality of the skin, both at cooler and warmer temperatures (Filingeri et al. 2017).

#### C-warm afferents

Konietzny (1984) described two putative types of warm fiber, namely 'low threshold warm receptors (LTWRs)' that fire at typical skin temperature ( $\sim 30^\circ\text{C}$ ) up to  $\sim 40^\circ\text{C}$ , and 'high threshold warm receptors (HTWRs)' that are excited at higher temperatures (from  $\sim 38^\circ\text{C}$ ) and fire up until the thermal heat pain threshold (Table 1). Although, considering the finding that C-cold fibers may also convey the sensation of warming, it is plausible that some warm sensations are also encoded by these afferents. Hence, C-cold afferents may also be HTWRs and underpin some hot, burning, and/or unpleasant heat sensations. Few studies have investigated LTWRs, yet it has been shown that they respond readily to warming, do not respond to touch, have punctate receptive fields, and are tonically active around or just above typical skin temperature (Konietzny and Hensel 1975, 1977; Hallin et al. 1981).

In the total of seven LTWR units studied, they have been found to conduct at  $\sim 0.7 \text{ m} \cdot \text{s}^{-1}$  (Konietzny and Hensel 1975; Konietzny 1984), which is relatively slow for a C-fiber. They fire from  $\sim 32^\circ\text{C}$  and their frequency of discharge is related to the rate of temperature increase, showing peak frequencies of up to  $35 \text{ spike} \cdot \text{s}^{-1}$ , between 5-10 s after the onset of stimulation (Konietzny and Hensel 1975, 1977; Konietzny 1984). After this initial increase in firing, the LTWR discharge decreases to a constant rate ( $\sim 5\text{-}10 \text{ spikes} \cdot \text{s}^{-1}$ ) to an adapted temperature, where the adapted value is proportionate to the initial peak response (Konietzny and Hensel 1977). It is thought that LTWRs contribute to sensing non-noxious warming, including the perception of static warmth (Konietzny 1984), where like the C-cold afferents, they could play a further role in thermoregulatory functions, and signal deviations from thermoneutrality. The activity dependent slowing properties of C-warm receptors have not yet been reported, so it is not currently possible to identify this population of afferents in multiunit recordings.

*C-mechanoreceptors*

C-tactile (CT) afferents signal gentle touch (Table 1) and, to date, have only been reported in hairy skin. CTs are defined by their responsiveness to light touch ( $< 5$  mN) and stroking of the skin (Vallbo et al. 1999; Löken et al. 2009; Ackerley et al. 2014; Watkins et al. 2017), and are differentiated from C-nociceptors based on mechanical responsiveness (Vallbo et al. 1999; Watkins et al. 2017). They are classed as intermediate adapting, have one or multiple small receptive fields, and show properties such as fatigue, delayed acceleration, and the propensity for after-discharges (Nordin 1990; Vallbo et al. 1999; Wessberg et al. 2003). They have conduction velocities of  $\sim 0.9$  m.s<sup>-1</sup> (Vallbo et al. 1999; Watkins et al. 2017). CTs show very little activity dependent slowing when studied using the 2 Hz protocol ( $< 1\%$ ; Fig. 2B; Campero et al. 2011; Watkins et al. 2017). This low level of slowing provides a clear distinction between CTs and all other identified C-fiber populations, and can be used to classify this population in the absence of natural skin stimulation.

CTs are very responsive to gentle mechanical stimulation, where slowly-moving touch is an effective stimulus for generating high firing frequencies (mean  $\sim 40$  spikes.s<sup>-1</sup>; Löken et al. 2009; Ackerley et al. 2014). The encoding of tactile velocity by CTs is non-linear, with responses following a quadratic (inverted-U shape) function, with a peak in firing frequency at stroking velocities of between 1-10 cm.s<sup>-1</sup>. The frequency of response is not strongly modulated by stimulus force (Löken et al. 2009); however, the responses to a moving stimulus are also modulated by stimulus temperature, with optimal responses around skin temperature (Ackerley et al. 2014). CTs are not thermoreceptors as such, as they are not sensitive to radiant heating or cooling, but their activity is decreased to heating, and they show complex responses to cooling (Nordin 1990; Ackerley et al. 2018). When a CT receptive field is cooled, then stroked, prolonged afterdischarges (of up to 30 s) can be found; however, these appear at a low frequency ( $\sim 5$  spikes.s<sup>-1</sup>) and there is no particular corresponding sensation that accompanies the discharge and it may be due the effect of visco-elastic changes of the skin (Ackerley et al. 2018).

CTs are hypothesized to convey positive affective touch and inter-personal, affiliative interactions (Morrison et al. 2010; McGlone et al. 2014), as their firing frequency to stroking correlates with subjective ratings of pleasantness (Löken et al. 2009; Ackerley et al. 2014). Their firing frequency appears to be critical in the signaling of sensations, as CTs readily respond during a very slow stroke (e.g. 0.3 cm.s<sup>-1</sup>) over its receptive field, producing numerous spikes, yet these are of lower frequency ( $\sim 25$  spikes.s<sup>-1</sup>; Löken et al. 2009; Ackerley et al. 2014). CTs do not seem to provide much sense of conscious touch, for example, individuals lacking fast-conducting, myelinated afferents state that they do not feel touch, yet they report a vague pressure sensation from a soft brush stroke, if they concentrate on a body area being stroked (Olausson et al. 2002). This sensation is reported to be pleasant, with no component of temperature, pain, itch, or tickle. Thus CTs may provide information that acts to 'color' the conscious sensations provided by other simultaneously activated afferents (i.e. A $\beta$  afferents), adding a positive emotional component to the sensation.

*The use of microneurography to study pathological mechanisms*

A number of microneurography studies have investigated pathological mechanisms in C-nociceptors and microneurography has been developed in animals, which has an impact on translational research (Serra et al. 2010). Many of the human microneurography studies find changes in C-nociceptor firing, which have been linked to aspects of chronic pain. In patients with neuropathy, Ørstavik et al. (2006) found that the proportion of CMs is decreased, where it seems that many CMs lose their sensitivity to heat and touch. C-nociceptors displaying spontaneous activity or mechanical sensitization have been regularly found in neuropathy patients (Ochoa et al. 2005; Ørstavik et al. 2006; Kleggetveit et al. 2012; Serra et al. 2012), and Kleggetveit et al. (2012) found that these were mainly accounted for by CMi afferents in patients with pain. Spontaneous CMi activity has been related to less pronounced activity dependent slowing, thus it seems that the afferents' axons had also become sensitized (Kleggetveit et al. 2012). Additional C-nociceptor spiking to a brief electrical stimulus has also been found in neuropathy patients (Bostock et al. 2005; Schmidt et al. 2012) (Bostock et al. 2005; Schmidt et al. 2012), which may be a mechanism by which C-nociceptor input to the central nervous system is amplified for a given intensity of stimulation. These lines of inquiry provide useful insights into the mechanisms of polyneuropathy and why it can be painful, which has implications for the developments of specific treatments.

Similar pathologies have been found in erythromelalgia patients suffering from allodynia and hyperalgesia. C-nociceptors were found to have significantly lowered conduction velocities, with increased activity dependent

slowing, where CMi nociceptors were particularly found to change properties, being spontaneously active or sensitized to mechanical stimuli (Ørstavik et al. 2003). Further, Namer et al. (2015) found that half of the CMi nociceptors recorded from in erythromelalgia patients were spontaneously active, which represents a much higher proportion than in patients with neuropathy. Fibromyalgia is a disease lacking obvious signs of damage or inflammation in peripheral tissues, but it is accompanied by sensitization and pathological activity in C-nociceptors (Serra et al. 2014). This research aids us in understanding how underlying pathologies differ between somatosensory conditions, may help us link particular symptoms of neurological disease to C-fiber pathology, and will help in producing novel therapeutic targets, such as membrane-stabilizing agents (Serra 2009).

Regarding physical nerve damage, Nyström and Hagbarth (1981) provided insights into peripheral nerve changes associated with phantom limb pain in amputees. They found pronounced spontaneous, bursting activity in both cutaneous and muscle nerve fascicles, which originated from both faster-conducting afferents and C-fibers. This demonstrates the peripheral changes found when trauma is experienced, where spontaneously-generated impulses were clearly linked to pain. Further, the authors concluded that mechanically-evoked pain also originated from hyperexcitable C-nociceptive afferents in the neuromata.

Microneurography has been used to explore changes in C-fibers with aging. Namer et al. (2009) explored C-fibers in younger (mean age 25 years) and older (mean age 56 years) participants and found that with aging, the C-nociceptor population distribution was changed, with a proportional increase in CM as compared to CMi nociceptors. Spontaneous nociceptor activity, sensitization, and loss of sensory function were shown with aging, and activity dependent slowing was more pronounced. However, these changes in C-nociceptors were not associated with any pain, and the proportion of pathological C-fibers was much lower than in patients with neuropathy. Hence, it seems that C-fibers naturally degrade over the lifespan and it is only when substantial changes are found that pathological pain occurs.

Further clinical investigations are warranted to investigate more of the complex mechanisms in various pain states, relating to peripheral and/or central disorders. One study investigated the possibility of altered C-nociceptor function in complex regional pain syndrome and found that spontaneous C-fiber responses were only in those with additional neuropathy (Campero et al. 2010). Thus, it is useful to see that in this pathology, it is likely that central mechanisms play a key role. In all clinical investigations using microneurography, great care must be taken in using patients, especially those with degraded nerves. Although microneurography is relatively painless and should not cause long-term damage, it is unknown whether the insertion of an electrode into an atypical peripheral nerve would cause further pathology to develop. Microneurography may be employed as a specialist clinical diagnostic tool, taking into account factors such as the expertise needed in conducting it, needing specialist equipment, the spatial resolution of the technique (i.e. you typically record from one or a few fibers at a time), and ethical requirements. In these situations, it may provide insights into aberrant firing in peripheral nerves that will lead to better ways to identify, manage, and treat somatosensory disorders. For example, microneurography is used to evaluate the effectiveness of novel pharmacological therapies on pain in patient groups, using spontaneous activity in C-nociceptors as a quantifiable marker of spontaneous pain (Serra et al. 2015).

### ***Future investigations and conclusions***

As well as the continuing use of microneurography for elucidating pathological mechanisms, there are many unresolved questions in the domain of C-fiber research. These questions include the investigation of afferents mediating temperature sensations in different human skin types, where there are no studies from glabrous skin, and how cutaneous afferents are involved in more complex stimulus interactions. When considering relationships between C-fiber afferent activity and perception, it is important to consider the integrative nature sensations generated. Natural stimulation of the skin will generate percepts that are mediated by C-fiber afferents, but a number of myelinated afferents will be co-activated. This is particularly important for complex sensations such as wetness or pleasantness, which are likely to involve the integration of A- and C-fiber activity (McGlone et al. 2014; Filingeri and Ackerley 2017). It is also of interest to look at how sensations from C-fibers can be modified to change somatosensory percepts, for example, can pleasantness be enhanced by activating CT fibers, which may also decrease pain and conversely, can pain be reduced by acting on nociceptors (for a review of such mechanisms, see Leknes and Tracey 2008). Concerning pain, chemicals that reduce or regulate CMi pathological activity in neuropathies may be beneficial, especially if such an approach did not act on CM

afference. From the work outlined above, several chemicals have already been identified that are more selective for CM or CMi nociceptors (Table 2), yet these all increase their firing, rather than control or reduce it. Identifying the underlying differences in C-fiber populations, such as the expression of different sodium channels that contribute to the non-overlapping differences seen with activity dependent slowing, may allow us to develop agents capable of modulating activity in specific populations.

In conclusion, microneurography presents a technically demanding, yet insightful, approach for studying C-fibers in humans. These thin-diameter fibers are numerous in the human afferent system in the periphery, where the information conducted is rich and varied. The present review highlights that we have learned a lot about C-fibers in humans, especially C-nociceptors, but there is plenty we have yet to uncover, for example little is known about thermoreceptor function. C-fiber microneurography continues to progress in both fundamental research and in understanding pathological mechanisms, where the knowledge gained may help the treatment of a range of debilitating somatosensory disorders.

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No conflicts of interest, financial or otherwise, are declared by the authors.

#### Author contributions

R.A. conceived the manuscript; R.A. and R.H.W. planned, prepared and wrote the manuscript together. Both authors approved the final version of the manuscript.

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#### New & noteworthy

The technique of microneurography allows us to directly investigate the functional roles of single C-fiber afferents in awake human beings. Here, we outline and discuss the current field of C-fiber research on this heterogeneous population of afferents in healthy subjects, in pathological states, and from a translational perspective. We cover C-fibers encoding touch, temperature, and pain, and provide perspectives on the future of C-fiber microneurography investigations in humans.

**Keywords:** C fibre, mechanoreceptor, microneurography, nociception, pain, touch, temperature