Optimal delineation of single C-tactile and C-nociceptive afferents in humans by latency slowing

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C-mechanoreceptive nociceptors, although the populations overlap in their responses to mechanical stimulation. This fundamental distinction demonstrates a divergence in affective touch signaling from the first stage of sensory processing, having implications for the processing of interpersonal touch.

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human C-mechanoreceptors, including CTs, although a case report has been made of a single low-threshold C fiber in a nerve-injured patient (Campero et al. 2011).

In animals, low-threshold C-mechanoreceptive afferents (CLTM) have been identified in a number of species and are typically distinguished from high-threshold C-mechanoreceptive afferents (CHTM) by their mechanical activation threshold (Bessou et al. 1971; Hoffmann et al. 2015; Kumazawa and Perl 1977; Leem et al. 1993). Studies of animal C-mechano-receptor latency changes using electrical stimulation have produced conflicting results, with some finding clear differentiation into CHTM and CLTM afferents (Gee et al. 1996; Taguchi et al. 2010) but others finding subtle or no differences (Hoffmann et al. 2015; Hulse 2016; Obreja et al. 2010). Akin to the human literature, it is unclear whether animal C-mechano-receptive afferents also consist of distinct populations, or how their response properties correspond.

Human C-mechanoreceptors may form a single population of mechanically responsive C-afferents, with CTs and CMs being those with greater and lesser mechanical sensitivity, respectively, or form two (or more) separate populations, divisible on the basis of their mechanically and/or electrically evoked responses. Understanding fundamental divisions between C-mechanoreceptor subpopulations will enhance our understanding of the distinctions between touch and pain signaling and may facilitate selective targeting of these systems. The present study aimed at characterizing a range of C-mechanoreceptive afferents with differing mechanical sensitivities, using latency changes during electrical stimulation. Specific hypotheses were tested, based on the following questions: 1) Can all C-mechanoreceptive afferents be identified and distinguished by the marking technique? 2) Can they be differentiated and categorized into separate populations by profiles of latency changes during repetitive electrical stimulation? 3) How do these responses correspond with categorization by natural stimulation across afferents with different mechanical response properties?

**MATERIALS & METHODS**

The experiment was approved by the University of Gothenburg ethics committee and performed in accordance with the Declaration of Helsinki, and written informed consent was obtained. Microneurographic axonal recordings were made from the left antebrachial cutaneous nerve (lateral or dorsal branch) in 20 sessions from 19 healthy human participants (25 ± 6 yr; 9 men, 10 women). Single-unit recordings were gained from CT and CM afferents in the forearm through an insulated, high-impedance tungsten recording electrode (FHC). Nerve signals were amplified, band-pass filtered (0.2–4 kHz), and digitized (20.8 kHz) with a Power1401 and Spike2 software (CED). C-mechanoreceptive units were identified by stroking or pinching the skin innervation territory, and single-unit waveforms were identified online with a combination of threshold crossing and template matching (Spike2).

The point of maximal tactile sensitivity in the receptive field was identified, and the monofilament with minimal bending force that reliably evoked a response from the unit was determined. To assess unit responsiveness to innocuous mechanical stimulation, a soft brush was stroked slowly across the receptive field. Units were initially classified as CT if they responded to the brush stimulus, typically with a burst of spikes, and CM if they did not respond (Valbo et al. 1999). Spike shape measurements were obtained for all units identified with marking stimulation and were generated by taking the first spike evoked by sensory stimulation after a period of >10 s without stimulation during the first 5 min of recording.

**Classification of C-mechanoreceptive units from their physiological properties.** Single-unit recordings were obtained from 36 C-mechanoreceptive afferents. On the basis of their responses to brush stroking (Fig. 1, C and D), 19 were classi-
C-mechanoreceptors was predominantly separated into brush-responsive low-threshold (<5 mN) CTs (Fig. 1C) and brush-unresponsive high-threshold (>10 mN) CMs (Fig. 1D). Notably, three CM afferents initially appeared to be low-threshold units on the basis of mechanical threshold obtained (<5 mN), although they lacked vigorous responses to brushing. These low-threshold CM afferents had a single point of high mechanical sensitivity, and one afferent was able to generate a single spike to the brush stimulus but not the typical burst of >10 spikes seen in CTs (Fig. 1C). Nine CT and 10 CM single-unit recordings were maintained to allow identification by electrical stimulation of the skin and the marking technique as well as their spike shape. The threshold for electrical stimulation, conduction velocity, and spike width were not significantly different between CTs and CMs (Fig. 1, F, G, and I, respectively), although CTs had significantly smaller spike amplitudes (P < 0.05; Fig. 1J).

\[ \text{C-mechanoreceptor classification using conditioning of electrical responses with mechanical stimulation (marking).} \]

The marking technique (Fig. 2, A and B) provided positive identification of all C-mechanoreceptive units. Spike latency changes to electrical stimulation were seen when delivering

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**Fig. 1.** Physiological properties of CM and CT afferents. A and B: indicators of receptive field locations of CT (A) and CM (B) units on the arm. Arrow indicates 2 CT units with overlapping receptive field locations. C and D: responses to brushing and suprathreshold indentation from a CT (monofilament threshold 0.04 mN) (C) and a CM (monofilament threshold 12 mN) (D) and overlaid spikes from all displayed responses. Spike times are marked above the trace. Multiunit discharges occurred in myelinated afferents during the brush stimulation (C and D), with a long-latency response in the CT unit, with a typical afterdischarge outlasting the stimulus (C), and no response in the CM unit (D). E: a bimodal distribution of monofilament thresholds was seen, but with some overlap between CTs and CMs. F and G: intracutaneous electrical thresholds (F) and conduction velocities (G) were not significantly different between CTs and CMs. H: spike shape measurements. I and J: there was no significant difference between CT and CM spike widths (I), but spike amplitude was significantly smaller in CTs (J; *P < 0.05).

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**Fig. 2.** Marking responses in CT and CM afferents. A: during electrical stimulation at 0.25 Hz, physiologically stimulating the CT unit (between electrical stimuli 2 and 4) increased the latency to electrical stimulation. The relative timings of sensory and electrical stimuli are shown on the original trace in B. C: extent of latency changes to 7.4-mN monofilament (open arrows) and brush (filled arrow) stimulation in a CT unit is related to the number of evoked spikes (numbers above arrows). Some shorter-latency spikes were seen, presumably originating from a shorter axonal branch, and were excluded from the analysis of latency shifts. D: number of evoked spikes correlated significantly with the observed latency changes in both CT (P < 0.001) and CM (P < 0.001) units, with significantly different slopes (P < 0.001). Inset: the same data for the unit shown in C (P < 0.001). Data presented are from 36 recordings from 8 CTs and 16 recordings from 8 CMs.
natural stimulation concomitantly. The number of spikes evoked by natural stimulation was strongly correlated with the observed change in latency to electrical stimulation for both CTs (slope = 0.05, \( R^2 = 0.80, P < 0.001 \); 36 markings from 8 units; see Fig. 2, C and D for an individual CT unit and the group, respectively) and CMs (slope = 0.54, \( R^2 = 0.91, P < 0.001 \); 16 markings from 8 units; Fig. 2D). The dependence of latency changes on the number of evoked spikes was strikingly diverse between CT and CM units, with a significant difference in the slopes of the linear regressions (\( P < 0.001 \)). The slopes show that each additional spike caused a latency shift of 0.5% in CMs but only a 0.05% shift in CTs.

**Classification of C-mechanoreceptive units by latency changes during 2-Hz electrical stimulation.** During 2-Hz electrical stimulation, a dramatically different pattern of spike latency changes was seen between two populations of C-mechanoreceptors, corresponding to CT and CM units (Fig. 3C). An example of a simultaneous CT and CM recording is shown in Fig. 3A, B; in this recording the CT readily responded to low-intensity (5.4 mN) monofilament stimulation, and both units responded to a stronger (78 mN) monofilament stimulation (Fig. 3A). The CT showed a 0.5% latency increase during 2-Hz stimulation, whereas the CM showed a 20% increase (Fig. 3B). This difference was reflected in the population, where the slowing after 40 pulses delivered at 2 Hz could be used to unequivocally classify all afferents in the sample and the difference after 360 pulses was even more pronounced, with little variance seen in the CT population (Fig. 3, C and D). Both CMs with low mechanical thresholds (2.5 mN) showed typical CM latency changes (>20%) during 2-Hz stimulation. A subpopulation of CTs (\( n = 4 \)) generated additional spikes that were not time-locked to the 2-Hz electrical stimulus, and the most pronounced example of this is shown in Fig. 3E. In these units, there was a delay of 15–60 pulses before the additional spike burst generation. All units recovered and showed a plateau in latency after the additional firing stopped, and the only two CTs showing >1% latency change during the 2-Hz stimulation were the CTs with most pronounced firing (>10 spikes/s).

**Classification of C-mechanoreceptive units through their response to high-frequency electrical stimulation.** Few differences were observed in the ability of CT and CM units to follow high-frequency (>50 Hz) electrical stimulation (Fig. 4A), and the onlyafferent following all pulses at 200 Hz was a CM with a low mechanical threshold. There were significantly smaller latency increases in CTs compared with CMs during 10-, 20-, and 50-Hz stimulation (all \( P < 0.001 \)), but no significant differences were observed at 100 Hz. CTs showed significantly less accumulative slowing (the difference between the second and third pulse and between the third and fourth pulse) at 10, 20, and 50 Hz (all \( P < 0.01 \)) but only between the third and fourth pulse at 100 Hz (\( P < 0.05 \)).

**DISCUSSION**

We found that human C-mechanoreceptive afferents could be separated unequivocally into two populations on the basis of...
their responses to electrical stimulation. Mechanical stimulation produced some inconsistencies with the classification. Brush stimulation separated the C-mechanoreceptors into a population of CTs (brush responsive) and CMs (brush unresponsive), although a few CMs responded weakly to the brush. Mechanical monofilament thresholds separated the populations, where CTs had thresholds of <5 mN but a few CMs fell into this range. These findings demonstrate the similarities and differences between properties of C-mechanoreceptive afferents; therefore care must be taken when classifying C-mechanoreceptors.

The marking technique was sensitive enough to detect the latency shift evoked by a single spike in CMs (Schmelz et al. 1995), but to achieve a detectable latency shift in a CT 10 spikes need to be evoked. This suggests that CT thresholds and weak responses cannot be assessed with this technique, although suprathreshold brush responses may be characterizable (Fig. 2C).

CT responses to 2-Hz electrical stimulation were distinct from CMs and all other C-fiber afferent and efferents (Obreja et al. 2010; Serra et al. 1999). There are large differences in mechanical activation thresholds between human CTs (Nordin et al. 2006; Vallbo et al. 1993, 1999) and animal CLTMs (Leem et al. 1993; Seal et al. 2009); thus electrical stimulation seems superior for classification and comparison. The previously unidentified “type 3” fibers with latency slowing <1% during 2-Hz stimulation in human and rat recordings (George et al. 2007; Serra et al. 1999) likely correspond to CTs and an animal equivalent of CTs, respectively. Repetitive 2-Hz stimulation may be used to compare CTs with genetically identified CLTMs (Seal et al. 2009; Vrontou et al. 2013) and other novel CLTM populations (Djouhri et al. 2016). Half of the CTs recorded produced additional discharges during 2-Hz stimulation; the cause of this is unclear. Afferents showing this pattern may be detectable as CTs in multunit recordings and may form a subpopulation of CT afferents. Animal CLTMs slowing >10% with 2-Hz stimulation (Hoffmann et al. 2015; Hulse 2016; Obreja et al. 2010) likely correspond to a population of CM fibers with low mechanical thresholds (cf. the low-threshold CMs found here).

Latency changes in mechanosensitive C fibers reflect changes in mechanical thresholds and excitability (De Col et al. 2012). We predict that the smaller latency changes in CTs than in CMs during stimulation at physiological rates (10–50 Hz) allow the generation and maintenance of higher spiking rates to mechanical stimulation, enabling the rates observed in CTs in response to mechanical stimuli (up to 100 spikes/s; Vallbo et al. 1999). The physiological upper firing capability appears similar between CMs and CTs from the minimal differences found between entrainment and latency changes at higher frequencies. Absolute refractory period may be governed by similar processes in these two populations, despite repetitive conditioning revealing different axonal mechanisms.

Disparities in conduction latency changes between CTs and CMs during repetitive stimulation are likely produced by differences in the expression of ion channels or ionic transporting mechanisms. A difference in voltage-gated ion channels is suggested by the narrower somatic spike widths in CLTMs than in CHTMs in the rat (Fang et al. 2005), and we find that CTs had significantly smaller spike amplitudes (Fig. 1H). The expression of voltage-gated sodium channel subtypes, which play a role in C-fiber latency changes (De Col et al. 2008; Kankel et al. 2012; Obreja et al. 2012), may be different between CTs and CMs, for example, with CTs expressing a subtype less prone to slow inactivation.

Identifying and characterizing fundamental divisions between C-fiber populations in the periphery provides opportunities to selectively modulate affect at the first stage of encoding. Targeting receptors at the periphery is particularly advantageous to avoid systemic side effects. Thus abhorrent pain signaling, as seen in various neuropathies (Kleggetveit et al. 2012; Serra et al. 2012, 2014), may be treated directly in the periphery without altering affective touch. Furthermore, it may be possible to explore the role of CTs in pain and enhance pleasure in touch, without impacting on normal nociceptive functioning. This has implications for the function of CTs in pathologies, where they are proposed to contribute to mechanical (Liljencrantz et al., 2013; Nagi et al. 2011) and cold (Samour et al. 2015) allodynia, as well as understanding their role in gating pain (Krahé et al. 2016; Liljencrantz et al. 2014). The propensity of some low-threshold CMs to fire in response to gentle mechanical stimulation must be considered in future human behavioral and brain imaging studies, especially in pathological situations where response properties may be altered. Further investigations into the potential subgroups of CTs and CMs are warranted to understand their roles in signaling affective touch and pain, which has implications for the targeting of specific types of C fibers in translational animal studies.

The marking technique and 2-Hz electrical stimulation protocols provide unequivocal separation of C-mechanoreceptors into putative gentle touch-signaling CT and nociceptive-signaling CM populations; however, we show overlap in some C-mechanoreceptive afferent physiological response properties. Our findings enable the unambiguous identification of CTs, which can be applied to future human multunit microneurography recordings.

Fig. 4. Latency changes during high-frequency stimulation in CM and CT afferents. A: similar proportions of CT and CM units followed all the pulses of the higher-frequency electrical stimulation. B: CT units showed significantly less latency increase than CM units during stimulation at 10–50 Hz (***P < 0.001 in all cases). Symbols and error bars indicate means ± SE, with the number of units in parentheses; error bars are masked by the symbols in most cases.
and provides a framework for comparing animal CLTMs to human CTs. The intrinsic axonal conduction differences highlight the fundamental differences between peripheral afferents signaling positive (CT) and negative (CM) affective touch. The underlying mechanisms may be pharmacologically targetable for control over the selective modulation and excitability of CT and CM firing, especially in pathological situations involving tactile dysfunction, such as allodynia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

R.H.W., J.W., H.B.W., J.P.D., and R.A. conceived and designed research; R.H.W., J.W., H.B.W., J.P.D., and R.A. performed experiments; R.H.W., J.W., H.B.W., J.P.D., H.O., R.D.J., and R.A. analyzed data; R.H.W., J.W., H.B.W., J.P.D., H.O., R.D.J., and R.A. drafted manuscript; R.H.W., J.P.D., and R.A. revised manuscript; R.H.W., J.W., H.B.W., J.P.D., H.O., R.D.J., and R.A. edited and prepared figures; R.H.W., J.W., H.B.W., J.P.D., H.O., R.D.J., and R.A. drafted and approved final version of manuscript.

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