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Differential effects of radiant and mechanically applied thermal stimuli on human C-tactile afferent firing patterns

Rochelle Ackerley^{1, 2}, Katarina Wiklund Fernström², Helena Backlund Wasling², Roger H. Watkins², Richard D. Johnson^{2,3}, Åke Vallbo², Johan Wessberg²

¹Aix Marseille Univ, CNRS, LNSC (Laboratoire de Neurosciences Sensorielles et Cognitives – UMR 7260), Marseille, France

²Department of Physiology, University of Gothenburg, Gothenburg, 40530, Sweden.

³Department of Physiological Sciences, University of Florida College of Veterinary Medicine, Florida 32610-0144, USA.

C-tactile (CT) afferents respond to gentle tactile stimulation, but only a handful of studies in humans and animals have investigated whether their firing is modified by temperature. We describe the effects of radiant thermal stimuli, and of stationary and very slowly moving mechano-thermal stimuli, on CT afferent responses. We find that CT afferents are primarily mechanoreceptors, as they fired little during radiant thermal stimuli, but they exhibited different patterns of firing during combined mechano-cool stimulation, as compared to warming. CTs fired optimally to gentle, very slowly moving or stationary mechano-thermal stimuli delivered at neutral temperature (~32°C, normal skin temperature), but they responded with fewer spikes (median 67% decrease) and at significantly lower rates (47% decrease) during warm (~42°C) tactile stimuli. During cool tactile stimuli (~18°C), their mean instantaneous firing frequency significantly decreased by 35%, but they often fired a barrage of afterdischarge spikes at a low frequency (~5 Hz) that outlasted the mechanical stimulus. These effects were observed under a variety of stimulus conditions, including during stationary and slowly moving touch (0.1 cm.s⁻¹), and we complemented these tactile approaches using a combined electrical-thermal stimulation experiment, where we found a suppression of spiking during warming. Overall, CT afferents are exquisitely sensitive to tactile events and we show that their firing is modulated with touch temperatures above and below neutral skin temperature. Warm touch consistently decreased their propensity to fire, whereas cool touch produced lower firing rates, but afterdischarge spiking.

Introduction

C-tactile (CT) afferents, and the animal homolog C-low threshold mechanoreceptors (CLTMs), are unmyelinated mechanoreceptive afferents that respond to gentle touch and are found exclusively in hairy skin (Nordin 1990; Vallbo et al. 1993, 1999; Wessberg et al. 2003; Liu et al. 2007; Löken et al. 2009; Li et al. 2011; Lou et al. 2013; Vrontou et al. 2013; Ackerley et al. 2014; McGlone et al. 2014; Watkins et al. 2017). They are characterized by their exquisite sensitivity to low-force skin indentations (<5 mN; Vallbo et al. 1999; Watkins et al. 2017) and a slow stroke over the unit's receptive field evokes optimal responses (Vallbo et al. 1999; Löken et al. 2009; Ackerley et al. 2014). Further, their mean instantaneous firing frequency during skin stroking stimuli correlates well with ratings of pleasantness (Löken et al. 2009; Ackerley et al. 2014), where their responses are optimal to stroking touch applied around human skin temperature (Ackerley et al. 2014). Hence, due to their response properties, CTs have been implicated in social bonding and affiliation (Morrison et al. 2010; Vrontou et al. 2013; McGlone et al. 2014).

An under-explored property of CT afferents is their sensitivity to changes in stimulus temperature, which may play a role in shaping affective processing. In humans, a handful of studies have tested the pure thermal sensitivity of CT afferents. Nordin (1990) found that two CTs responded with a short burst of spikes to cooling, whereas Nordin (1990) and Vallbo et al. (1999) both observed that heating CT receptive fields produced no or very little activity. Animal studies have shown that CLTMs respond somewhat to cooling, especially when it is rapid (Hensel et al. 1960; Iggo 1960; Bessou et al. 1971; Kumazawa and Perl 1977; Shea and Perl 1985; Seal et al. 2009), and one study showed very weak CLTM responses to heating (Shea and Perl 1985). Bessou et al. (1971) and Hahn (1971) conducted fuller comparisons between the responses evoked from cooling and those through mechanical stimulation. They both found that touch was a much more effective stimulus for eliciting CLTM responses, where responses to rapid cooling only approached 20% of the maximal response to mechanical stimulation. Both studies also found that mechanical stimulation and cooling evoked similar rates of fatigue in CLTMs and that there was

some interaction between prior non-congruent somatosensory stimuli, for example, a CLTM fatigued by a mechanical stimulus was less excitable during subsequent cooling. Thus, it is clear that the preferred stimulus for CTs and CLTMs is mechanical activation, although cooling can elicit responses, but the mechanisms behind the mechanical and cooling responses are still unclear, but may be semi-independent.

Only two studies have used combined mechano-thermal stimulation to activate CTs/CLTMs, presenting the opportunity to understand how touch and temperature stimuli interact simultaneously, which occurs often in everyday life. Hahn (1971) showed that a warm stationary mechano-thermal stimulus resulted in decreased CLTM discharge in cats, as compared to only mechanical stimulation, and that a cool stationary mechano-thermal stimulus elicited CLTM responses, but these were inferior to the sum of separately-applied mechanical and cool stimuli. Ackerley et al. (2014) used various speeds ($0.3\text{--}30\text{ cm}\cdot\text{s}^{-1}$) of moving cool (18°C), neutral (32°C ; typical human skin temperature, Arens and Zhang, 2006), and warm (42°C) probe temperatures to elicit responses in human CTs. This combined mechano-thermal stimulus excited CT afferents most strongly at stroking velocities of $1\text{--}10\text{ cm}\cdot\text{s}^{-1}$, which was correlated with pleasantness ratings (cf. Löken et al., 2009), but that CT firing frequency was significantly lower during moving touch at cool (33% decrease) and warm (27% decrease) temperatures, as compared to neutral, over all stroking velocities, apart from at $30\text{ cm}\cdot\text{s}^{-1}$. Thus, touch with a thermal component deviant from skin temperature reduced firing in CLTMs and CTs. However, for cooling, the responses may be more complicated and depend on the exact nature of the stimulation, where cooling alone can elicit low-level firing in CTs/CLTMs, but that combined mechano-cool stimulation may interact, although the mechanisms behind this are unknown.

The previous literature has shown that CTs are primarily mechanoreceptors that likely signal positive affective (pleasant) touch, for example to ameliorate conspecific bonding, and that deviations from typical skin temperature are generally perceived as unpleasant (Greenspan et al. 2003; Ackerley et al. 2014). Therefore, we aimed to investigate the role of CT afferents in coding changes in both thermal and tactile stimuli, both of which are pertinent in affective interactions. In line with the previous literature, we hypothesized that CT afferent firing would be diminished during heating, whereas the responses to cooling may involve more complex mechanisms. Specifically, two mechanisms may be at work, where CT firing frequency may be suppressed during combined mechano-cool stimulation, but that additional low frequency and long-lasting spiking may be induced by cooling. Stationary and moving stimuli were used to investigate differences between modes of tactile application, where moving stimuli evoke prolonged barrages of CT responses that may be more pertinent in social and affective touch.

Material and Methods

The study was performed using 19 healthy, human participants (age range 21-42 years, 4 males), who gave written, informed consent and were paid for their time. The experimental procedures were approved by the University of Gothenburg ethics committee and performed in accordance with the Declaration of Helsinki. The technique of microneurography (Vallbo and Hagbarth 1968) was used to record from single CT afferents in the left antebrachial cutaneous nerve, from either the lateral or dorsal branch, which innervates the hairy skin of the forearm. To start, the participants were seated comfortably in an adjustable dental chair, with their left arm immobilized using a vacuum airbag. The experiments were conducted at a controlled, ambient room temperature of 23°C . A custom-built preamplifier (Department of Physiology, Umeå University, Sweden) was taped to their upper arm and a ground electrode plate placed under their forearm. The skin just above the cubital fold was palpated to find the ideal place for insertion of the stimulating and recording electrodes, and an uninsulated reference electrode was inserted $\sim 5\text{ cm}$ from this site.

To locate the small diameter antebrachial nerve, an electrical search procedure was used. An uninsulated stimulating electrode (35 or 50 mm length, $200\text{ }\mu\text{m}$ shaft diameter, $\sim 5\text{ }\mu\text{m}$ tip diameter; FHC, Bowdoin, ME) was used to deliver $200\text{ }\mu\text{s}$ square, negative, 1 Hz pulses until the participant reported sensations spreading down the arm (maximum current used $\sim 1.5\text{ mA}$). Once an ideal depth and location was found, an insulated recording electrode of the same specifications was inserted just distal to the stimulating electrode. The recording electrode was used to access the nerve directly.

Once the recording electrode had penetrated the nerve, the experimenter used rapid strokes of the participant's arm to locate single CT units. The mass activity of fast-conducting, myelinated A β afferents was typically heard as the stroking occurred, but the experimenter listened for a delayed response to the stroke, which corresponded to slowly-conducting C fiber afferents. Single spikes were identified online by the spike detection algorithms of the data acquisition system, sampled at 12.8 kHz, band-pass filtered (0.2-4 kHz) and stored (using SC/ZOOM; Department of Physiology, Umeå University, Sweden or Spike2; CED, Cambridge, UK). When a single C unit had been located, its responses were assessed by the auditory feedback (via a loudspeaker) and the visualization of spikes (via the traces on a screen) during mechanical stimulation of its receptive field, to determine whether it was a low-threshold (CT) or high-threshold (C-nociceptor) unit.

Single CT units were classified by showing a major deflection in the negative direction (relating to the unmyelinated axon), delayed responses to mechanical stimulation of the skin receptive field (relating to the slow conduction velocity), responding to monofilament indentations of 2.5 mN or less, and prominent responsivity (typically a short barrage of spikes) to gentle brushing, which does not typically activate the high-threshold C mechanoreceptive afferents (Vallbo et al. 1999; Watkins et al. 2017). The conduction velocity of CT units was estimated using a hand-held, blunt strain gauge. Responses were recorded to a short, mechanical tap to the center of a unit's receptive field and the conduction velocity was calculated using the distance from this location to the recording electrode (cf. Vallbo et al. 1999; Watkins et al. 2017). Once a CT unit had been located and verified, it was subject to a number of tests involving thermal, mechano-thermal, and electro-thermal stimulation.

Pure thermal (radiant) stimulation

Two different types of stimulation devices were used to deliver radiant thermal stimulation, with no mechanical aspects. Pure cool stimulation was delivered by holding a standard cold pack 3-10 mm above the receptive field, for 30-60 s. Participants reported feeling cooling sensations of the skin. We tested the timecourse of the cooling in 5 separate participants using a calibrated thermistor on the skin in the middle of the stimulus area. The mean detection threshold for cooling was 27°C and the mean temperature of the skin at 30 s into the stimulation was 22°C. After 60 s, the final mean skin temperature was 19°C, hence it is clear that the cooling was an effective stimulus. Pure warm stimulation was delivered using a gold-coated reflector lamp (OSRAM HLX Xenophot 34835, 15V/150W). The power was set to 98 W. The lamp was positioned 8 cm from the participant's forearm. A metal shield with a circular aperture of 1 cm diameter mounted in front of the lamp limited the warmed area to a 9 cm diameter area. The temperature was ramped up and a calibrated thermistor was used to determine the rate of temperature increase at skin level, which was found to be 2°C/s. The participants readily felt a warming sensation and the heat ramp continued until the participant reported pain. We also tested the timecourse of the warming in 5 separate participants, where the mean detection threshold was 37°C, the mean temperature of the skin at 30 s was 47°C, and the mean pain threshold was 49°C.

Stationary mechano-thermal stimulation

Different devices were used to deliver stationary mechano-thermal stimulation to the skin. The order of stimulation of the different temperatures was randomized and the stimulation duration lasted 1-17 s. Up to 8 repeats of stationary mechano-thermal stimulation were applied, per recorded unit. A circular thermode (skin contact area 660 mm²) was used to deliver thermal stimulation to the unit's receptive field at constant cool (15°C), neutral (32°C, typical human arm skin temperature; Arens and Zhang 2006), or warm (42°C) temperatures. The thermode was constructed of Peltier elements (Melcor CP Series thermoelectric module; Laird, London, UK) mounted on a steel block, which acted as an effective heat sink to maintain the desired temperature. The weight of the device was ~300 g. An accelerometer (EGAX-5; Entran Sensors and Electronics, Fairfield, NJ) was placed on the steel block to indicate skin contact onset and offset. The thermode was held perpendicular to the receptive field, by hand. To keep the thermode in contact with the skin the experimenter supported the thermode, allowing it to exert its inherent load on the receptive field.

A further type of stationary mechano-thermal stimulation was used, namely a lightweight steel metal spatula (15 x 140 x 1 mm), applied to the receptive field. The spatula was held horizontally over the receptive field and the experimenter was trained to deliver a load of ~6.5 g, as measured with a high precision balance. Between stimuli, spatulas were kept in ice water or hot water, and before being applied to the skin, the spatula was dried and left at

room temperature to adjust to a cool (18°C) or neutral (32°C) temperature, as measured with an infra-red thermometer.

Directly after the stationary thermal stimulation, manual brush strokes were often applied to assess the responsiveness of the unit, while the skin temperature was either cool or warm. This was to further assess whether afterdischarges, i.e. additional impulses that outlast the duration of the stimulus, could be generated, as stroking produces more prominent afterdischarges, compared to static indentation of the receptive field (in CLTMs: Iggo 1960; in CTs: Nordin 1990). Occasionally, the receptive field was cooled with an ice cube, then dried and brushed to evoke spikes, in a similar manner to generate afterdischarges.

Slowly moving mechano-thermal stimulation

A rotary tactile stimulator (Dancer Design, Wirral, UK) was used to move a mechano-thermal probe (contact surface ~5 cm²) across a CT unit's receptive field. The data were obtained in an earlier study (Ackerley et al. 2014), where this device was used to collect CT firing to three constant probe temperatures (cool (18°C), neutral (32°C), and warm (42°C)), delivered over six stroking velocities (0.1, 0.3, 1, 3, 10, and 30 cm.s⁻¹). Ackerley et al. (2014) presented the thermal responses over velocities 0.3-30 cm.s⁻¹ and we currently present the results at the stroking velocity of 0.1 cm.s⁻¹, over all three temperatures. This slow stroking velocity was used as it had a moving element (which is an effective stimulus for eliciting CT responses; Vallbo et al. 1999; Löken et al. 2009), but that it had a much longer time course while stroking the CT's receptive field. This allowed more detailed analyses of CT firing, as with velocities over 1 cm.s⁻¹, far fewer spikes are elicited (Löken et al. 2009).

The contact surface of the probe was a rounded, smooth metallic plate, where its temperature was controlled using a custom-designed thermode consisting of probe-mounted Peltier elements (Melcor CP Series thermoelectric module; Laird, London, UK) interfaced to Melcor PR-59 programmable control modules and thermocouples (0.05°C resolution). This robotic stimulator provided high-precision computer control over the velocity of stroking and temperatures, delivering a calibrated normal force (0.4 N). The temperatures were presented in a block design, where the presentation order was pseudo-randomized. In each block, three repeats of the 0.1 cm.s⁻¹ stroking velocity were presented that were inter-leaved with other stroking velocities presented in Ackerley et al. (2014). There was an inter-stimulus-interval of 30 seconds to allow for recovery of CT afferent response that can show fatigue with repeated stimulation (Zotterman 1939; Iggo 1960; Bessou et al. 1971; Hahn 1971; Iggo and Kornhuber 1977; Nordin 1990; Vallbo et al. 1999; Watkins et al. 2017). Our previous study showed that there was no overall effect of the repetitive thermal stimulation on ambient skin temperature with this period of recovery (Ackerley et al. 2014).

Combined electro-thermal stimulation

The responses of a single CT afferent were recorded during the electrical stimulation of its receptive field, combined with radiant temperature stimuli. This protocol has been found to induce additional spiking which is not time locked to, but presumably indirectly induced by, the electrical stimulation in around half of CT afferents studied (Watkins et al. 2017). The same radiant heat lamp was used to warm the skin to a stable temperature of 44°C and a piece of dry ice was held a few millimeters above the skin to provide cooling to a stable temperature of 24°C. In the protocol, the CT unit was first tested twice at neutral skin temperature (35°C), then warming was tested, then cooling, and after, the protocol was re-tested during neutral skin temperature once more. The temperature of the skin was measured throughout using a thermocouple attached just next to the CT receptive field. In each test, constant current electrical stimulation (0.5 ms pulse width) was delivered through two uninsulated microneurography electrodes that were inserted obliquely into the skin, just around the receptive field of the CT (Watkins et al. 2017). Electrical stimulation of the CT showed that it had a threshold of 1.6 mA for activation and a level of two times the threshold was set throughout the protocols. A standard 2 Hz latency identification protocol was used for each temperature investigation. This was composed of a baseline stimulation period of 0.25Hz repetitive electrical stimulation, 2 Hz stimulation for 3 minutes, followed by 10 pulses of recovery at 0.25 Hz (Watkins et al. 2017). A period of at least 2 minutes of rest was used between protocols, to allow for the recovery of spike conduction to baseline levels.

CT spike analysis

The nerve data were pre-processed to verify the single-unit nature of all units with an offline pattern-matching algorithm and the recorded spikes were inspected in expanded time-scale, using custom-written software implemented in MATLAB (The Mathworks, Natick, MA). Analyses for the electro-thermal stimulation were also carried about in Spike2 (CED; Cambridge, UK). Single spikes were time-stamped and descriptive statistics were gained on the number of spikes generated and their instantaneous firing frequency, over temperature conditions. In the combined electro-thermal stimulation, electrically evoked spikes were identified by virtue of their constant latency using a custom-written script in Spike2 and additional spikes generated during the stimulation that were not time locked to the electrical stimulus were quantified for the 2 Hz stimulation train (Watkins et al. 2017). Non-parametric statistical comparisons (Wilcoxon signed Rank tests for comparing medians to a hypothetical value, equal to zero, for no evoked activity and Friedman ANOVAs, with Dunn's multiple comparison post-hoc tests for group comparisons) were made using GraphPad Prism (La Jolla, CA), as some of the data were not normally distributed (using Shapiro-Wilk tests) and significances were sought below the $p < 0.05$ level (p values are given for significance to three decimal places).

Results

A total of 35 CT units were studied (locations in Fig. 1). All units were highly sensitive to innocuous skin deformation and responded readily to slow stroking of the skin. Their force activation thresholds ranged between 0.05-2.5 mN (median 0.7 mN) and they had slow conduction velocities between 0.4-1.5 $\text{m}\cdot\text{s}^{-1}$ (mean 0.9 $\text{m}\cdot\text{s}^{-1}$). They responded with a mean peak instantaneous frequency of 70 $\text{spikes}\cdot\text{s}^{-1}$ (range 41-90 $\text{spikes}\cdot\text{s}^{-1}$) to these innocuous stimuli. No spontaneous activity was observed in any of the units during these mechanical identification procedures.

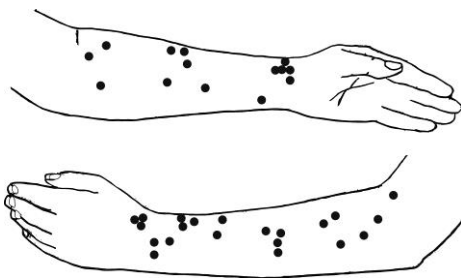


Figure 1: Location of C-tactile (CT) units.

Locations of 35 CT units during the radiant and stationary mechano-thermal stimuli ($n = 24$), the slowly moving mechano-thermal stimuli ($n = 10$), and the combined electro-thermal stimulation ($n = 1$).

Pure thermal (radiant) stimulation

CT units were tested for their responses to pure thermal stimuli, with no concomitant mechanical contact. A total of 5 units were tested to radiant cooling, where a few spikes of low frequency (mean 0.4 $\text{spikes}\cdot\text{s}^{-1}$, range 0.1-0.9) occurred during the test period. The responses were not modulated by the onset and termination of cooling, when the dynamic change in skin temperature was greatest. Responses to radiant warming were tested in 17 units and as with the responses to cooling, only infrequent spikes were seen (mean 1.1 $\text{spikes}\cdot\text{s}^{-1}$, range 0.03-3.8). The majority of the units (14/17) produced firing frequencies of $<1.5 \text{ spikes}\cdot\text{s}^{-1}$, whereas 3 units showed a slightly higher rate between 2.4-3.8 $\text{spikes}\cdot\text{s}^{-1}$. As per the cooling stimulus, the majority of units were not modulated by the onset and termination of the thermal stimulus. Only one CT showed a modulation to the timing of the warming, where it responded with a low mean discharge rate of 0.1 $\text{spikes}\cdot\text{s}^{-1}$ during the first 10 s of warming, which increased to a mean of 3.3 $\text{spikes}\cdot\text{s}^{-1}$ for its entire observation period of 25 s. This unit also showed a brief spike afterdischarge when the thermal stimulus was removed, consisting of 14 spikes over 1.8 seconds. Hence, responses to thermal stimuli were far inferior, as compared to responses to light mechanical stimuli.

Stationary mechano-thermal stimulation

Combined stationary mechano-thermal stimulation demonstrated that CT responses were modulated markedly by temperature. A total of 15 CT units were tested with the stationary mechano-thermal stimulation, at cool, neutral and warm probe temperatures. Figure 2A-C shows example responses from a single CT afferent to cool (15°C), neutral (32°C), and warm (42°C) stationary mechano-thermal stimulation, respectively. Here, consistent responses were elicited from each mechanical contact with the skin; however, at the warm temperature, the responses were much fewer, in terms of the number and duration of spiking, and the instantaneous firing frequency (peak 11 $\text{spikes}\cdot\text{s}^{-1}$; Fig. 2C). In contrast, CT spiking duration was longer and consisted of more spikes at higher frequencies for both cool (peak 18 $\text{spikes}\cdot\text{s}^{-1}$; Fig. 2A) and neutral (peak 30 $\text{spikes}\cdot\text{s}^{-1}$; Fig. 2B) stationary touch.

Temperature modulation of C-tactile responses

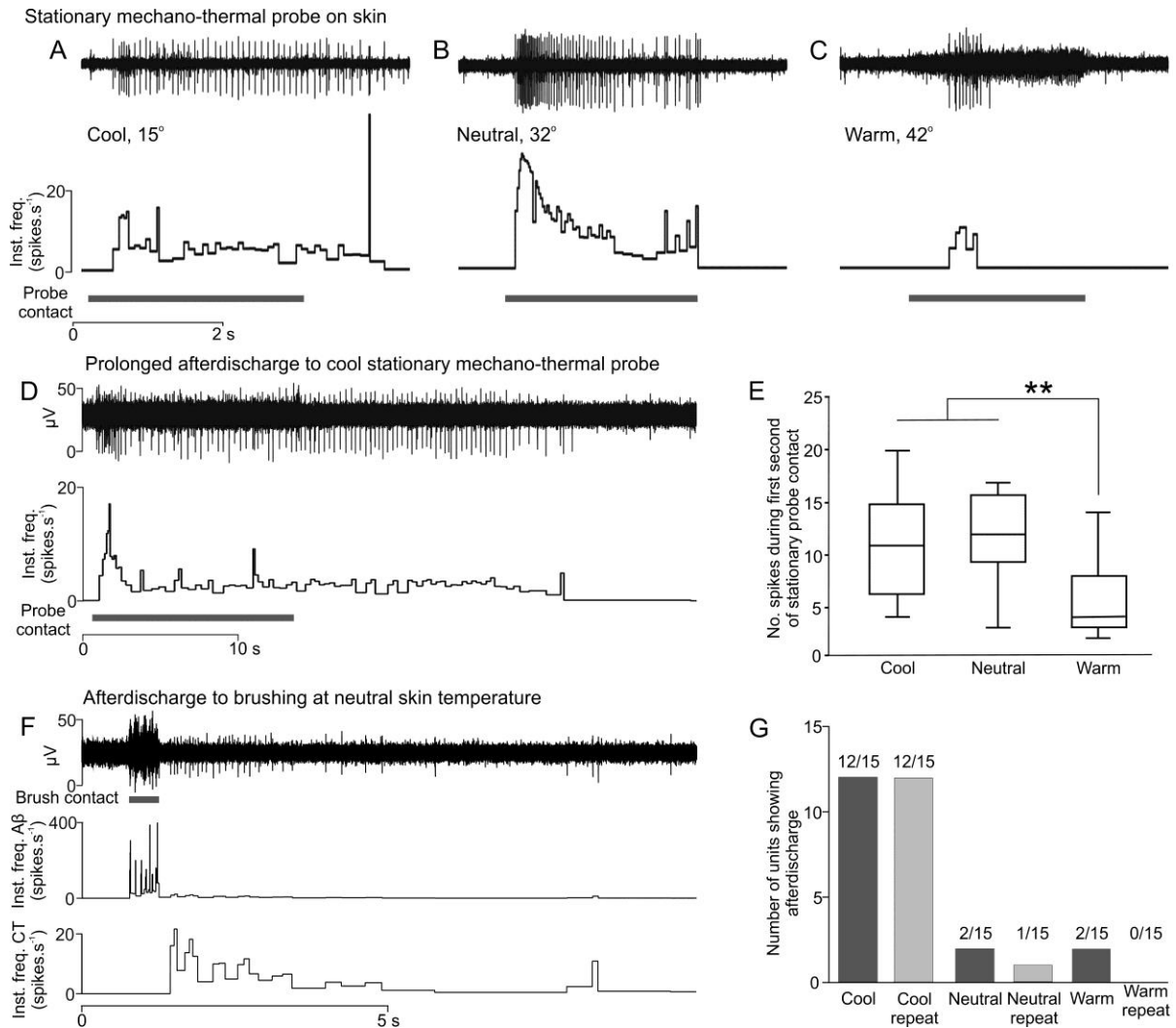


Figure 2: C-tactile (CT) firing to stationary mechano-thermal stimuli over all temperatures.

Example spikes evoked and instantaneous frequencies of a single CT, to the (A) cool (15o), (B) neutral (32o), and (C) warm (42o) stationary mechano-thermal probe. (A) The cool stationary mechano-thermal stimulus evoked high initial firing frequencies, which then levelled off. Afterdischarge was observed, where the CT continued to fire after the stimulus had been removed. (B) The neutral stationary mechano-thermal stimulation evoked a strong response that declined rapidly within 1.5 s. (C) The warm stationary mechano-thermal stimulation gave a short-duration and weak response, with no afterdischarge. (D) An example of a prolonged afterdischarge in response to 13 s of stationary cool mechano-thermal stimulation. The stimulation evoked an afterdischarge that lasted ~17 s on removal of the probe. The inset shows 20 overlaid CT spikes (horizontal scale, 1 ms; vertical scale, 40 μ V). (E) A box-and-whisker plot (median, quartile, minimum-maximum) for the number of CT spikes generated in the first second of contact with the stationary mechano-thermal probe stimulation ($n = 15$ units) for cool, neutral and warm temperatures. There was a significant decrease in the number of spikes generated during warm stimuli compared to the cool and neutral stimuli. (F) An example of afterdischarge during neutral skin temperature stroking. The inset shows 20 overlaid CT spikes (horizontal scale, 1 ms; vertical scale, 20 μ V). N.B. The instantaneous frequency for a myelinated unit in the recording, which responds almost instantaneously, is shown below the delayed CT instantaneous frequency response (scale same as for CT). (G) Overview of the tendency for afterdischarge in CTs and its reproducibility. In $n = 15$ CT units, the number of CTs responding with an afterdischarge is shown, and whether this occurred again in a subsequent test.

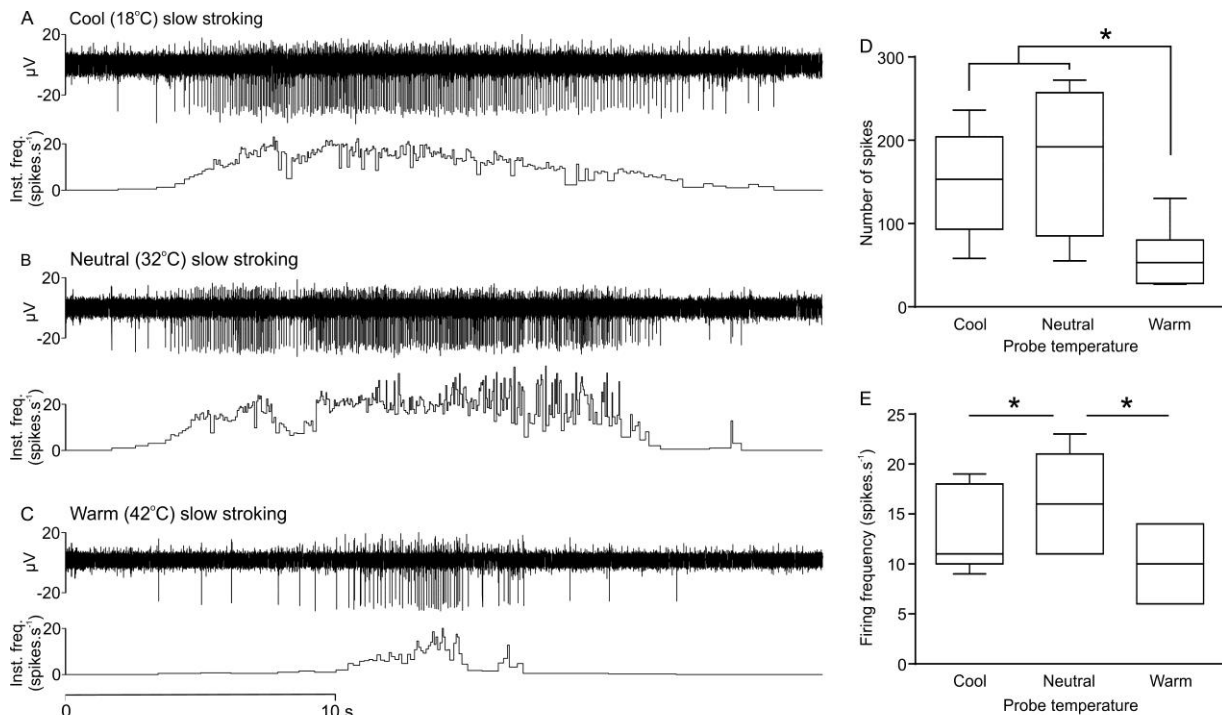
To quantify the group thermo-modulatory effect, the number of spikes during the first second of stimulation were counted, pooled, and compared. A significant effect was found (Friedman ANOVA, $F = 17.16$, $p < 0.001$), where a decrease in the number of spikes was seen during the warm, as compared to both the neutral (median decrease 67%; $p < 0.001$) and cool (median decrease 64%, $p = 0.001$) mechano-thermal stimuli (Fig. 2E). No significant difference was found between the responses at cool and neutral temperatures (median 8% decrease during cool touch, $p = 0.411$).

A recurring observation during the microneurography experiments was that CTs often produced afterdischarges (cf. Nordin 1990) after the removal of the mechano-thermal stimulus. The most prominent afterdischarges were observed after skin cooling and Figure 2D shows an example where the CT response started during stationary mechano-thermal cooling and continued after the stimulus had left the skin. The afterdischarges from CTs in cooled skin could last many seconds (e.g. ~30 s in Fig. 2D) and occurred at an instantaneous firing rate of ~5 Hz frequency (Fig. 2A, D). The tendency for afterdischarge was quantified in 15 CT units at cool, neutral and warm temperatures, using the stationary mechano-thermal probe. On the group level, the afterdischarge from mechano-cool stimulation lasted for an average of $5.6 \text{ s} \pm 2.9 \text{ SEM}$ (range: 0.5-31 s, $n = 12$ units), at an instantaneous frequency of $5.3 \text{ Hz} \pm 0.5 \text{ SEM}$. The mean coefficient of variation for the afterdischarge frequency was 0.81, which indicates regular neuronal firing (when this value is <1 , Taube, 2010). Afterdischarge was also observed in 3/5 units tested with the cool spatula, and the application of an ice cube then brushing the skin readily evoked a barrage of afterdischarge.

With neutral, as well as warm, mechano-thermal stimulation only small and short-lasting afterdischarges were seen. It consisted of only 1-3 spikes and terminated directly after the removal of the probe. In the 15 units tested, afterdischarges with the mechano-neutral temperature probe were found in only 2 units; moreover these afterdischarges were irreproducible. Similarly, no afterdischarges were observed when the units were tested with the neutral temperature spatula; however, these 2 units showed afterdischarges to cooling. Incidentally, brief afterdischarges were common after stroking the skin at neutral skin temperature (Fig. 2F), although these consisted of only a few spikes that lasted a couple of seconds at most. With warm mechano-thermal stimulation, only 2/15 units showed brief afterdischarge responses, yet neither of these units responded to mechano-cool or -neutral stimulation. Akin to the lack of reproducibility from neutral temperature stimuli, these units did not show reproducible afterdischarge on re-warming (Fig. 2G).

Slowly moving mechano-thermal stimulation

In a separate paradigm, we tested the effects of a slowly moving mechano-thermal stimulus (at a velocity of $0.1 \text{ cm}\cdot\text{s}^{-1}$) on 10 CT units. Figure 3 shows example responses of CT firing to slowly moving cool (18°C ; Fig. 3A), neutral (32°C ; Fig. 3B), and warm (42°C ; Fig. 3C) temperatures.



(Figure 3 legend on next page)

Temperature modulation of C-tactile responses

Figure 3: C-tactile (CT) responses to a slowly moving mechano-thermal stimulus at cool, neutral, and warm temperatures.

Example responses of a single CT, to (A) cool (18°C), (B) neutral (32°C), and (C) warm (42°C) slowly moving (0.1 cm.s⁻¹) mechano-thermal stimuli delivered with the rotary tactile stimulator. Note that the stroking stimulation occurred the whole time in the presented windows, where the probe was also in contact with the skin surrounding the CT, and that the time windows are aligned to the middle of the movement. Box-and-whisker plots (median, quartile, minimum-maximum) for (D) the number of spikes and (E) the mean instantaneous firing frequency for cool, neutral and warm temperatures (n = 10 units). There was a significant decrease in the number of spikes generated during warm stimulation, as compared to cool and neutral stimuli in (D), whereas there was a significant decrease between the neutral and both the cool and warm condition for the mean instantaneous firing frequency in (E) (all p < 0.05). The insets show 20 overlaid CT spikes for each condition (scale bar in C and same for all; horizontal scale, 1 ms; vertical scale, 30 μV).

It can be seen that numerous spikes were evoked to the very slow mechanical stimulus, yet these were not of particularly high firing frequency. Figure 3D and E show the number of spikes and the mean instantaneous firing frequency, respectively, for grouped data from 10 CT units tested at the different temperatures. Significant effects of temperature were found (Friedman ANOVA, F = 14.00, p = 0.002), where decreased numbers of spikes were elicited during slowly moving warm stimulation, as compared to neutral (median decrease 67%, p = 0.001) and cool (median decrease 60%, p = 0.014) temperatures (Fig. 3D). No significant difference was found between the number of evoked spikes during the cool vs. neutral temperature touch (median 16% decrease during cool touch, p = 0.480). Further, a different effect was seen in the mean instantaneous firing frequency of CTs (Friedman ANOVA, F = 10.12, p = 0.003), where an increase was found for the neutral temperature condition, as compared to the cool (median decrease 35%, p = 0.004) and warm (median decrease 47%, p = 0.049) conditions (Fig. 3E). No significant difference was observed between firing frequency in the cool and warm conditions (median 18% decrease during warm touch, p = 0.239).

Combined electro-thermal stimulation

A further paradigm was tested in a single CT to test whether temperature-dependent effects were also found during electrical stimulation, which by-passes the mechanical transduction and encoding of a stimulus. It has been shown that this kind of electrical stimulation may induce background firing in addition to the single spike time-locked to the electrical stimuli (Watkins et al. 2017). Figure 4 shows that during the warm stimulus, markedly less additional spikes were generated than at neutral or cool temperatures. There was no marked effect on the time course or duration of spiking, in relation to the electrical pulse, in any condition (not shown), and warming acted to reduce the number of spikes by decreasing the mean rate of additional spiking in this unit.

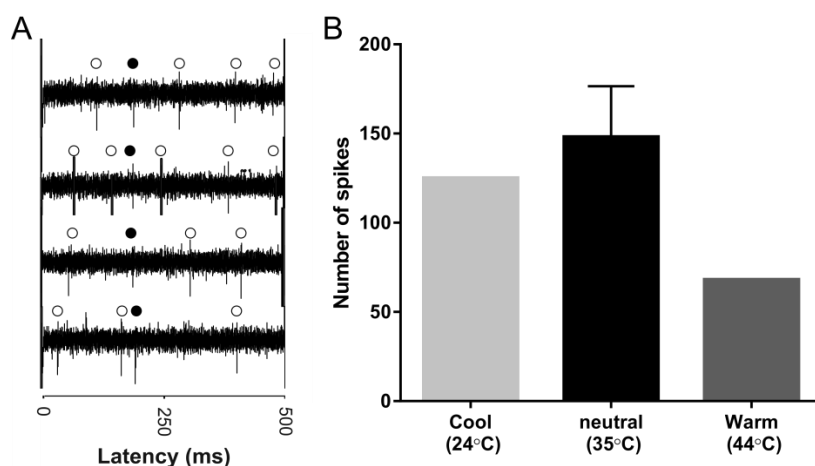


Figure 4: Effect of temperature on the non-time locked spikes evoked during 2 Hz electrical stimulation.

Spike responses from the electrical stimulation of a CT receptive field during different temperatures (n = 1 unit). (A) Illustration of four successive responses during electrical stimulation at a rate of 2 Hz with the CT spikes marked above the trace, at neutral temperature. Filled circles represent electrically-evoked spike responses and open circles represent the non-electrically evoked spikes. (B) Quantification of the total number of non-time locked spikes evoked during 2 Hz electrical stimulation for 3 mins, showing a marked decrease in the number of spikes evoked during warming, as compared to neutral temperature (which was repeated and hence has error bars, see Methods). The insets show 20 overlaid CT spikes (1 ms; vertical scale, 30 μV).

Discussion

We investigated the responses of CT afferents during pure thermal stimulation and during both mechano-thermal and electro-thermal stimulation. CT afferents were not spontaneously active and showed negligible or weak responses to cool or warm radiant stimulation, hence CTs are not thermoreceptors. Combined mechano-thermal

stimulation modulated CT firing differentially, where maximal responses were consistently produced during neutral (skin) temperature touch, as compared to warm touch that reduced CT firing. A different and more complex relationship was found with cool touch. The impulse rate was lower during cool touch, than during neutral temperature touch, and a continued afterdischarge of spikes was often seen on termination of the mechano-cool stimulation, which was rarely found from neutral temperature or warm touch. The long-lasting response to cool stimuli was not related to any perceived pleasantness (cf. Ackerley et al. 2014) and the precise mechanism of afterdischarge modulation is unknown, but may be a result of visco-elastic changes in the skin (cf. Iggo and Kornhuber 1977).

In our present results, CT afferents were readily activated by gentle mechanical stimulation, which is in agreement with the literature defining them as low-threshold mechanoreceptors (Nordin 1990; Vallbo et al. 1993, 1999; Wessberg et al. 2003; Löken et al. 2009; Ackerley et al. 2014; Watkins et al. 2017). Previous work on both CTs (Nordin 1990; Vallbo et al. 1999) and CLTMs (Iggo 1960; Hahn 1971; Kumazawa and Perl 1977) generally agrees that their responses are weak or reduced during warming, hence it is likely that CTs (and CLTMs) do not possess mechanisms to directly encode *increases* in temperature. This is in line with the hypothesis that CTs are involved in encoding positive affective touch, as heating the skin much over typical skin temperature is not pleasant (Greenspan et al. 2003; Ackerley et al. 2014), may lead to damage, and such stimuli are readily encoded by nociceptors and thermoreceptors (Ackerley and Watkins 2018). Conversely, CTs (Nordin 1990) and CLTMs (Hensel et al. 1960; Iggo 1960; Bessou et al. 1971; Hahn 1971; Kumazawa and Perl 1977; Shea and Perl 1985; Seal et al. 2009) respond somewhat to cooling. There is, however, little detail in the literature about the exact responses and we advance this presently by showing that the number of spikes elicited during cooling is not reflected in an increased firing frequency. CTs do not seem to encode *decreases* in temperature *per se*, but their firing may be modulated by the subsequent physical effects from decreasing skin temperature.

An earlier study, using combined moving mechano-thermal stroking at faster velocities ($0.3\text{-}10\text{ cm}\cdot\text{s}^{-1}$), found similar maximal instantaneous frequency responses around human skin temperature, as compared to cool ($\sim 18^\circ\text{C}$) or warm ($\sim 42^\circ\text{C}$) touch (Ackerley et al. 2014). We extend the previous findings by showing the same effect for very slowly moving thermal touch ($0.1\text{ cm}\cdot\text{s}^{-1}$) and for stationary thermal touch. We found a general decrease in the propensity for CTs to fire (in both the number of spikes and instantaneous firing frequency) during warm touch, whereas for cool touch, only the firing frequency was reduced. Therefore, the modulatory effect of temperature on CT responses to mechanical stimuli is complex and deviations from neutral skin temperature in either direction diminish the response. However, CT responses were fundamentally different for cool and warm temperatures and a striking feature in response to mechano-cool stimulation was the promotion of afterdischarges.

The propensity for afterdischarges, i.e. a response that outlasts a (mechanical) stimulus, has been demonstrated in CTs (Nordin 1990) and CLTMs (Zotterman 1939; Iggo 1960; Bessou et al. 1971; Iggo and Kornhuber 1977; Lynn and Carpenter 1982). Iggo (1960) found that these brief afterdischarges were prominent when the skin was stroked and occurred at a low frequency ($\sim 5\text{ Hz}$, similar to the present findings). Iggo and Kornhuber (1977) postulated that such afterdischarges were due to restorative movements of the skin, where visco-elastic mechanisms prolong CLTM responses after removal of a (mechanical) stimulus. We found that CTs showed similar afterdischarge properties to CLTMs, but we extend the work by demonstrating that these afterdischarges were far more pronounced when the skin was cooled, which may relate to the tendency for CLTMs to be activated in general by cooling. It seems that small changes in the mechanics of the skin are sufficient to activate CTs/CLTMs and the prolonged afterdischarge in cooling may be a clear consequence of this, where contractile movements during skin cooling, and then re-warming, have a direct and extended effect on the CT receptor. The contraction and expansion of the skin may be related to changes in internal and external temperature, where it also follows that the warmer the skin, the more it expands, and the CT receptor would be less sensitive to the decreased skin movement, as we found presently. However, due to the time lags in thermal changes of the skin, it is likely that CTs do not have a strong relationship between absolute temperatures and their firing (i.e. that only stronger or longer-lasting heating or cooling may change their responses).

As well as using combined mechano-thermal stimuli, we tested a repetitive electrical stimulation paradigm during radiant thermal stimuli, by-passing the mechanotransduction process, and found concurrent results. During cooling

and at neutral (skin) temperature, additional spiking (cf. CTs in Watkins et al. 2017) was induced, whereas in warming, there was less spiking as compared to neutral. Although we present data here from only one CT, the results were congruent with our other findings, in that temperature changes shape CT responses in the presence of other stimuli. Hence, the electro-thermal stimulation showed that these effects were not simply due to a mechanical stimulus and the additional spiking may relate to visco-elastic changes of the skin affecting the CT receptor, which may also be the case with the afterdischarge phenomenon.

Our findings are in accordance with the affective touch hypothesis (Olausson et al. 2010; McGlone et al. 2014), which proposes that CTs play a central role in positive somatosensory affective experiences, especially in reinforcing social and inter-personal bonds. Our finding that CTs respond optimally to touch at human skin temperature adds to their role in conveying skin-to-skin interactions (cf. Ackerley et al. 2014). CTs fired at significantly lower frequencies to warm or cool touch, than touch at neutral temperature, and the cooling afterdischarges occurred only at low rates (~5 Hz). It is believed that the firing frequency of C-fibers determines their coding intensity, where the actual spike timing is less important. Therefore, the low firing rates seen during cool touch may not provide substantive somatosensory information and/or that low rates may actually correlate with decreases in pleasantness (cf. Löken et al. 2009; Ackerley et al. 2014).

In conclusion, we find that CT afferents fire optimally to touch, but are sensitive to changes in stimulus temperature. Vigorous responses were generated to combined mechanical-thermal stationary and slowly-moving stimuli at neutral skin temperature (~32°C). Warm touch (~42°C) consistently decreased their responses, in terms of mean instantaneous firing frequency and number of spikes. Cool touch (~18°C) was more complex: CTs fired at a lower instantaneous frequency, but significantly more spikes were generated than for warm touch. An afterdischarge of spikes was also regularly seen with cool touch that outlasted the skin contact, which may relate to visco-elastic mechanisms in the skin modifying the propensity of CTs to fire. In general, human CT and animal CLTM afferents show similar properties in their responses to applied mechanical and thermal stimuli. These findings reinforce the role of CTs in coding innocuous, pleasant touch, but their afterdischarge responses to cool touch should be investigated further.

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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: 2879–83, 2014.
- 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.* in review.
- Arens E, Zhang H.** The skin's role in human thermoregulation and comfort. In: *Thermal and Moisture Transport in Fibrous Materials*, edited by Pan N, Gibson P. Cambridge, UK: Woodhead Publishing Ltd, 2006, p. 560–602.
- Bessou P, Burgess PR, Perl ER, Taylor CB.** Dynamic properties of mechanoreceptors with unmyelinated (C) fibers. *J Neurophysiol* 34: 116–131, 1971.
- Greenspan JD, Roy EA, Caldwell PA, Farooq NS.** Thermosensory intensity and affect throughout the perceptible range. *Somatosens Mot Res* 20: 19–26, 2003.
- Hahn J.** Thermal-mechanical stimulus interactions in low-threshold C-fiber mechanoreceptors of cat. *Exp Neurol* 33: 607–617, 1971.
- Hensel H, Iggo A, Witt I.** A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. *J Physiol* 153: 113–126, 1960.
- Iggo A.** Cutaneous mechanoreceptors with afferent C fibres. *J Physiol* 152: 337–353, 1960.
- Iggo A, Kornhuber HH.** A quantitative study of C-mechanoreceptors in hairy skin of the cat. *J Physiol* 271: 549–65, 1977.
- Kumazawa T, Perl ER.** Primate cutaneous sensory units with unmyelinated (C) afferent fibers. *J Neurophysiol* 40: 1325–38, 1977.

- Li L, Rutlin M, Abraira VE, Cassidy C, Kus L, Gong S, Jankowski MP, Luo W, Heintz N, Koerber HR, Woodbury CJ, Ginty DD.** The functional organization of cutaneous low-threshold mechanosensory neurons. *Cell* 147: 1615–27, 2011.
- Liu Q, Vrontou S, Rice FL, Zylka MJ, Dong X, Anderson DJ.** Molecular genetic visualization of a rare subset of unmyelinated sensory neurons that may detect gentle touch. *Nat Neurosci* 10: 946–8, 2007.
- Löken LS, Wessberg J, Morrison I, McGlone F, Olausson H.** Coding of pleasant touch by unmyelinated afferents in humans. *Nat Neurosci* 12: 547–8, 2009.
- Lou S, Duan B, Vong L, Lowell BB, Ma Q.** Runx1 controls terminal morphology and mechanosensitivity of VGLUT3-expressing C-mechanoreceptors. *J Neurosci* 33: 870–82, 2013.
- Lynn B, Carpenter S.** Primary afferent units from the hairy skin of the rat hind limb. *Brain Res* 238: 29–43, 1982.
- McGlone F, Wessberg J, Olausson H.** Discriminative and affective touch: sensing and feeling. *Neuron* 82: 737–55, 2014.
- Morrison I, Löken LS, Olausson H.** The skin as a social organ. *Exp Brain Res* 204: 305–14, 2010.
- Nordin M.** Low-threshold mechanoreceptive and nociceptive units with unmyelinated (C) fibres in the human supraorbital nerve. *J Physiol* 426: 229–240, 1990.
- Olausson H, Wessberg J, Morrison I, McGlone F, Vallbo A.** The neurophysiology of unmyelinated tactile afferents. *Neurosci Biobehav Rev* 34: 185–91, 2010.
- Seal RP, Wang X, Guan Y, Raja SN, Woodbury CJ, Basbaum AI, Edwards RH.** Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. *Nature* 462: 651–5, 2009.
- Shea V, Perl E.** Sensory receptors with unmyelinated (C) fibers innervating the skin of the rabbit's ear. *J Neurophysiol* 54: 491–501, 1985.
- Taube JS.** Interspike Interval Analyses Reveal Irregular Firing Patterns at Short, But Not Long, Intervals in Rat Head Direction Cells. *J Neurophysiol* 104: 1635–1648, 2010.
- Vallbo Å, Hagbarth K.** Activity from skin mechanoreceptors recorded percutaneously in awake human subjects. *Exp Neurol* 289: 270–289, 1968.
- Vallbo Å, Olausson H, Wessberg J.** Unmyelinated afferents constitute a second system coding tactile stimuli of the human hairy skin. *J Neurophysiol* 81: 2753–2763, 1999.
- Vallbo A, Olausson H, Wessberg J, Norrsell U.** A system of unmyelinated afferents for innocuous mechanoreception in the human skin. *Brain Res* 628: 301–4, 1993.
- Vrontou S, Wong AM, Rau KK, Koerber HR, Anderson DJ.** Genetic identification of C fibres that detect massage-like stroking of hairy skin in vivo. *Nature* 493: 669–73, 2013.
- Watkins RH, Wessberg J, Wasling HB, Dunham JP, Olausson H, Johnson RD, Ackerley R.** Optimal delineation of single C-tactile and C-nociceptive afferents in humans using latency slowing. *J Neurophysiol* 117: 1608–1614, 2017.
- Wessberg J, Olausson H, Fernström KW, Vallbo AB.** Receptive field properties of unmyelinated tactile afferents in the human skin. *J Neurophysiol* 89: 1567–75, 2003.
- Zotterman Y.** Touch, pain and tickling: an electro-physiological investigation on cutaneous sensory nerves. *J Physiol* 95: 1–28, 1939.

Corresponding author: Rochelle Ackerley, Laboratoire de Neurosciences Sensorielles et Cognitives (LNSC – UMR 7260), Aix-Marseille Université - CNRS, Fédération 3C, Case B, 3 place Victor Hugo, CS 80249, 13331 Marseille CEDEX 03, France. E-mail: rochelle.ackerley@univ-amu.fr

New & Noteworthy:

C-tactile (CT) afferents are thought to underpin pleasant touch and previous work has shown that they respond optimally to a slow caress delivered at typical (neutral) skin temperature. Here, we show that, although CTs are primarily mechanoreceptive afferents, they are modified by temperature: warm touch decreases their firing, whereas cool touch produces lower firing rates, but long-lasting spiking, frequently seen as afterdischarges. This has implications for the encoding of affective sensory events in human skin.

Key words: Mechanical; thermal; moving touch; warm; cool; skin; unmyelinated; CT afferent

Abbreviations: CLTM – C-low threshold mechanoreceptor; CT – C-tactile;