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

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

Rochelle Ackerley,[1,2] Katarina Wiklund Fernström,[2] Helena Backlund Wasling,[2] Roger H. Watkins,[2] Richard D. Johnson,[2,3] Åke Vallbo,[2] and Johan Wessberg[2]

[1]*Aix Marseille Université, CNRS, LNSC (Laboratoire de Neurosciences Sensorielles et Cognitives – UMR 7260), Marseille, France;* [2]*Department of Physiology, University of Gothenburg, Gothenburg, Sweden; and* [3]*Department of Physiological Sciences, University of Florida College of Veterinary Medicine, Gainesville, Florida*

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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. First published July 25, 2018; doi:10.1152/jn.00940.2017.—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 mechanothermal 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 compared with warming. CTs fired optimally to gentle, very slowly moving, or stationary mechanothermal 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), 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.

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.

Address for reprint requests and other correspondence: R. 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).

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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 (Ackerley et al. 2014; Li et al. 2011; Liu et al. 2007; Löken et al. 2009; Lou et al. 2013; McGlone et al. 2014; Nordin 1990; Vallbo et al. 1993, 1999; Vrontou et al. 2013; Watkins et al. 2017; Wessberg et al. 2003). 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 (Ackerley et al. 2014; Löken et al. 2009; Vallbo et al. 1999). Furthermore, their mean instantaneous firing frequency during skin stroking stimuli correlates well with ratings of pleasantness (Ackerley et al. 2014; Löken et al. 2009), 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 (McGlone et al. 2014; Morrison et al. 2010; Vrontou et al. 2013).

An underexplored 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 (Bessou et al. 1971; Hensel et al. 1960; Iggo 1960; Kumazawa and Perl 1977; Seal et al. 2009; Shea and Perl 1985), 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 stimula-

tion. They both found that touch was a much more effective stimulus for eliciting CLTM responses, where responses to rapid cooling approached only 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 noncongruent 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, where the mechanisms behind the mechanical and cooling responses are still unclear but may be semi-independent.

Only two studies have used combined mechanothermal 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 mechanothermal stimulus resulted in decreased CLTM discharge in cats compared with only mechanical stimulation and that a cool stationary mechanothermal 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 (0.3–30 cm/s) 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 mechanothermal stimulus excited CT afferents most strongly at stroking velocities of 1–10 cm/s, 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 compared with neutral, overall stroking velocities apart from at 30 cm/s. 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.

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 (Ackerley et al. 2014; Greenspan et al. 2003). 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 might 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 evoked prolonged barrages of CT responses that might be more pertinent in social and affective touch.

MATERIALS AND METHODS

The study was performed using 19 healthy human participants (age range 21–42 yr, 4 men), 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 ~5 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 μm shaft diameter, ~5 μm tip diameter; FHC, Bowdoin, ME) was used to deliver 200 μs-square, negative, 1-Hz pulses until the participant reported sensations spreading down the arm (maximum current used ~1.5 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 Aβ 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 handheld, 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 subjected to a number of tests involving thermal, mechanothermal, and electrothermal 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 time course of the cooling in five separate participants by 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, 15 V/150 W). 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 time course of the warming in five 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 mechanothermal stimulation. Different devices were used to deliver stationary mechanothermal stimulation to the skin. The order of stimulation of the different temperatures was randomized, and the stimulation duration lasted 1–17 s. Up to eight repeats of stationary mechanothermal stimulation were applied per recorded unit. A circular thermode (skin contact area 660 mm^2) 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 mechanothermal stimulation was used, namely a lightweight steel metal spatula (15 × 140 × 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 infrared thermometer.

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

Slowly moving mechanothermal stimulation. A rotary tactile stimulator (Dancer Design, Wirral, UK) was used to move a mechanothermal probe (contact surface ~5 cm^2) 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). Ackerley et al. (2014) presented the thermal responses over velocities 0.3–30 cm/s, and we currently present the results at the stroking velocity of 0.1 cm/s, 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; Löken et al. 2009; Vallbo et al. 1999), but 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, far fewer spikes are elicited (Löken et al. 2009).

The contact surface of the probe was a rounded, smooth metallic plate, whose temperature was controlled using a custom-designed thermode consisting of probe-mounted Peltier elements (Melcor CP Series thermoelectric module) 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 stroking velocity were presented that were interleaved with other stroking velocities presented in Ackerley et al. (2014). There was an inter-stimulus-interval of 30 s to allow for recovery of CT afferent response that could show fatigue with repeated stimulation (Bessou et al. 1971; Hahn 1971; Iggo 1960; Iggo and Kornhuber 1977; Nordin 1990; Vallbo et al. 1999; Watkins et al. 2017; Zotterman 1939). 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 electrothermal 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 one-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 afterward, the protocol was retested 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 0.25-Hz repetitive electrical stimulation, 2-Hz stimulation for 3 min, followed by 10 pulses of recovery at 0.25 Hz (Watkins et al. 2017). A period of at least 2 min of rest was used between protocols to allow for the recovery of spike conduction to baseline levels.

CT spike analysis. The nerve data were preprocessed 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 electrothermal 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 electrothermal stimulation, electrically evoked spikes were identified by virtue of their constant latency by 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). Nonparametric 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 and 2.5 mN (median 0.7 mN), and they had slow conduction velocities between 0.4 and 1.5 m/s (mean 0.9 m/s). They responded with a mean peak instantaneous frequency of 70 spikes/s (range 41–90 spikes/s) to these innocuous stimuli. No spontaneous activity was observed in any of the units during these mechanical identification procedures.

Pure thermal (radiant) stimulation. CT units were tested for their responses to pure thermal stimuli with no concomitant mechanical contact. Five units were tested to radiant cooling, where a few spikes of low frequency (mean 0.4 spikes/s, 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 spikes/s, range 0.03–3.8). The majority of the units (14/17) produced firing frequencies of <1.5 spikes/s, whereas three units showed a slightly higher rate between 2.4 and 3.8 spikes/s. 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 spikes/s during the first 10 s of warming, which increased to a mean of 3.3 spikes/s 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 s. Hence, responses to thermal stimuli were far inferior compared with responses to light mechanical stimuli.

Stationary mechanothermal stimulation. Combined stationary mechanothermal stimulation demonstrated that CT responses were modulated markedly by temperature. Fifteen CT units were tested with the stationary mechanothermal stimulation at cool, neutral, and warm probe temperatures. Figure 2, *A–C*, shows example responses from a single CT afferent to cool (15°C), neutral (32°C), and warm (42°C) stationary mechanothermal 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 spikes/s; Fig. 2*C*). In contrast, CT spiking duration was longer and consisted of more spikes at higher frequencies for both cool (peak 18 spikes/s; Fig. 2*A*) and neutral (peak 30 spikes/s; Fig. 2*B*) stationary touch.

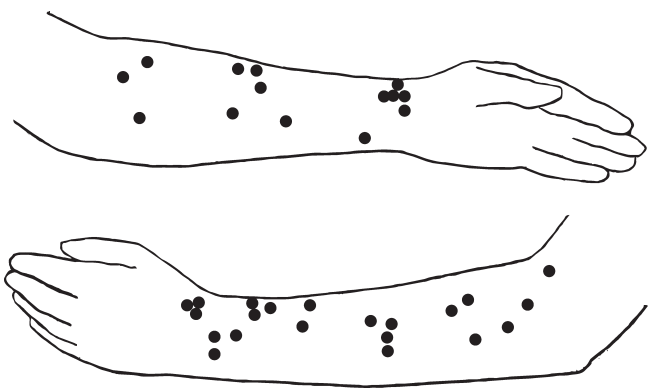

Fig. 1. Location of C-tactile (CT) units. Locations of 35 CT units during the radiant and stationary mechanothermal stimuli ($n = 24$), the slowly moving mechanothermal stimuli ($n = 10$), and the combined electrothermal stimulation ($n = 1$).

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 compared with both the neutral (median decrease 67%; $P < 0.001$) and cool (median decrease 64%, $P = 0.001$) mechanothermal stimuli (Fig. 2*E*). 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 mechanothermal stimulus. The most prominent afterdischarges were observed after skin cooling, and Fig. 2*D* shows an example where the CT response started during stationary mechanothermal 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. 2*D*) and occurred at an instantaneous firing rate of ~5 Hz frequency (Fig. 2, *A* and *D*). The tendency for afterdischarge was quantified in 15 CT units at cool, neutral, and warm temperatures, using the stationary mechanothermal probe. On the group level, the afterdischarge from mechano-cool stimulation lasted for an average of 5.6 s ± 2.9 SE (range: 0.5–31 s, $n = 12$ units), at an instantaneous frequency of 5.3 Hz ± 0.5 SE. 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 three of five 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, mechanothermal stimulation only small and short-lasting afterdischarges were seen. It consisted of only one to three 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 two units; moreover, these afterdischarges were irreproducible. Similarly, no afterdischarges were observed when the units were tested with the neutral temperature spatula; however, these two units showed afterdischarges to cooling. Incidentally, brief afterdischarges were common after stroking of the skin at neutral skin temperature (Fig. 2*F*), although these consisted of only a few spikes that lasted a couple of seconds at most. With warm mechanothermal stimulation, only 2 of 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. 2*G*).

Slowly moving mechanothermal stimulation. In a separate paradigm, we tested the effects of a slowly moving mechanothermal stimulus (at a velocity of 0.1 cm/s) on 10 CT units. Figure 3 shows example responses of CT firing to slowly moving cool (18°C; Fig. 3*A*), neutral (32°C; Fig. 3*B*), and

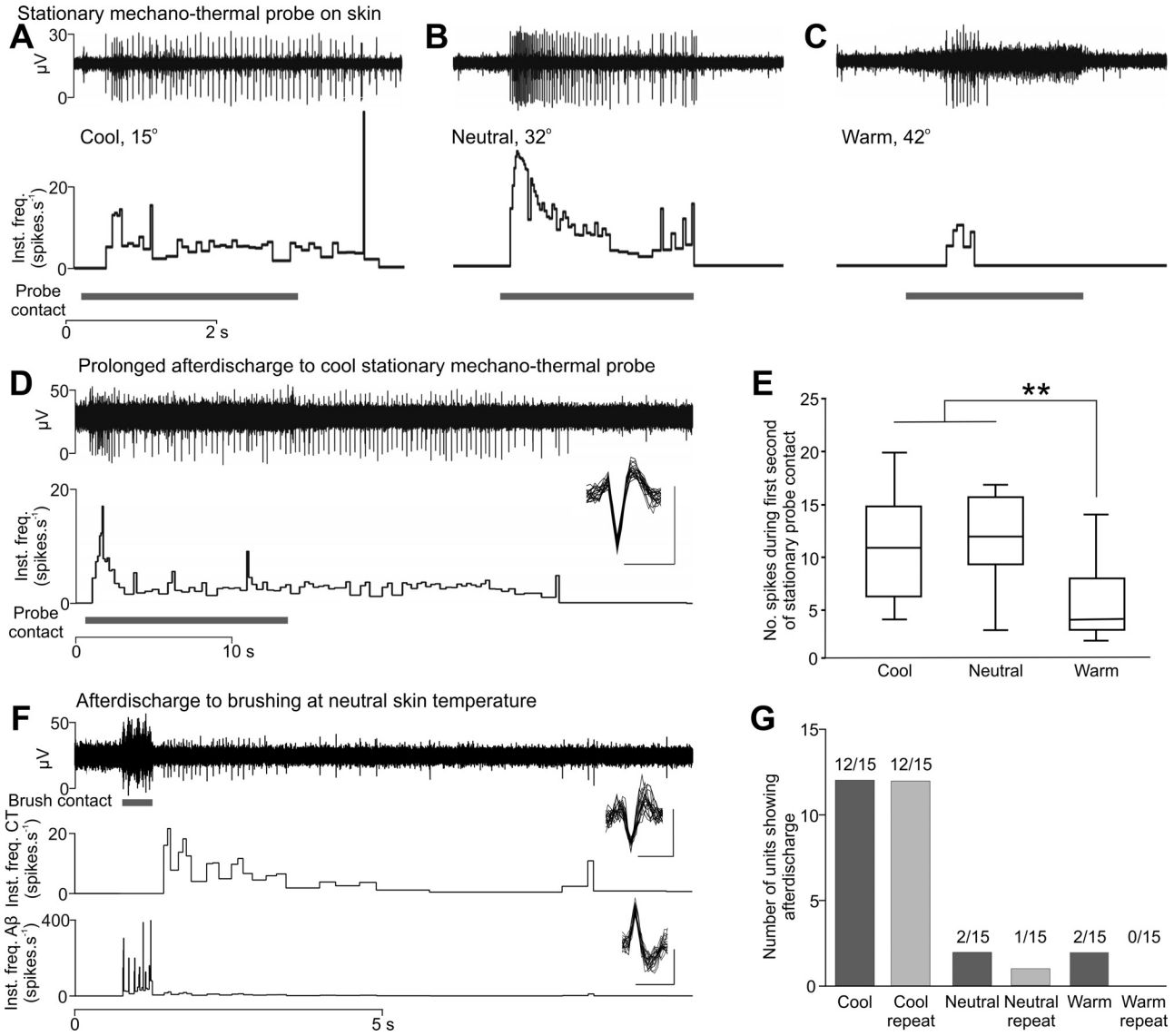

Fig. 2. C-tactile (CT) firing to stationary mechanothermal stimuli over all temperatures. Example spikes evoked and instantaneous frequencies of a single CT to the cool (15°; *A*), neutral (32°; *B*), and warm (42°; *C*) stationary mechanothermal probe. *A*: cool stationary mechanothermal stimulation evoked high initial firing frequencies, which then leveled off. Afterdischarge was observed, where the CT continued to fire after the stimulus had been removed. *B*: neutral stationary mechanothermal stimulation evoked a strong response that declined rapidly within 1.5 s. *C*: warm stationary mechanothermal stimulation gave a short-duration and weak response, with no afterdischarge. D: example of a prolonged afterdischarge in response to 13 s of stationary cool mechanothermal stimulation. Stimulation evoked an afterdischarge that lasted ~17 s on removal of the probe. *Inset*: 20 overlaid CT spikes (horizontal scale, 1 ms; vertical scale, 40 μV). *E*: box-and-whisker plot (median, quartile, minimum-maximum) for the number of CT spikes generated in the first second of contact with the stationary mechanothermal 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 vs. the cool and neutral stimuli. *F*: example of afterdischarge during neutral skin temperature stroking. *Inset*: 20 overlaid CT spikes (horizontal scale, 1 ms; vertical scale, 20 μV). Note 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.

warm (42°C; Fig. 3*C*) temperatures. 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 3, *D* 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 compared with neutral (median decrease 67%, $P = 0.001$) and cool (median decrease 60%, $P = 0.014$) temperatures (Fig. 3*D*). No significant difference was found between the number of evoked spikes during the cool vs. neutral temperature touch (median 16% decrease during cool touch, $P = 0.480$). Furthermore, 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 compared with the cool (median decrease 35%, $P = 0.004$) and warm (median decrease 47%, $P = 0.049$) conditions (Fig. 3*E*). No significant difference was observed be-

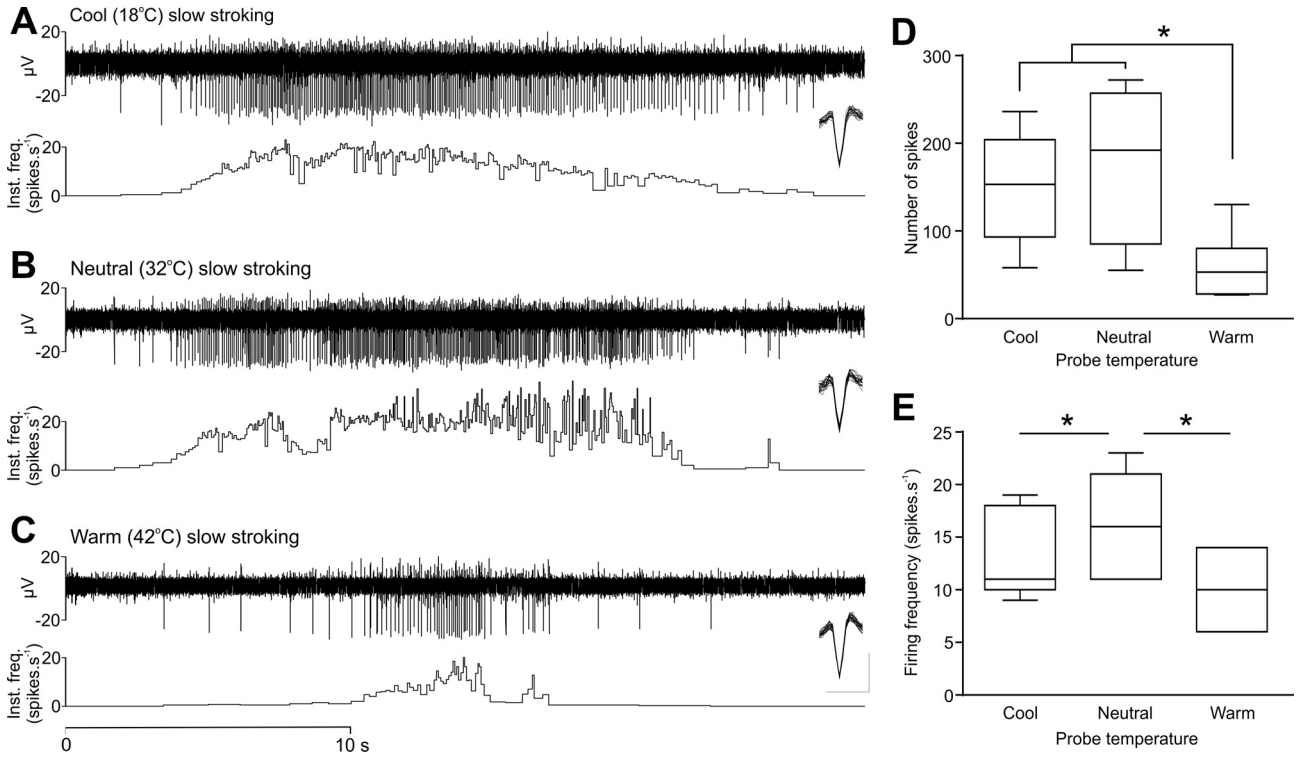

Fig. 3. C-tactile (CT) responses to a slowly moving mechanothermal stimulus at cool, neutral, and warm temperatures. Example responses of a single CT to the cool (15°; *A*), neutral (32°; *B*), and warm (42°; *C*) slowly moving (0.1 cm/s) mechanothermal stimuli delivered with the rotary tactile stimulator. 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 the number of spikes (*D*) and the mean instantaneous firing frequency (*E*) for cool, neutral, and warm temperatures ($n = 10$ units). There was a significant decrease in the number of spikes generated during warm stimulation vs. 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 $P < 0.05$). *Insets*: 20 overlaid CT spikes for each condition (scale bar in *C* and same for all; horizontal scale, 1 ms; vertical scale, 30 μV).

tween firing frequency in the cool and warm conditions (median 18% decrease during warm touch, $P = 0.239$).

Combined electrothermal stimulation. A further paradigm was tested in a single CT to test whether temperature-dependent effects were also found during electrical stimulation that bypassed 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 fewer 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.

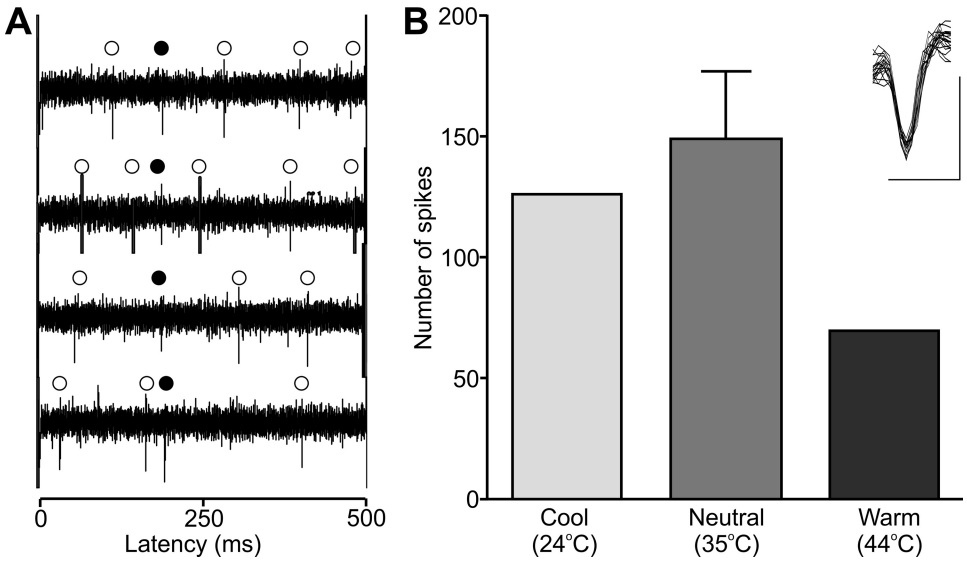

Fig. 4. Effect of temperature on the non-time-locked spikes evoked during 2-Hz electrical stimulation. Spike responses from electrical stimulation of a CT receptive field during different temperatures ($n = 1$ unit). *A*: illustration of 4 successive responses during electrical stimulation at a rate of 2 Hz, with the CT spikes marked above the trace, at neutral temperature. Filled circles represent electrically evoked spike responses; open circles represent the nonelectrically evoked spikes. *B*: quantification of the total number of non-time-locked spikes evoked during 2-Hz electrical stimulation for 3 min, showing a marked decrease in the number of spikes evoked during warming vs. neutral temperature (which was repeated and, hence, has error bars, see MATERIALS AND METHODS). *Insets*: 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 mechanothermal and electrothermal 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 mechanothermal stimulation modulated CT firing differentially, where maximal responses were consistently produced during neutral (skin) temperature touch compared with 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 viscoelastic 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 (Ackerley et al. 2014; Löken et al. 2009; Nordin 1990; Vallbo et al. 1993, 1999; Watkins et al. 2017; Wessberg et al. 2003). Previous works on both CTs (Nordin 1990; Vallbo et al. 1999) and CLTMs (Hahn 1971; Iggo 1960; Kumazawa and Perl 1977) generally agree 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 (Ackerley et al. 2014; Greenspan et al. 2003) and may lead to damage, and such stimuli are readily encoded by nociceptors and thermoreceptors (R. Ackerley and R. H. Watkins In press). Conversely, CTs (Nordin 1990) and CLTMs (Bessou et al. 1971; Hahn 1971; Hensel et al. 1960; Iggo 1960; Kumazawa and Perl 1977; Seal et al. 2009; Shea and Perl 1985) 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 mechanothermal stroking at faster velocities (0.3–10 cm/s), found similar maximal instantaneous frequency responses around human skin temperature compared with cool (~18°C) or warm (~42°C) touch (Ackerley et al. 2014). We extend the previous findings by showing the same effect for very slowly moving thermal touch (0.1 cm/s) 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 (Bessou et al. 1971; Iggo 1960; Iggo and Kornhuber 1977; Lynn and Carpenter 1982; Zotterman 1939). Iggo (1960) found that these brief afterdischarges were prominent when the skin was stroked and occurred at a low frequency (~5 Hz, similar to the present findings). Iggo and Kornhuber (1977) postulated that such afterdischarges were due to restorative movements of the skin, where viscoelastic mechanisms prolong CLTM responses after removal of a (mechanical) stimulus. We found that CTs showed similar afterdischarge properties to CLTMs, but we extended 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, wherein contractile movements during skin cooling, and then rewarming 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 mechanothermal stimuli, we tested a repetitive electrical stimulation paradigm during radiant thermal stimuli, bypassing the mechanotransduction process, and found concordant 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 compared with 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 electrothermal stimulation showed that these effects were not simply due to a mechanical stimulus, and the additional spiking may relate to viscoelastic changes of the skin affecting the CT receptor, which may also be the case with the afterdischarge phenomenon.

Our findings are in accord with the affective touch hypothesis (McGlone et al. 2014; Olausson et al. 2010), which proposes that CTs play a central role in positive somatosensory affective experiences, especially in reinforcing social and interpersonal 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 to 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 low rates may

actually correlate with decreases in pleasantness (cf. Ackerley et al. 2014; Löken et al. 2009).

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 slow-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 viscoelastic 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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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

R.A., K.W.F., H.B.W., R.H.W., R.D.J., Å.V., and J.W. conceived and designed research; R.A., K.W.F., H.B.W., R.H.W., R.D.J., Å.V., and J.W. performed experiments; R.A., K.W.F., H.B.W., and R.H.W. analyzed data; R.A., K.W.F., H.B.W., R.H.W., R.D.J., Å.V., and J.W. interpreted results of experiments; R.A., K.W.F., and R.H.W. prepared figures; R.A., K.W.F., and R.D.J. drafted manuscript; R.A., H.B.W., R.H.W., R.D.J., Å.V., and J.W. edited and revised manuscript; R.A., K.W.F., H.B.W., R.H.W., R.D.J., Å.V., and J.W. approved final version of manuscript.

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