J Neurophysiol 120: 2834–2846, 2018.
First published September 26, 2018; doi:10.1152/jn.00109.2018.

REVIEW | *50 Years of Microneurography: Insights into Neural Mechanisms in Humans*

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

Rochelle Ackerley[1,2] and Roger Holmes Watkins[2]

[1]*Aix Marseille University, CNRS, LNSC (Laboratoire de Neurosciences Sensorielles et Cognitives - UMR 7260), Marseille, France; and* [2]*Department of Physiology, University of Gothenburg, Gothenburg, Sweden*

Submitted 13 February 2018; accepted in final form 25 September 2018

Ackerley R, Watkins RH. Microneurography as a tool to study the function of individual C-fiber afferents in humans: responses from nociceptors, thermoreceptors, and mechanoreceptors. *J Neurophysiol* 120: 2834–2846, 2018. First published September 26, 2018; doi:10.1152/jn.00109.2018.—The technique of microneurography—recording neural traffic from nerves in awake humans—has provided us with unrivaled 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%). With the use of microneurography in humans, C-fiber afferents have been differentiated into discrete subclasses 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.

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.

C-fiber; mechanoreceptor; microneurography; nociception; pain; temperature; touch

INTRODUCTION

Microneurography is a technique involving the insertion of a microelectrode into a peripheral nerve 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 m/s), unmyelinated C-fibers, where combinations of peripheral stimuli (e.g., touch, temper-

Address for reprint requests and other correspondence: R. Ackerley, Laboratoire de Neurosciences Sensorielles et Cognitives (LNSC–UMR 7260), Centre Saint-Charles, Fédération de Recherche 3C–Case B, 3 place Victor Hugo, Aix-Marseille Université, 13331 Marseille Cedex 03, France (e-mail: rochelle.ackerley@univ-amu.fr).

ature, electrical stimulation) can be used to group C-fibers into well-defined, discrete populations subserving different sensory functions. Several classes of C-afferents 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 *1*) provide a background for how microneurography can be used to study C-fibers; *2*) 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 *3*) 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 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, that involve 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, and 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 to register electrical potentials from axons (see Fig. 1, *A* and *B*, for a typical setup). This is far less invasive than surgical techniques, as the electrode is gently pushed through the skin and underlying tissue, with the electrode position adjusted until an intraneural 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 et al. 1979; Vallbo and Hagbarth 1968).

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 because of the relatively low signal amplitudes of C-fibers. Subsequent work showed that it was possible to register unitary potentials from afferent unmyelinated C-fibers with microneurography (Torebjörk and Hallin 1974a). This was surprising, as it was initially believed that single C-fibers could not be recorded from with microneurography, because of the ratio between the small-diameter C-fiber axons (<2 μm) and the larger needle electrode (tip ~5 μm), 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 with 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 nonglabrous skin that covers the majority of the body. Most studies have recorded from afferents in the hairy arm (e.g., responses in Fig. 1, *B* and *C*) or leg skin, with a few recordings from the face (e.g., Nordin 1990). Conversely, the majority of studies into Aβ 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 to localize recordings from C-fibers compared with 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, in terms of both receptor type and function, it is possible that the work described below may not be fully applicable to C-fibers in the glabrous skin.

Table 1. *Main classifications of C-fiber afferents*

Classification	Afferent Type, Nomenclature	Subpopulations	Preferred Stimulus
Nociceptor	C-mechanosensitive (CM) nociceptor, type 1A, polymodal	C-mechano-heat nociceptors (CMH) C-mechano-heat-cold nociceptors (CMHC)	Noxious touch (CM); the subclasses also respond to noxious temperature.
Nociceptor	C-mechanoinsensitive nociceptors (CMi or C-MIA), type 1B	C-mechanoinsensitive-heat-insensitive nociceptors (CMiHi) C-mechanoinsensitive histamine-positive [CMi(His+)], C-pruritic, C-itch	Noxious heat, little mechanical sensitivity within measurable limits. CMiHi have little thermal sensitivity, either. CMi(His+) are pruriceptors and sensitive to histamine.
Thermoreceptor	C-cold afferent, type 2 (C2)	No defined subpopulations	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) High-threshold warm receptors (HTWRs)	Warming, with no sensitivity to touch.
Mechanoreceptor	C-tactile afferent (CT; C-low-threshold mechanoreceptor, CLTM), type 3	No defined subpopulations	Responds preferentially to slow, gentle, stroking touch delivered at skin temperature.

C-fibers comprise 3 main subclasses of nociceptors, thermoreceptors, and mechanoreceptors, although they often respond to >1 type of stimulus (mechanical/thermal/chemical). Note that C-sympathetic efferents (not included) are described as "type 4."

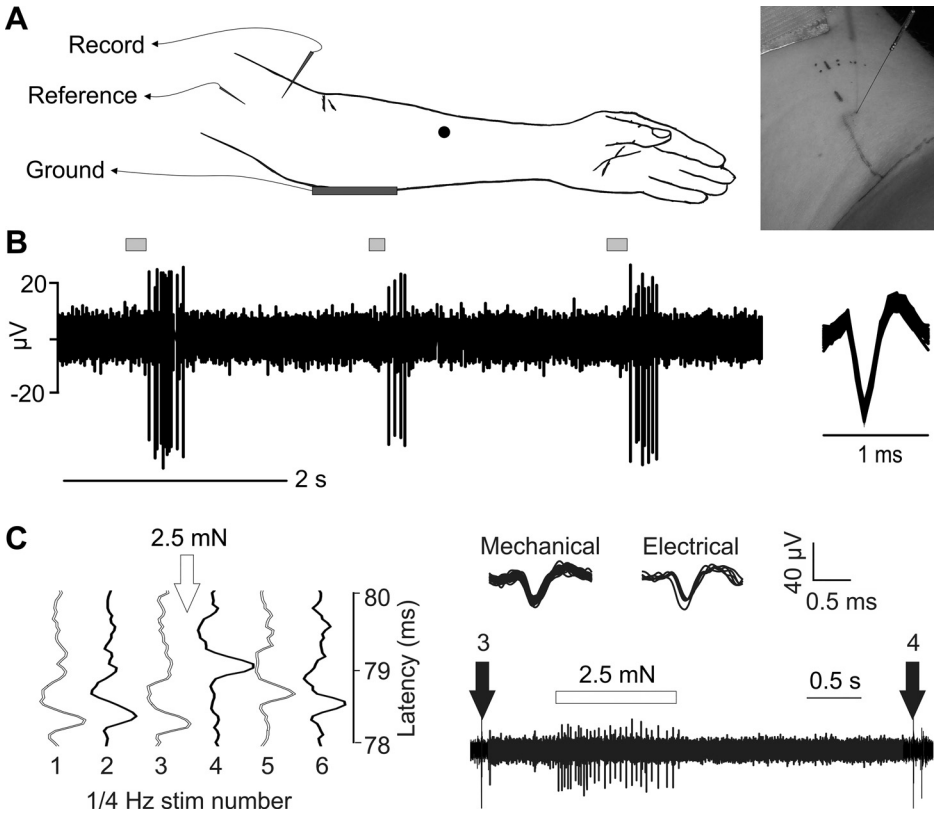

Fig. 1. An example recording from a C-tactile (CT) fiber. *A*, *left*: a typical experimental setup for microneurography recordings is presented diagrammatically, showing a recording in the lateral antebrachial nerve, from a single CT unit, represented as a black dot on the arm. *Right*: an electrode inserted into this nerve. *B*, *left*: an example recording from the CT, positioned as in *A*. Negative-going spikes are shown to 4 indentations of the skin (shown by gray boxes at *top*). *Right*: overlaid CT spikes ($n = 40$) from the recording. *C*: marking responses in a (different) CT afferent. *Left*: 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. *Bottom right*: the relative timings of electrical stimuli and mechanical stimuli delivered between *electrical stimuli 3* and *4* are shown on the original recording. *Top right*: overlaid CT spikes from the mechanical and electrical stimuli.

METHODS FOR STUDYING C-FIBERS WITH MICRONEUROGRAPHY

To study C-fibers with 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. 1*A*, *left*, for a typical setup for recording from the left lateral antebrachial nerve in the arm and Fig. 1*A*, *right*, for a photograph of the electrode in the nerve). 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; Dunham et al. 2018; Granata et al. 2016).

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 with feedback from near-instantaneous myelinated Aβ mechanoreceptive afferent discharges, which typically respond to the stroking of a wide area of skin (Schmidt et al. 1995; Serra et al. 2012; Vallbo et al. 1999; Vallbo and Hagbarth 1968; Watkins et al. 2017). Alternatively, the peripheral innervation of a fascicle may be defined by the use of intraneural microstimulation (INMS; detailed below) to define the region of projected sensation for further study (Serra et al. 1999, 2004; Simone et al. 1994). Microadjustments of the electrode can then be performed to identify and register unitary potentials.

Identifying C-Fibers in Microneurography Recordings

C-fibers may be initially identified on the basis of 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 compared with myelinated fibers (Vallbo et al. 1999). Single C-fiber units can also be identified by the spike shape, which is predominantly triphasic with a major negative deflection (Fig. 1, *B* and *C*), compared with 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 recoding 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 (Campero et al. 2009; Konietzny and Hensel 1977; Serra et al. 1999; Vallbo et al. 1999; Van Hees and Gybels 1981; 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). 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, the use of 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 of C-fibers. A method that may be used in the identification of C-fiber receptive fields, particularly to identify C-fibers with high mechanical thresholds, is INMS (Konietzny et al. 1981; Ochoa and Torebjörk 1989; Torebjörk and Ochoa 1990), which stimulates small bundles of grouped C-fibers. Depending on 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 et al. 1987; Torebjörk and Ochoa 1980; Vallbo 1981), as several lines of evidence suggest that 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 with the use of electrical stimulation of the skin with the latency separation of evoked spikes (Serra et al. 1999; Torebjörk and Hallin 1974a). This method can be used to classify most C-fiber populations on the basis of their distinct response profile (Obreja et al. 2010; Serra et al. 1999) and may be used in comparative translational studies to identity 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 compared with 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 (Serra et al. 1999; Torebjörk and Ochoa 1990), 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 INMS (Bostock et al. 2003; Serra et al. 1999). This method can be used 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; Schmelz et al. 1995; Schmidt et al. 1995; Torebjörk and Hallin 1974b). 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 on 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. 1*C*). Additional natural stimuli are then applied to the receptive field in the skin (e.g., mechanical stimuli between the electrical pulses; Fig. 1*C*), 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. 1*C*), the magnitude of which is dependent on the intensity of evoked activity (Hallin and Torebjörk 1974; Schmidt et al. 1995; Watkins et al. 2017).

With 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 (Schmelz et al. 1995; Schmidt et al. 1995), the receptive field structure (Schmelz 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 nonhuman 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 while 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 on both the stimulation frequency and the type of C-fiber. The original mechanism proposed for 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. 2015a). 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 (~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. 2*A*; Obreja et al. 2010; Serra et al. 1999; Weidner et al. 1999). CM nociceptors show low latency increases during low-frequency stimulation, whereas CMi nociceptors show higher latency increases (Serra et al. 2004; Weidner et al. 1999). Low-frequency stimulation provides unequivocal separation of C-nociceptive afferents compared with intermediate-frequency stimulation, which pro-

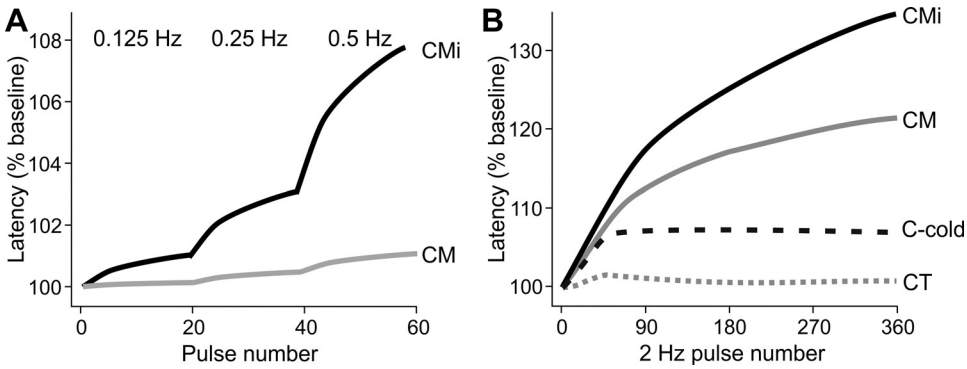

Fig. 2. Schematic of activity-dependent slowing profiles. Activity-dependent slowing protocols induce latency changes in afferent responses over time. Latency changes are dependent on both the stimulation frequency and the type of C-fiber, where characteristic profiles are found. *A*: activity-dependent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

duces more modest differences between CM and CMi nociceptors (Obreja et al. 2010; Serra et al. 2004; Weidner et al. 1999). Latency changes during intermediate-frequency stimulation are monitored during 2-Hz stimulation (usually 3 min in duration; Fig. 2*B*), followed by recovery pulses delivered at 0.25 Hz (Campero et al. 2001; Obreja et al. 2010; Serra et al. 1999; 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 (Campero et al. 2001; Obreja et al. 2010; Serra et al. 1999, 2004; Watkins et al. 2017; Weidner et al. 1999), and can be additionally used to separate C-fiber afferents from sympathetic efferents (Obreja et al. 2010; Serra et al. 2004). High-frequency stimulation protocols can complement the classification of different fiber types, as defined by the previous stimulation protocols (Bostock et al. 2003; Weidner et al. 2000), and may provide insights into the excitability and adaptation of C-fibers at firing frequencies observed during natural stimulation (Watkins et al. 2017; Weidner et al. 2002).

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 on 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 based on their responsiveness to a forceful mechanical stimulus (~750 mN; Serra et al. 2004; Weidner et al. 1999), 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 (Kleggetveit et al. 2012; Ørstavik et al. 2003; Serra et al. 2004, 2012, 2014).

C-mechanosensitive 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 fields of CMs are ~1 cm^2 (ranging from 0.1 cm^2 to 3 cm^2) and are relatively uniform in shape, although some CMs display more complex multispot receptive fields (Nordin 1990; Schmelz et al. 1994; Schmidt et al. 1997; Torebjörk 1974; Torebjörk and Hallin 1974a; Van Hees and Gybels 1972). Their mechanical thresholds, as assessed with monofilaments, range from 10 to 300 mN, with a median of ~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 pinprick, may evoke a maximal instantaneous firing frequency of ~60 spikes/s (Nordin 1990; Torebjörk and Hallin 1974a). CM neurons have conduction velocities of ~1 m/s and electrical activation thresholds at the skin of $<$10 mA when studied with a monopolar skin surface stimulation (Watkins et al. 2017; Weidner et al. 1999). 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-afferents 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 (CMHs) and C-mechano-heat-cold nociceptors (CMHCs) (Campero et al. 1996; Schmelz et al. 1995) (Table 1). Heat stimulation activates 60–80% of CMs (Schmidt et al. 1995), with thresholds for firing having a mean

temperature of ~40°C (CMHs; Schmidt et al. 1997; Weidner et al. 1999; Yarnitsky et al. 1992). 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°C (Hallin et al. 1982; Van Hees and Gybels 1972; Yarnitsky et al. 1992). A subdivision of nonhuman 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°C; Campero et al. 1996). The CMHCs likely encode extremes of temperature, where activation by very hot or very cold stimuli is perceived as a burning pain sensation. The maximal response frequency of CMHCs at cold temperatures is much lower (<0.5 spikes/s) compared with mechanical or heat stimulation (>15 spikes/s) (Campero et al. 1996; Yarnitsky et al. 1992). Thus, signaling of cold pain via this population of afferents may be via a mechanism separate from 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 (Elam et al. 1999; Handwerker et al. 1991; Olausson 1998), 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 (Namer et al. 2008a; Schmelz et al. 1997, 2003); and chemicals inducing mixed pain and itch sensations: endothelin-1 (Namer et al. 2008b), prostaglandin E_2, acetylcholine, and serotonin, which all induce activity in a proportion of CM neurons (Namer et al. 2015b; Schmelz et al. 2003). Table 2 shows these sensitivities and an overview of the sensations elicited by these chemicals.

When studied with activity-dependent slowing, CMs show moderately low latency changes during low-frequency stimulation [<2% during 5 min of 0.125-, 0.25-, and 0.5-Hz stimulation (Fig. 2*A*) (Obreja et al. 2010; Weidner et al. 1999) or <1% during 6 min of 0.25-Hz stimulation (Serra et al. 2004)] and high (>10%) latency changes during 2-Hz stimulation (Fig. 2*B*; Namer et al. 2009; Obreja et al. 2010; Ørstavik et al. 2006; Serra et al. 1999, 2004; 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 (Bostock et al. 2003; Weidner et al. 2000). 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 (Kleggetveit et al. 2012; Ørstavik et al. 2003; 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 noninjured 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). Furthermore, 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 noninjured 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 in CMH nociceptors (Van Hees and Gybels 1972), although dynamic firing rates evoked

Table 2. *Responsiveness of different C-nociceptor afferents to applied chemicals*

Chemical	Predominant Sensation	CM Response	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 E_2	Mixed	Yes (weak)	Yes (His+) No (His−)
Endothelin 1	Mixed, mostly pain	Yes (sustained)	None

An overview of the responsiveness of 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. His+ refers to afferents that respond to histamine.

by temperature ramps and contact heat stimuli may evoke firing rates of >5 spikes/s (Yarnitsky et al. 1992). The rate of C-nociceptor firing relates to the intensity of heat pain (Van Hees and Gybels 1972; Yarnitsky et al. 1992), 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. Furthermore, CMHs are likely involved in the detection of heat pain (i.e., signaling heat pain threshold) and in signaling nonhistaminergic itch, as determined by selective nerve blocking experiments (Weinkauf et al. 2016).

C-mechanoinsensitive nociceptors. The first studies using electrical stimulation and latency marking to identify receptive properties of C-fiber afferents identified a group lacking responses to even strong mechanical stimulation (Schmelz et al. 1995; Schmidt et al. 1995). These neurons were easily differentiated from CM nociceptors on the basis of 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 > 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) and 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^2 (Schmidt et al. 2002; Weidner et al. 1999). 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, and electrical activation thresholds at the skin of >30 mA, 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) (Namer et al. 2008b; Schmelz et al. 1997, 2003), and chemicals with mixed pain and itch sensations: prostaglandin E_2, acetylcholine, serotonin, and bradykinin, which induce activity in a proportion of CMi neurons (Namer et al. 2015b; Schmelz et al. 2003). However, unlike CMs, they do not respond to cowhage (Namer et al. 2008a), which produces the sensation of itch via nonhistaminergic 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 does 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 (Kleggetveit et al. 2012; Ørstavik et al. 2003; 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. 2*A*) (Obreja et al. 2010; Weidner et al. 1999) or >2% during 0.25-Hz stimulation (Serra et al. 2004)] and high (>10%) latency changes during 2-Hz stimulation protocols (Fig. 2*B*; Namer et al. 2009; Obreja et al. 2010; Ørstavik et al. 2006; Serra et al. 2004). CMi nociceptors show progressive latency increases at higher instantaneous stimulation rates, up to an instantaneous stimulation frequency of ~20 Hz, where a relative latency decrease/acceleration of subsequent spikes is seen (Bostock et al. 2003; Weidner et al. 2000). 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 (Bostock et al. 2003; Serra et al. 2004; Weidner et al. 1999, 2000) 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 (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 ensuing 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) correlate 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 by 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) and there is an absence of cowhage-induced itch (both signaled by CMs) yet a maintenance of histamine-induced itch (Schley et al. 2013; Weinkauf et al. 2016), further implicating CMi afferents in conveying the sensation of itch.

Because of 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 with touch than with temperature, and confounding effects of temperature stimulation on evoked spike latency complicate the interpretation of responses identified by 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 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 (~1 spike/s) around typical skin temperature of ~30°C and are very sensitive to changes in skin temperature, where higher discharges are seen at <29°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°C, with peak sensitivity ~20°C, and thus underpin cutaneous cold sensations. The firing rates of C-cold afferents are variable, but in general they all respond with a phasic 2- to 3-s increase in firing during cold stimulation that decays to an adapted tonic response. They have punctate receptive fields and conduct at ~1 m/s, and some C-cold units also respond paradoxically to heating from ~40°C (Campero et al. 2001, 2009; Konietzny 1984). C-cold fibers slow ~5% with repetitive 2-Hz electrical stimulation, tend to plateau in their response, and recover quickly (Fig. 2*B*; Campero et al. 2001; Serra et al. 1999). 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 of the receptive field to 20°C a unit may fire consistently around 15 spikes/s; however, on rewarming 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 et al. 2017; Filingeri and Ackerley 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 nonnoxious cooling well but it is not a particularly pleasant perception. This fits with a possible role in sensing dynamic thermoneutrality of the skin, at both 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 (~30°C) up to ~40°C and "high-threshold warm receptors (HTWRs)" that are excited at higher temperatures (from ~38°C) and fire up to 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 (Hallin et al. 1982; Konietzny and Hensel 1975, 1977).

In the total of seven LTWR units studied, they have been found to conduct at ~0.7 m/s (Konietzny 1984; Konietzny and Hensel 1975), which is relatively slow for a C-fiber. They fire from ~32°C, and their frequency of discharge is related to the rate of temperature increase, showing peak frequencies of up to 35 spike/s between 5 and 10 s after the onset of stimulation (Konietzny 1984; Konietzny and Hensel 1975, 1977). After this initial increase in firing, the LTWR discharge decreases to a constant rate (~5–10 spikes/s) 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 nonnoxious 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 (Ackerley et al. 2014; Löken et al. 2009; Vallbo et al. 1999; Watkins et al. 2017) and are differentiated from C-nociceptors on the basis of 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 afterdischarges (Nordin 1990; Vallbo et al. 1999; Wessberg et al. 2003). They have conduction velocities of ~0.9 m/s (Vallbo et al. 1999; Watkins et al. 2017). CTs show very little activity-dependent slowing when studied with the 2 Hz protocol (<1%; Fig. 2*B*; 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 ~40 spikes/s; Ackerley et al. 2014; Löken et al. 2009). The encoding of tactile velocity by CTs is nonlinear, with responses following a quadratic (inverted-U shape) function, with a peak in firing frequency at stroking velocities of between 1 and 10 cm/s. 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 (Ackerley et al. 2018; Nordin 1990). When a CT receptive field is cooled and then stroked, prolonged afterdischarges (of up to 30 s) can be found; however, these appear at a low frequency (~5 spikes/s), and there is no particular corresponding sensation that accompanies the discharge and it may be due to the effect of viscoelastic changes of the skin (Ackerley et al. 2018).

CTs are hypothesized to convey positive affective touch and interpersonal, affiliative interactions (McGlone et al. 2014; Morrison et al. 2010), as their firing frequency to stroking correlates with subjective ratings of pleasantness (Ackerley et al. 2014; Löken et al. 2009). 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) over the receptive field, producing numerous spikes, yet these are of lower frequency (~25 spikes/s; Ackerley et al. 2014; Löken et al. 2009). 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., Aβ afferents), adding a positive emotional component to the sensation.

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 (Kleggetveit et al. 2012; Ochoa et al. 2005; Ørstavik et al. 2006; 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), 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). Furthermore, 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. Furthermore, 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 yr) and older (mean age 56 yr) partic-

ipants and found that with aging the C-nociceptor population distribution was changed, with a proportional increase in CM as compared with 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 life span 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, the need for 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 of the 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 coactivated. 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 (Filingeri and Ackerley 2017; McGlone et al. 2014). 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 does 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 controlling or reducing it. Identifying the underlying differences in C-fiber populations, such as the expression of different sodium channels that contribute to the nonoverlapping 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 both in fundamental research and in understanding pathological mechanisms, where the knowledge gained may help the treatment of a range of debilitating somatosensory disorders.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

R.A. and R.H.W. prepared figures; R.A. and R.H.W. drafted manuscript; R.A. and R.H.W. edited and revised manuscript; R.A. and R.H.W. approved final version of manuscript.

REFERENCES

Ackerley R, Backlund Wasling H, Liljencrantz J, Olausson H, Johnson RD, Wessberg J. Human C-tactile afferents are tuned to the temperature of a skin-stroking caress. *J Neurosci* 34: 2879–2883, 2014. doi:10.1523/JNEUROSCI.2847-13.2014.

Ackerley R, Wiklund Fernström K, Backlund Wasling H, Watkins RH, Johnson RD, Vallbo Å, Wessberg J. Differential effects of radiant and mechanically applied thermal stimuli on human C-tactile afferent firing patterns. *J Neurophysiol* 120: 1885–1892, 2018. doi:10.1152/jn.00940.2017.

Adriaensen H, Gybels J, Handwerker HO, Van Hees J. Suppression of C-fibre discharges upon repeated heat stimulation may explain characteristics of concomitant pain sensations. *Brain Res* 302: 203–211, 1984. doi:10.1016/0006-8993(84)90232-4.

Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol* 9: 807–819, 2010. doi:10.1016/S1474-4422(10)70143-5.

Bini G, Cruccu G, Hagbarth KE, Schady W, Torebjörk E. Analgesic effect of vibration and cooling on pain induced by intraneural electrical stimulation. *Pain* 18: 239–248, 1984. doi:10.1016/0304-3959(84)90819-4.

Bostock H, Campero M, Serra J, Ochoa J. Velocity recovery cycles of C fibres innervating human skin. *J Physiol* 553: 649–663, 2003. doi:10.1113/jphysiol.2003.046342.

Bostock H, Campero M, Serra J, Ochoa JL. Temperature-dependent double spikes in C-nociceptors of neuropathic pain patients. *Brain* 128: 2154–2163, 2005. doi:10.1093/brain/awh552.

Bove GM, Dilley A. The conundrum of sensitization when recording from nociceptors. *J Neurosci Methods* 188: 213–218, 2010. doi:10.1016/j.jneumeth.2010.02.010.

Campero M, Baumann TK, Bostock H, Ochoa JL. Human cutaneous C fibres activated by cooling, heating and menthol. *J Physiol* 587: 5633–5652, 2009. doi:10.1113/jphysiol.2009.176040.

Campero M, Bostock H, Baumann TK, Ochoa JL. A search for activation of C nociceptors by sympathetic fibers in complex regional pain syndrome.

Clin Neurophysiol 121: 1072–1079, 2010. doi:10.1016/j.clinph.2009.12.038.

Campero M, Bostock H, Baumann TK, Ochoa JL. Activity-dependent slowing properties of an unmyelinated low threshold mechanoreceptor in human hairy skin. *Neurosci Lett* 493: 92–96, 2011. doi:10.1016/j.neulet.2011.02.012.

Campero M, Serra J, Bostock H, Ochoa JL. Slowly conducting afferents activated by innocuous low temperature in human skin. *J Physiol* 535: 855–865, 2001. doi:10.1111/j.1469-7793.2001.t01-1-00855.x.

Campero M, Serra J, Bostock H, Ochoa JL. Partial reversal of conduction slowing during repetitive stimulation of single sympathetic efferents in human skin. *Acta Physiol Scand* 182: 305–311, 2004. doi:10.1111/j.1365-201X.2004.01357.x.

Campero M, Serra J, Ochoa JL. C-polymodal nociceptors activated by noxious low temperature in human skin. *J Physiol* 497: 565–572, 1996. doi:10.1113/jphysiol.1996.sp021789.

Curry TB, Charkoudian N. The use of real-time ultrasound in microneurography. *Auton Neurosci* 162: 89–93, 2011. doi:10.1016/j.autneu.2011.03.007.

De Col R, Messlinger K, Carr RW. Conduction velocity is regulated by sodium channel inactivation in unmyelinated axons innervating the rat cranial meninges. *J Physiol* 586: 1089–1103, 2008. doi:10.1113/jphysiol.2007.145383.

Douglas WW, Ritchie JM. Nonmedullated fibres in the saphenous nerve which signal touch. *J Physiol* 139: 385–399, 1957. doi:10.1113/jphysiol.1957.sp005899.

Dunham JP, Sales AC, Pickering AE. Ultrasound-guided, open-source microneurography: approaches to improve recordings from peripheral nerves in man. *Clin Neurophysiol* 129: 2475–2481, 2018. doi:10.1016/j.clinph.2018.07.011.

Elam M, Olausson B, Skarphedinsson JO, Wallin BG. Does sympathetic nerve discharge affect the firing of polymodal C-fibre afferents in humans? *Brain* 122: 2237–2244, 1999. doi:10.1093/brain/122.12.2237.

Filingeri D, Ackerley R. The biology of skin wetness perception and its implications in manual function and for reproducing complex somatosensory signals in neuroprosthetics. *J Neurophysiol* 117: 1761–1775, 2017. doi:10.1152/jn.00883.2016.

Filingeri D, Zhang H, Arens EA. Characteristics of the local cutaneous sensory thermoneutral zone. *J Neurophysiol* 117: 1797–1806, 2017. doi:10.1152/jn.00845.2016.

Granata G, Giambattistelli F, Padua L, Coraci D, Petrini FM. High-frequency ultrasound in guiding needle insertion for microneurography. *Clin Neurophysiol* 127: 970–971, 2016. doi:10.1016/j.clinph.2015.04.061.

Green BG, Pope JV. Innocuous cooling can produce nociceptive sensations that are inhibited during dynamic mechanical contact. *Exp Brain Res* 148: 290–299, 2003. doi:10.1007/s00221-002-1280-9.

Gybels J, Handwerker HO, Van Hees J. A comparison between the discharges of human nociceptive nerve fibres and the subject's ratings of his sensations. *J Physiol* 292: 193–206, 1979. doi:10.1113/jphysiol.1979.sp012846.

Hagbarth KE, Vallbo AB. Mechanoreceptor activity recorded percutaneously with semi-microelectrodes in human peripheral nerves. *Acta Physiol Scand* 69: 121–122, 1967. doi:10.1111/j.1748-1716.1967.tb03498.x.

Hagbarth KE, Vallbo AB. Pulse and respiratory grouping of sympathetic impulses in human muscle-nerves. *Acta Physiol Scand* 74: 96–108, 1968. doi:10.1111/j.1365-201X.1968.tb10904.x.

Hagbarth KE, Vallbo AB. Single unit recordings from muscle nerves in human subjects. *Acta Physiol Scand* 76: 321–334, 1969. doi:10.1111/j.1748-1716.1969.tb04475.x.

Hallin RG, Torebjörk HE. Methods to differentiate electrically induced afferent and sympathetic C unit responses in human cutaneous nerves. *Acta Physiol Scand* 92: 318–331, 1974. doi:10.1111/j.1748-1716.1974.tb05750.x.

Hallin RG, Torebjörk HE, Wiesenfeld Z. Nociceptors and warm receptors innervated by C fibres in human skin. *J Neurol Neurosurg Psychiatry* 45: 313–319, 1982. doi:10.1136/jnnp.45.4.313.

Handwerker HO. Microneurography of pruritus. *Neurosci Lett* 470: 193–196, 2010. doi:10.1016/j.neulet.2009.06.092.

Handwerker HO, Forster C, Kirchhoff C. Discharge patterns of human C-fibers induced by itching and burning stimuli. *J Neurophysiol* 66: 307–315, 1991. doi:10.1152/jn.1991.66.1.307.

Hensel H, Boman KK. Afferent impulses in cutaneous sensory nerves in human subjects. *J Neurophysiol* 23: 564–578, 1960. doi:10.1152/jn.1960.23.5.564.

Hilliges M, Weidner C, Schmelz M, Schmidt R, Ørstavik K, Torebjörk E, Handwerker H. ATP responses in human C nociceptors. *Pain* 98: 59–68, 2002. doi:10.1016/S0304-3959(01)00469-9.

Ikoma A, Steinhoff M, Ständer S, Yosipovitch G, Schmelz M. The neurobiology of itch. *Nat Rev Neurosci* 7: 535–547, 2006. doi:10.1038/nrn1950.

Jørum E, Lundberg LE, Torebjörk HE. Peripheral projections of nociceptive unmyelinated axons in the human peroneal nerve. *J Physiol* 416: 291–301, 1989. doi:10.1113/jphysiol.1989.sp017761.

Kankel J, Obreja O, Kleggetveit IP, Schmidt R, Jørum E, Schmelz M, Namer B. Differential effects of low dose lidocaine on C-fiber classes in humans. *J Pain* 13: 1232–1241, 2012. doi:10.1016/j.jpain.2012.09.008.

Kleggetveit IP, Namer B, Schmidt R, Helås T, Rückel M, Ørstavik K, Schmelz M, Jørum E. High spontaneous activity of C-nociceptors in painful polyneuropathy. *Pain* 153: 2040–2047, 2012. doi:10.1016/j.pain.2012.05.017.

Koltzenburg M, Handwerker HO. Differential ability of human cutaneous nociceptors to signal mechanical pain and to produce vasodilatation. *J Neurosci* 14: 1756–1765, 1994. doi:10.1523/JNEUROSCI.14-03-01756.1994.

Konietzny F. Peripheral neural correlates of temperature sensations in man. *Hum Neurobiol* 3: 21–32, 1984.

Konietzny F, Hensel H. Warm fiber activity in human skin nerves. *Pflugers Arch* 359: 265–267, 1975. doi:10.1007/BF00587384.

Konietzny F, Hensel H. The dynamic response of warm units in human skin nerves. *Pflugers Arch* 370: 111–114, 1977. doi:10.1007/BF00707956.

Konietzny F, Perl ER, Trevino D, Light A, Hensel H. Sensory experiences in man evoked by intraneural electrical stimulation of intact cutaneous afferent fibers. *Exp Brain Res* 42: 219–222, 1981. doi:10.1007/BF00236910.

Leem JG, Bove GM. Mid-axonal tumor necrosis factor-alpha induces ectopic activity in a subset of slowly conducting cutaneous and deep afferent neurons. *J Pain* 3: 45–49, 2002. doi:10.1054/jpai.2002.27138.

Leknes S, Tracey I. A common neurobiology for pain and pleasure. *Nat Rev Neurosci* 9: 314–320, 2008. doi:10.1038/nrn2333.

Löken LS, Wessberg J, Morrison I, McGlone F, Olausson H. Coding of pleasant touch by unmyelinated afferents in humans. *Nat Neurosci* 12: 547–548, 2009. doi:10.1038/nn.2312.

McGlone F, Wessberg J, Olausson H. Discriminative and affective touch: sensing and feeling. *Neuron* 82: 737–755, 2014. doi:10.1016/j.neuron.2014.05.001.

Morrison I, Löken LS, Olausson H. The skin as a social organ. *Exp Brain Res* 204: 305–314, 2010. doi:10.1007/s00221-009-2007-y.

Namer B, Barta B, Ørstavik K, Schmidt R, Carr R, Schmelz M, Handwerker HO. Microneurographic assessment of C-fibre function in aged healthy subjects. *J Physiol* 587: 419–428, 2009. doi:10.1113/jphysiol.2008.162941.

Namer B, Carr R, Johanek LM, Schmelz M, Handwerker HO, Ringkamp M. Separate peripheral pathways for pruritus in man. *J Neurophysiol* 100: 2062–2069, 2008a. doi:10.1152/jn.90482.2008.

Namer B, Hilliges M, Ørstavik K, Schmidt R, Weidner C, Torebjörk E, Handwerker H, Schmelz M. Endothelin 1 activates and sensitizes human C-nociceptors. *Pain* 137: 41–49, 2008b. doi:10.1016/j.pain.2007.08.008.

Namer B, Ørstavik K, Schmidt R, Kleggetveit IP, Weidner C, Mørk C, Kvernebo MS, Kvernebo K, Salter H, Carr TH, Segerdahl M, Quiding H, Waxman SG, Handwerker HO, Torebjörk HE, Jørum E, Schmelz M. Specific changes in conduction velocity recovery cycles of single nociceptors in a patient with erythromelalgia with the I848T gain-of-function mutation of Nav1.7. *Pain* 156: 1637–1646, 2015a. doi:10.1097/j.pain.0000000000000229.

Namer B, Schick M, Kleggetveit IP, Ørstavik K, Schmidt R, Jorum E, Torebjörk E, Handwerker H, Schmelz M. Differential sensitization of silent nociceptors to low pH stimulation by prostaglandin E2 in human volunteers. *Eur J Pain* 19: 159–166, 2015b. doi:10.1002/ejp.532.

Nordin M. Low-threshold mechanoreceptive and nociceptive units with unmyelinated (C) fibres in the human supraorbital nerve. *J Physiol* 426: 229–240, 1990. doi:10.1113/jphysiol.1990.sp018135.

Nyström B, Hagbarth KE. Microelectrode recordings from transected nerves in amputees with phantom limb pain. *Neurosci Lett* 27: 211–216, 1981. doi:10.1016/0304-3940(81)90270-6.

Obreja O, Hirth M, Turnquist B, Rukwied R, Ringkamp M, Schmelz M. The differential effects of two sodium channel modulators on the conductive properties of C-fibers in pig skin in vivo. *Anesth Analg* 115: 560–571, 2012. doi:10.1213/ANE.0b013e3182542843.

Obreja O, Ringkamp M, Namer B, Forsch E, Klusch A, Rukwied R, Petersen M, Schmelz M. Patterns of activity-dependent conduction velocity changes differentiate classes of unmyelinated mechano-insensitive afferents including cold nociceptors, in pig and in human. *Pain* 148: 59–69, 2010. doi:10.1016/j.pain.2009.10.006.

Ochoa J, Torebjörk E. Sensations evoked by intraneural microstimulation of C nociceptor fibres in human skin nerves. *J Physiol* 415: 583–599, 1989. doi:10.1113/jphysiol.1989.sp017737.

Ochoa JL, Campero M, Serra J, Bostock H. Hyperexcitable polymodal and insensitive nociceptors in painful human neuropathy. *Muscle Nerve* 32: 459–472, 2005. doi:10.1002/mus.20367.

Olausson B. Recordings of human polymodal single C-fiber afferents following mechanical and argon-laser heat stimulation of inflamed skin. *Exp Brain Res* 122: 55–61, 1998. doi:10.1007/s002210050490.

Olausson H, Lamarre Y, Backlund H, Morin C, Wallin BG, Starck G, Ekholm S, Strigo I, Worsley K, Vallbo AB, Bushnell MC. Unmyelinated tactile afferents signal touch and project to insular cortex. *Nat Neurosci* 5: 900–904, 2002. doi:10.1038/nn896.

Ørstavik K, Namer B, Schmidt R, Schmelz M, Hilliges M, Weidner C, Carr RW, Handwerker H, Jørum E, Torebjörk HE. Abnormal function of C-fibers in patients with diabetic neuropathy. *J Neurosci* 26: 11287–11294, 2006. doi:10.1523/JNEUROSCI.2659-06.2006.

Ørstavik K, Weidner C, Schmidt R, Schmelz M, Hilliges M, Jørum E, Handwerker H, Torebjörk E. Pathological C-fibres in patients with a chronic painful condition. *Brain* 126: 567–578, 2003. doi:10.1093/brain/awg060.

Schley M, Rukwied R, Blunk J, Menzer C, Konrad C, Dusch M, Schmelz M, Benrath J. Mechano-insensitive nociceptors are sufficient to induce histamine-induced itch. *Acta Derm Venereol* 93: 394–399, 2013. doi:10.2340/00015555-1513.

Schmelz M, Forster C, Schmidt R, Ringkamp M, Handwerker HO, Torebjörk HE. Delayed responses to electrical stimuli reflect C-fiber responsiveness in human microneurography. *Exp Brain Res* 104: 331–336, 1995. doi:10.1007/BF00242018.

Schmelz M, Schmid R, Handwerker HO, Torebjörk HE. Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. *Brain* 123: 560–571, 2000. doi:10.1093/brain/123.3.560.

Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjörk HE. Specific C-receptors for itch in human skin. *J Neurosci* 17: 8003–8008, 1997. doi:10.1523/JNEUROSCI.17-20-08003.1997.

Schmelz M, Schmidt R, Ringkamp M, Forster C, Handwerker HO, Torebjörk HE. Limitation of sensitization to injured parts of receptive fields in human skin C-nociceptors. *Exp Brain Res* 109: 141–147, 1996. doi:10.1007/BF00228635.

Schmelz M, Schmidt R, Ringkamp M, Handwerker HO, Torebjörk HE. Sensitization of insensitive branches of C nociceptors in human skin. *J Physiol* 480: 389–394, 1994. doi:10.1113/jphysiol.1994.sp020368.

Schmelz M, Schmidt R, Weidner C, Hilliges M, Torebjörk HE, Handwerker HO. Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. *J Neurophysiol* 89: 2441–2448, 2003. doi:10.1152/jn.01139.2002.

Schmidt R, Kleggetveit IP, Namer B, Helås T, Obreja O, Schmelz M, Jørum E. Double spikes to single electrical stimulation correlates to spontaneous activity of nociceptors in painful neuropathy patients. *Pain* 153: 391–398, 2012. doi:10.1016/j.pain.2011.10.041.

Schmidt R, Schmelz M, Forster C, Ringkamp M, Torebjörk E, Handwerker H. Novel classes of responsive and unresponsive C nociceptors in human skin. *J Neurosci* 15: 333–341, 1995. doi:10.1523/JNEUROSCI.15-01-00333.1995.

Schmidt R, Schmelz M, Ringkamp M, Handwerker HO, Torebjörk HE. Innervation territories of mechanically activated C nociceptor units in human skin. *J Neurophysiol* 78: 2641–2648, 1997. doi:10.1152/jn.1997.78.5.2641.

Schmidt R, Schmelz M, Torebjörk HE, Handwerker HO. Mechano-insensitive nociceptors encode pain evoked by tonic pressure to human skin. *Neuroscience* 98: 793–800, 2000. doi:10.1016/S0306-4522(00)00189-5.

Schmidt R, Schmelz M, Weidner C, Handwerker HO, Torebjörk HE. Innervation territories of mechano-insensitive C nociceptors in human skin. *J Neurophysiol* 88: 1859–1866, 2002. doi:10.1152/jn.2002.88.4.1859.

Serra J. Re-emerging microneurography. *J Physiol* 587: 295–296, 2009. doi:10.1113/jphysiol.2008.167858.

Serra J, Bostock H, Navarro X. Microneurography in rats: a minimally invasive method to record single C-fiber action potentials from peripheral nerves in vivo. *Neurosci Lett* 470: 168–174, 2010. doi:10.1016/j.neulet.2009.09.061.

Serra J, Bostock H, Solà R, Aleu J, García E, Cokic B, Navarro X, Quiles C. Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats. *Pain* 153: 42–55, 2012. doi:10.1016/j.pain.2011.08.015.

Serra J, Campero M, Bostock H, Ochoa J. Two types of C nociceptors in human skin and their behavior in areas of capsaicin-induced secondary hyperalgesia. *J Neurophysiol* 91: 2770–2781, 2004. doi:10.1152/jn.00565.2003.

Serra J, Campero M, Ochoa J, Bostock H. Activity-dependent slowing of conduction differentiates functional subtypes of C fibres innervating human skin. *J Physiol* 515: 799–811, 1999. doi:10.1111/j.1469-7793.1999.799ab.x.

Serra J, Collado A, Solà R, Antonelli F, Torres X, Salgueiro M, Quiles C, Bostock H. Hyperexcitable C nociceptors in fibromyalgia. *Ann Neurol* 75: 196–208, 2014. doi:10.1002/ana.24065.

Serra J, Duan WR, Locke C, Solà R, Liu W, Nothaft W. Effects of a T-type calcium channel blocker, ABT-639, on spontaneous activity in C-nociceptors in patients with painful diabetic neuropathy: a randomized controlled trial. *Pain* 156: 2175–2183, 2015. doi:10.1097/j.pain.0000000000000249.

Simone DA, Marchettini P, Caputi G, Ochoa JL. Identification of muscle afferents subserving sensation of deep pain in humans. *J Neurophysiol* 72: 883–889, 1994. doi:10.1152/jn.1994.72.2.883.

Stevens JC, Choo KK. Temperature sensitivity of the body surface over the life span. *Somatosens Mot Res* 15: 13–28, 1998. doi:10.1080/08990229870925.

Thalhammer JG, Raymond SA, Popitz-Bergez FA, Strichartz GR. Modality-dependent modulation of conduction by impulse activity in functionally characterized single cutaneous afferents in the rat. *Somatosens Mot Res* 11: 243–257, 1994. doi:10.3109/08990229409051392.

Torebjörk HE. Afferent C units responding to mechanical, thermal and chemical stimuli in human non-glabrous skin. *Acta Physiol Scand* 92: 374–390, 1974. doi:10.1111/j.1748-1716.1974.tb05755.x.

Torebjörk HE, Hallin RG. Identification of afferent C units in intact human skin nerves. *Brain Res* 67: 387–403, 1974a. doi:10.1016/0006-8993(74)90489-2.

Torebjörk HE, Hallin RG. Responses in human A and C fibres to repeated electrical intradermal stimulation. *J Neurol Neurosurg Psychiatry* 37: 653–664, 1974b. doi:10.1136/jnnp.37.6.653.

Torebjörk HE, Ochoa JL. Specific sensations evoked by activity in single identified sensory units in man. *Acta Physiol Scand* 110: 445–447, 1980. doi:10.1111/j.1748-1716.1980.tb06695.x.

Torebjörk HE, Ochoa JL. New method to identify nociceptor units innervating glabrous skin of the human hand. *Exp Brain Res* 81: 509–514, 1990. doi:10.1007/BF02423499.

Torebjörk HE, Vallbo ÅB, Ochoa JL. Intraneural microstimulation in man. Its relation to specificity of tactile sensations. *Brain* 110: 1509–1529, 1987.

Vallbo AB. Sensations evoked from the glabrous skin of the human hand by electrical stimulation of unitary mechanosensitive afferents. *Brain Res* 215: 359–363, 1981. doi:10.1016/0006-8993(81)90517-5.

Vallbo ÅB. Microneurography: how it started and how it works. *J Neurophysiol* 120: 1415–1427, 2018. doi:10.1152/jn.00933.2017.

Vallbo AB, Hagbarth KE. Activity from skin mechanoreceptors recorded percutaneously in awake human subjects. *Exp Neurol* 21: 270–289, 1968. doi:10.1016/0014-4886(68)90041-1.

Vallbo AB, Hagbarth KE, Torebjörk HE, Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* 59: 919–957, 1979. doi:10.1152/physrev.1979.59.4.919.

Vallbo AB, Hagbarth KE, Wallin BG. Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol (1985)* 96: 1262–1269, 2004. doi:10.1152/japplphysiol.00470.2003.

Vallbo AB, Johansson RS. Properties of cutaneous mechanoreceptors in the human hand related to touch sensation. *Hum Neurobiol* 3: 3–14, 1984.

Vallbo AB, Olausson H, Wessberg J. Unmyelinated afferents constitute a second system coding tactile stimuli of the human hairy skin. *J Neurophysiol* 81: 2753–2763, 1999. doi:10.1152/jn.1999.81.6.2753.

Van Hees J, Gybels J. C nociceptor activity in human nerve during painful and non painful skin stimulation. *J Neurol Neurosurg Psychiatry* 44: 600–607, 1981. doi:10.1136/jnnp.44.7.600.

Van Hees J, Gybels JM. Pain related to single afferent C fibers from human skin. *Brain Res* 48: 397–400, 1972. doi:10.1016/0006-8993(72)90198-9.

Watkins RH, Wessberg J, Backlund Wasling H, Dunham JP, Olausson H, Johnson RD, Ackerley R. Optimal delineation of single C-tactile and C-nociceptive afferents in humans by latency slowing. *J Neurophysiol* 117: 1608–1614, 2017. doi:10.1152/jn.00939.2016.

Weidner C, Schmelz M, Schmidt R, Hammarberg B, Ørstavik K, Hilliges M, Torebjörk HE, Handwerker HO. Neural signal processing: the underestimated contribution of peripheral human C-fibers. *J Neurosci* 22: 6704–6712, 2002. doi:10.1523/JNEUROSCI.22-15-06704.2002.

Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjörk HE. Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. *J Neurosci* 19: 10184–10190, 1999. doi:10.1523/JNEUROSCI.19-22-10184.1999.

Weidner C, Schmidt R, Schmelz M, Hilliges M, Handwerker HO, Torebjörk HE. Time course of post-excitatory effects separates afferent human C fibre classes. *J Physiol* 527: 185–191, 2000. doi:10.1111/j.1469-7793.2000.00185.x.

Weinkauf B, Dusch M, van der Ham J, Benrath J, Ringkamp M, Schmelz M, Rukwied R. Mechano-sensitive nociceptors are required to detect heat pain thresholds and cowhage itch in human skin. *Eur J Pain* 20: 215–222, 2016. doi:10.1002/ejp.710.

Wessberg J, Olausson H, Fernström KW, Vallbo AB. Receptive field properties of unmyelinated tactile afferents in the human skin. *J Neurophysiol* 89: 1567–1575, 2003. doi:10.1152/jn.00256.2002.

Wooten M, Weng HJ, Hartke TV, Borzan J, Klein AH, Turnquist B, Dong X, Meyer RA, Ringkamp M. Three functionally distinct classes of C-fibre nociceptors in primates. *Nat Commun* 5: 4122, 2014. doi:10.1038/ncomms5122.

Yarnitsky D, Simone DA, Dotson RM, Cline MA, Ochoa JL. Single C nociceptor responses and psychophysical parameters of evoked pain: effect of rate of rise of heat stimuli in humans. *J Physiol* 450: 581–592, 1992. doi:10.1113/jphysiol.1992.sp019144.

Zimmermann K, Hein A, Hager U, Kaczmarek JS, Turnquist BP, Clapham DE, Reeh PW. Phenotyping sensory nerve endings in vitro in the mouse. *Nat Protoc* 4: 174–196, 2009. doi:10.1038/nprot.2008.223.