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Slow brushing reduces heat pain in humans

J. Liljencrantz[1], I. Strigo[2,3], D.M. Ellingsen[4,5], H.H. Krämer[6], L.C. Lundblad[1], S.S. Nagi[7,8], S. Leknes[1,5], H. Olausson[1,7]

1 Institute of Neuroscience and Physiology, University of Gothenburg, Sweden
2 VA San Francisco Healthcare System, USA
3 University of California San Francisco, USA
4 MGH/HST Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, USA
5 Department of Psychology, University of Oslo, Norway
6 Department of Neurology, Justus Liebig University, Giessen, Germany
7 Center for Social and Affective Neuroscience, Linköping University, Sweden
8 School of Medicine, Western Sydney University, NSW, Australia

Correspondence
Saad S. Nagi
E-mail: saad.nagi@liu.se

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Conflicts of interest
None declared.

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Abstract

Background: C-tactile (CT) afferents are unmyelinated low-threshold mechanoreceptors optimized for signalling affective, gentle touch. In three separate psychophysical experiments, we examined the contribution of CT afferents to pain modulation.
Methods: In total, 44 healthy volunteers experienced heat pain and CT optimal (slow brushing) and CT sub-optimal (fast brushing or vibration) stimuli. Three different experimental paradigms were used: Concurrent application of heat pain and tactile (slow brushing or vibration) stimulation; Slow brushing, applied for variable duration and intervals, preceding heat pain; Slow versus fast brushing preceding heat pain.
Results: Slow brushing was effective in reducing pain, whereas fast brushing or vibration was not. The reduction in pain was significant not only when the CT optimal touch was applied simultaneously with the painful stimulus but also when the two stimuli were separated in time. For subsequent stimulation, the pain reduction was more pronounced for a shorter time interval between brushing and pain. Likewise, the effect was more robust when pain was preceded by a longer duration of brush stimulation. Strong CT-related pain reduction was associated with low anxiety and high calmness scores obtained by a state anxiety questionnaire.
Conclusions: Slow brushing – optimal for CT activation – is effective in reducing pain from cutaneous heating. The precise mechanisms for the pain relief are as yet unknown but possible mechanisms include inhibition of nociceptive projection neurons at the level of the dorsal horn as well as analgesia through cortical mechanisms.
Significance: Slow brushing stimuli – optimal for activation of C-tactile fibres – can reduce pain from cutaneous heating. No such effect was seen with fast brushing or vibration. These observations indicate the role of C-tactile fibres in pain modulation.

1. Introduction

Touch is commonly used to console, as in a parent's soft touch, and to diminish pain, as in the natural response to rub or stroke a wounded body part. A neurophysiological explanation for this phenomenon is the gate control theory (Melzack and Wall, 1965)

which proposes that activation of myelinated mechanoreceptive (Aβ) afferents at the site of an injury activates inhibitory interneurons in the spinal cord that in turn decreases the amount of nociceptive information signalled to the brain. At the time when this theory was presented, human touch was thought to be signalled exclusively by Aβ afferents. The C-low-threshold mechanoreceptive (C-LTMR) afferents found in animals were long believed to be missing in humans (Kumazawa and Perl, 1977). However, using the technique of microneurography, C-LTMRs were later found to exist also in humans (Nordin, 1990; Vallbo et al., 1993), and they were termed C-tactile (CT) afferents. CTs have distinctly different characteristics compared to Aβs: CTs are unmyelinated and thus have a slow conduction velocity (akin to C-nociceptors); a phenotypically identical class to hairy-skin CTs has thus far *not* been found in human glabrous skin; CTs respond optimally to skin stroking with velocities in the range of 1–10 cm/s (in contrast to Aβs whose activation increases with velocity) (Vallbo et al., 1993, 1999; Wessberg et al., 2003; Löken et al., 2009). There is a robust positive correlation between the firing frequency of CT afferents and the perceived pleasantness of touch (Löken et al., 2009; Ackerley et al., 2014), and CTs are posited to mediate emotional, interpersonal aspects of touch.

In rats, C-LTMR-targeted input inhibits C-nociceptive signalling in the dorsal horn (Lu and Perl, 2003). Recent evidence suggests that C-LTMRs, when activated, release the chemokine-like secreted protein TAFA4 that has analgesic effects (Delfini et al., 2013). In addition, pharmacogenetic activation of Mas-related G protein-coupled receptor B4 ($MRGPRB4^+$)-expressing neurons, thought to be a sub-class of C-LTMRs, promotes conditioned place preference in mice (Vrontou et al., 2013). This behaviour indicates that activation of these neurons is positively reinforcing and/or anxiolytic.

In the present study, we hypothesized that CT optimal stimuli would be effective in reducing pain perception in humans. This was tested using three different experimental paradigms. The first paradigm compared the effects of *concurrent* application of slow brushing or vibration on heat pain intensity. In the second paradigm, slow brushing and heat pain stimuli were applied separately – at varying intervals – in order to account for any potential distraction component of touch on pain. Furthermore, the duration of brushing (preceding heat pain stimulation) was varied in order to test the temporal aspect of pain reduction by slow brushing. That is, whether the magnitude of pain reduction increased with the duration of brushing. The third paradigm was made up of the combined parameter information gained from the first two paradigms and aims to further explain our findings by examining the sources of interpersonal variance. In this paradigm, both slow and fast brushing stimuli were used.

2. General Methods

2.1 Overview

Three experiments were conducted (see Fig. 1 for an overview of the experimental paradigms) that asked unique questions and differed in many respects including stimulation protocol, number of study participants and body area stimulated. The methods shared by all experiments are outlined in this section, whereas the details specific to each experiment are described separately. The results from each experiment informed the design of the subsequent study protocol. The experiments were performed in accordance with the Declaration of Helsinki, and approved by the ethical review board at the University of Gothenburg.

2.2 Participants

All participants were healthy, right-handed volunteers ($n = 44$ median age 23 years, range 19–36 years, 21 men) who gave informed and written consent to participate. The participants were prevented from seeing the stimulated skin area through the use of a curtain.

2.3 Heat pain stimuli

The static heat pain stimuli were delivered using a Peltier thermode (3 × 3 cm, Medoc, TSA 2001, Thermosensory Analyzer, Rimat Yishai, Israel) that was attached to the skin during the entire experimental session. At the start of the experiment, a moderately painful heat stimulus (corresponding to an approximate numeric rating of 4 on a scale with anchors 0 = no pain, 1 = pain threshold, and 10 = most intense pain) was determined for each participant. Participants were not informed that the same temperature was used for all stimuli in the experimental session, and nor were they instructed to base their ratings on the initial test. They were asked to focus on their perception of each individual heat pain stimulus and rate accordingly.

2.4 Tactile stimuli

Three types of tactile stimuli were used: slow brushing, fast brushing and focal vibration. CT optimal

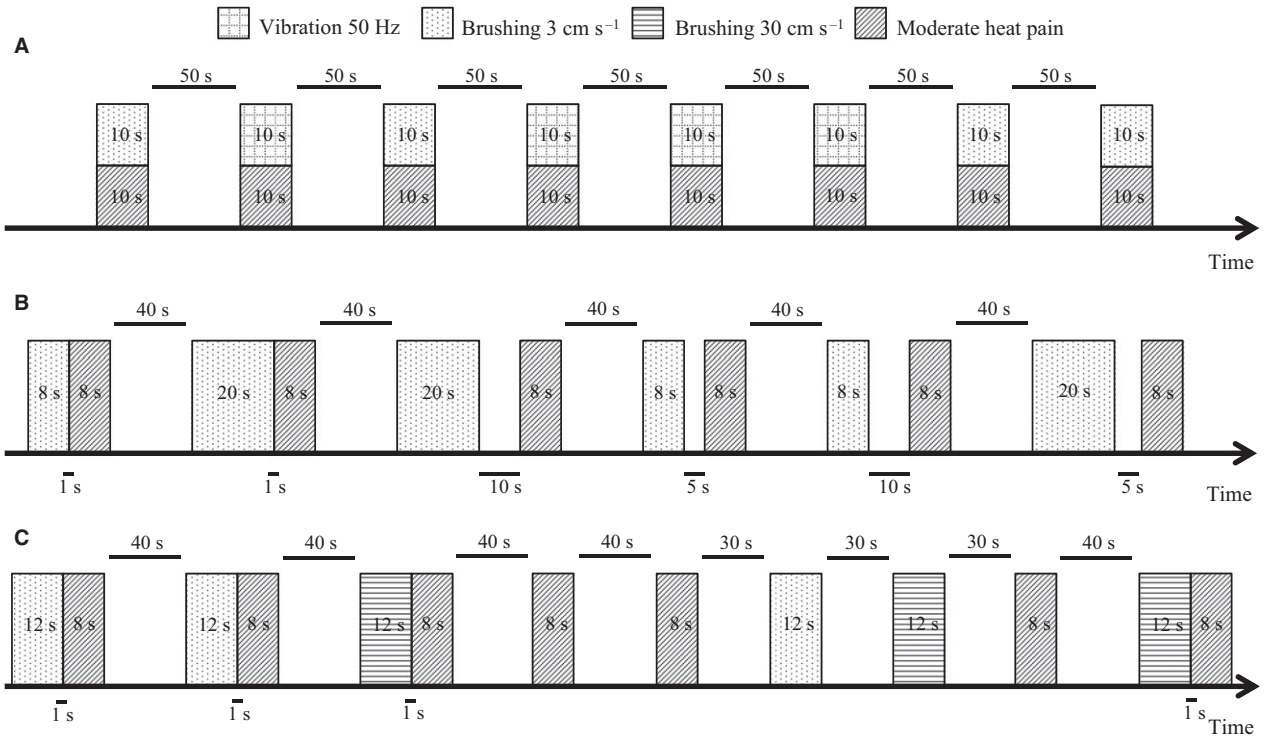

Figure 1 Experimental designs. (A) Experiment 1: Simultaneous heat pain and tactile stimuli. (B) Experiment 2: Temporal spacing of skin stroking and heat pain. (C) Experiment 3: Slow versus fast skin stroking preceding heat pain.

stimulation consisted of slow brush stroking in proximal to distal direction, with a velocity of approximately 3 cm/s and an approximate indentation force of 0.3 N (Liljencrantz et al., 2013). All stroking stimuli were delivered manually by an experimenter trained to apply the strokes with constant force and velocity. CT afferent firing is insensitive to changes in indentation force in the range 0.2–0.4 N and/or velocity in the range 1–10 cm/s (Löken et al., 2009), so any unintentional variation in force or velocity is unlikely to influence the activation of these afferents. The tactile stimuli and the heat pain were applied within the same dermatome. Stimuli were delivered in a pseudo-randomized order (Fig. 1).

2.5 Pain ratings

Participants rated the derived pain intensity continuously on a 10-cm-long computerized visual analogue scale (CoVAS, 0–100, anchors 'no pain' and 'worst pain imaginable', Medoc TSA-II Accessories, Ramat Yishai, Israel; sampling rate: 0.11 s).

2.6 Data analysis

Three variables were determined from the continuous pain ratings: (1) area under the curve (AUC), extracted as the sum of all pain ratings when temperature was higher than baseline; (2) peak pain rating for each thermal stimulus; and (3) time to pain rating onset (time from reached target temperature to pain rating onset). Statistical comparisons were made in SPSS (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc., Chicago, Ill., USA) using ANOVAs and planned *t*-tests. Kolmogorov–Smirnov tests of normality were run for all data and parametric or non-parametric statistics were used accordingly as described in the experiment-specific methods. For certain comparisons, individual pain ratings following tactile stimulation were normalized to the pain only condition using the following computation [(mean pain following slow or fast brushing or vibration/mean pain only)*100].

3. Experiment 1

3.1 Methods

We used simultaneous heat pain and tactile stimulation to assess the analgesic effects of slow brushing and vibration.

3.1.1 Experimental procedure

Each participant (n = 14, median age 25, range 22–27 years, seven men) was tested with an individually determined temperature ranging from 45 to 50 °C (median 48 °C; procedure as explained above). The stimuli were applied for 10 s followed by 50 s of rest. The heat pain stimulus was presented (1) with simultaneously applied slow brushing (3 cm/s), (2) with vibration or (3) without touch stimulation (Fig. 1A). The heat pain was applied to the right thigh, 10 cm proximal to the patella in the L4 dermatome; a skin area with dense CT innervation (Edin, 2001). The thermode baseline temperature was set to 32 °C and the ramp rate to 10 °C/s. The brushing (soft goat's hair brush, 7-cm wide) was applied as three strokes starting 12 cm proximal to the thermode over a distance of 7 cm. There was a 1.5-s pause between each of the strokes giving a total duration of 10 s for each stimulation block. Vibration was applied 7 cm proximal to the thermode at 50 Hz (4.0 cm × 1.2 cm × 0.7 cm of balsa wood connected to a piezo-element, Piezo Systems, Inc., Cambridge, MA, USA) for 10 s. Each condition was repeated 10 times, in pseudo-randomized order (Fig. 1A).

3.2 Results

3.2.1 Simultaneous heat pain and tactile stimuli

Effects of slow stroking touch or vibration on pain perception were investigated using a one-way ANOVA (repeated measures, three levels: pain only, pain + slow stroking and pain + vibration) and indicated a significant difference between the AUC measurements (Wilks' Lambda = 0.33, $F(2, 12) = 12$, $p = 0.001$, Fig. 2A). The AUC for pain with simultaneous stroking was significantly smaller than that of pain with simultaneous vibration (slow stroking = 33 69 ± 648, mean AUC ± SEM; vibration = 414 8 ± 752; $p < 0.001$, t-test) and significantly smaller than that of heat pain only (4168 ± 714, $p = 0.01$, t-test). The AUC for pain with simultaneous vibration was not significantly separable from that of heat pain only ($p = 0.94$, t-test).

For the peak pain ratings, there was also a significant difference between conditions (Wilks' Lambda = 0.42, $F(2, 12) = 8.3$, $p = 0.005$, Fig. 2B). Peak ratings for heat pain with simultaneous slow stroking were significantly lower than those of pain with simultaneous vibration (slow stroking = 52 ± 7.7, mean peak pain ± SEM; vibration = 58 ± 7.9; $p = 0.001$, t-test) and significantly lower than those of heat pain only (59 ± 7.5; $p = 0.04$, t-test). Peak pain ratings during simultaneous vibration were not significantly different from those of heat pain only ($p = 0.6$, t-test). Fig. 2D shows the individual peak pain ratings across conditions. Given the wide range of ratings, we investigated if there was a correlation between the stimulus temperature and the degree of pain relief for CT-targeted touch but there was no significant correlation (Spearman's rho = 0.24, $p = 0.40$; Fig. 3B). We also investigated if the stimulus temperature correlated with the peak heat pain only ratings (Experiment 1 and 3 data collapsed) but there was no significant correlation (Spearman's rho = −0.26, $p = 0.13$; Fig. 3A). To test for any heat de/sensitization effect, we compared the second and the last peak pain ratings for each individual and performed a two-tailed paired samples t-test between these values. No significant difference ($p = 0.4$) was found. We used the second rating rather than the first to avoid any pain anticipatory effect.

Although a trend was indicated, there was no significant difference between conditions on time to pain rating onset (heat pain only = 2.3 ± 0.5 mean s to pain onset ± SEM; slow stroking = 2.9 ± 0.6; vibration = 2.4 ± 0.4, repeated measures ANOVA: Wilks' Lambda = 0.63, $F(2, 20) = 3.6$, $p = 0.06$, Fig. 2C).

In summary, there was a significant analgesic effect of CT optimal stroking touch on simultaneously applied heat pain, as evidenced by reduced AUC, reduced peak pain rating and a trend towards a delay in pain onset. However, simultaneous tactile stimulation may also cause analgesia through unspecific attentional mechanisms (distraction).

4. Experiment 2

4.1 Methods

We employed variable temporal spacing of CT optimal stroking touch and heat pain to assess the analgesic effects of brushing independent of unspecific effects of distraction. Pain and stroking touch stimuli were now applied to the forearm which, similar to the thigh, is densely innervated by CT afferents (Wessberg et al., 2003; Löken et al., 2009).

4.1.1 Experimental procedure

Each participant (n = 8, median age 25, range 21–36 years, six men) was tested with an individually determined temperature ranging from 46.5 to

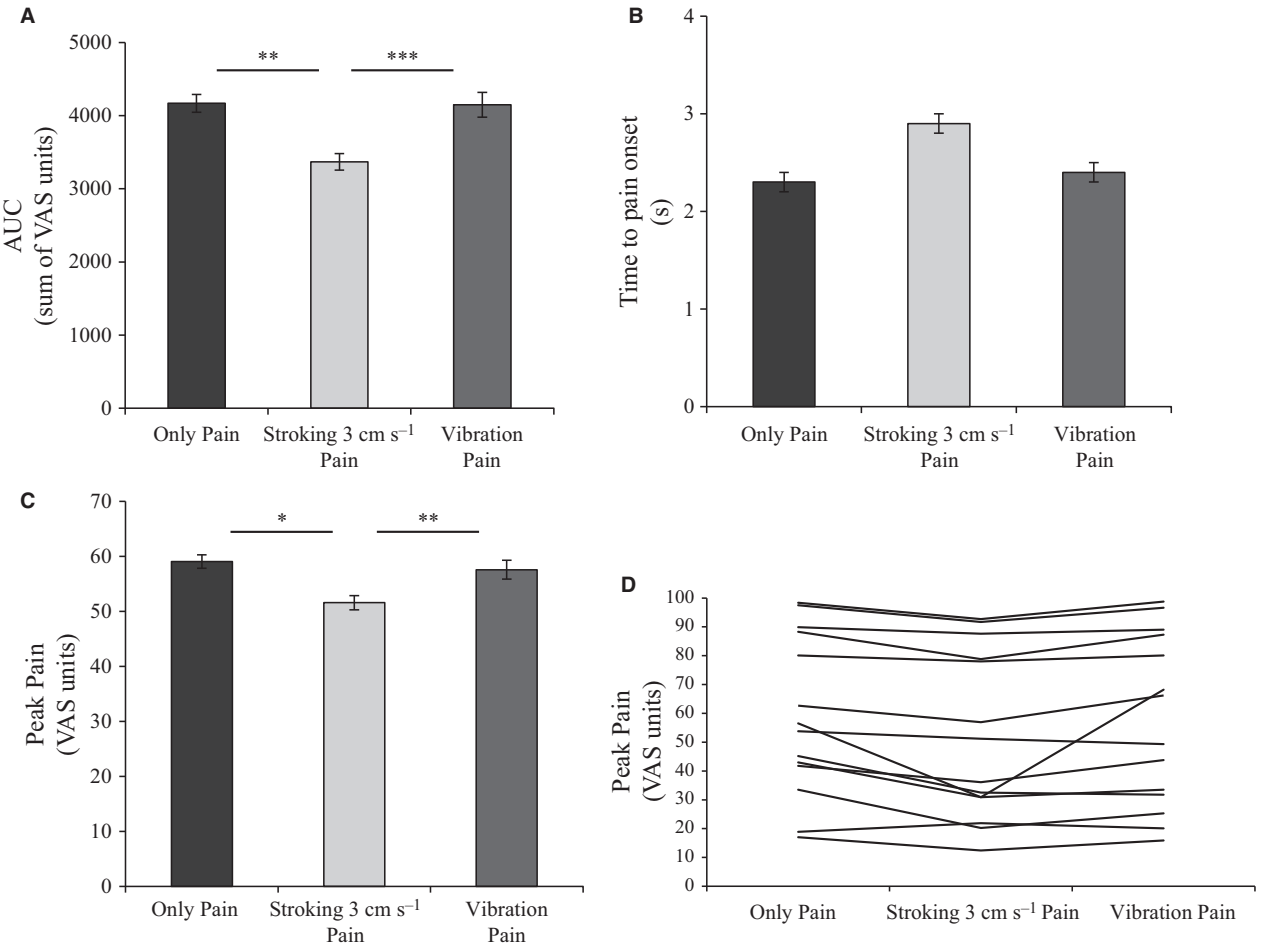

Figure 2 Experiment 1: Simultaneous heat pain and tactile stimuli. Group mean data and within subject error bars, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. (A) The area under the curve (AUC) for pain with simultaneous slow stroking was significantly smaller than that of pain with simultaneous vibration ($p < 0.001$) and significantly smaller than that of heat pain only ($p = 0.01$). The AUC for pain with simultaneous vibration was not significantly separable from that of heat pain only. (B) Peak pain ratings for heat pain with simultaneous slow stroking were significantly smaller than those of pain with simultaneous vibration ($p = 0.001$) and significantly smaller than those of heat pain only ($p = 0.04$). Peak pain ratings during simultaneous vibration were not significantly separable from that of heat pain only. (C) There was no significant effect on time to pain rating onset for slow stroking or vibration compared to heat pain only. (D) Mean peak pain ratings for each individual subject across conditions.

49.5 °C (median 49 °C; procedure as explained above). The thermode was positioned on the radial left forearm, just distal to the elbow in the C7 dermatome. The thermode baseline temperature was set to 33 °C, the ramp rate to 10 °C/s and the duration of the heat plateau was 5 s. The duration of the brush stroking at 3 cm/s was either 8 or 20 s and the stimulation distance was 12 cm, i.e. either two brush strokes (8-s duration) or five brush strokes (20 s) preceding the heat stimulation (Fig. 1B). The stroking was applied just distal of the heat probe. The inter-stimulus interval (ISI), i.e. between the stroking offset and the heat pain onset, was 1, 5 or 10 s. The 18 stimulus pairs (stroking + pain) were delivered in a pseudo-randomized order (Fig. 1B). The inter-trial interval (ITI), i.e. the time between the end of one pain stimulus to the start of the subsequent touch stimulus, was 40 s.

4.2 Results

4.2.1 Temporal spacing of skin stroking and heat pain

A repeated measures ANOVA of the AUC measurements, with main factors ISI (1, 5, 10 s) and stroking

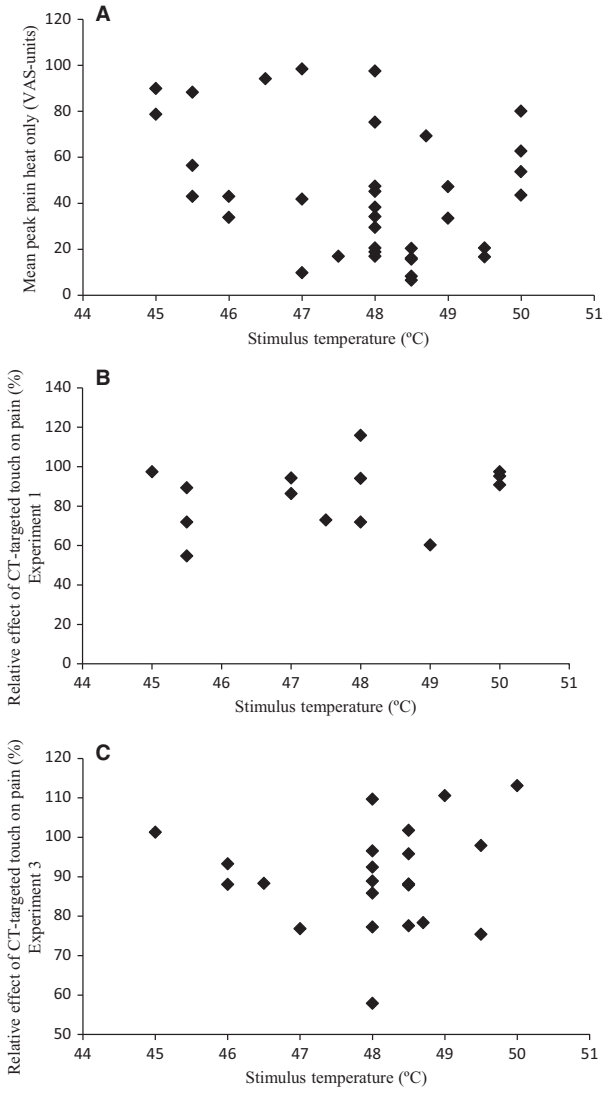

Figure 3 The stimulus temperature did not affect the pain ratings or the degree of pain relief. (A) For experiment 1 and 3, there is no correlation ($r = -0.26$) between the stimulus temperature, predetermined by each subject to correspond to moderate heat pain (approximately 4 on a numeric rating scale), and the mean peak pain rating for the heat pain only condition. (B) For experiment 1, there is no correlation ($r = 0.24$) between the stimulus temperature and the degree of pain relief of CT-targeted touch (%) [calculated as (peak pain rating for heat pain with simultaneous stroking at 3 cm/s/peak pain rating for heat pain only) × 100]. (C) For experiment 3, there is no correlation ($r = 0.14$) between the stimulus temperature and the degree of pain relief of CT-targeted touch (%) [calculated as (peak pain rating for heat pain preceded by stroking at 3 cm/s/peak pain rating for heat pain only) × 100].

duration (8, 20 s) indicated a main effect of ISI (Wilks' Lambda = 0.27, $F(2, 6) = 8.2$, $p = 0.02$). AUC measurements were significantly lower for the 1 s compared to the 5- and 10-s ISIs (ISI 1 vs. 5 s, $p = 0.009$, ISI 1 vs. 10 s, $p = 0.003$, ISI 5 vs. 10 s, $p = 0.091$; *t*-test, Fig. 4A). There was no significant difference in AUC measurements related to the duration of the stimulation (Wilks' Lambda = 0.66, $F(1, 7) = 3.5$, $p = 0.102$, Fig. 4A). There was no significant interaction between ISI and duration of stroking on AUC measurements ($p = 0.6$).

Peak pain ratings were also significantly lower for the 1-s compared to the 5- and 10-s ISIs (Wilks' Lambda = 0.25, $F(2, 6) = 8.9$, $p = 0.016$, *t*-test; ISI 1 vs. 5 s, $p = 0.007$, ISI 1 vs. 10 s, $p = 0.003$, ISI 5 vs. 10 s, $p = 0.487$; Fig. 4B). The peak pain ratings were lower when the heat pain was preceded by a 20-s compared to an 8-s stroking stimulus (Wilks' Lambda = 0.54, $F(1, 7) = 5.9$, $p = 0.045$, *t*-test; 8 s vs. 20 s duration, $p = 0.045$; Fig. 4B). There was no significant interaction between ISI and duration of stroking on peak pain ratings ($p = 0.4$).

The mean delay of pain rating onset was significantly longer for the 1-s ISI (Wilks' Lambda = 0.1, $F(2, 6) = 20.2$, $p = 0.002$, *t*-test: ISI 1 compared to 5 s $p = 0.001$, and 10 s $p < 0.001$, ISI 5 s vs. 10 s $p = 0.08$; Fig. 4C). There was a trend towards a significant difference in time to pain onset related to the duration of the stimulus (Wilks' Lambda = 0.6, $F(1, 7) = 5.1$, $p = 0.058$; Fig. 4C). There was no significant interaction between ISI and duration of stroking on time to pain rating onset ($p = 0.2$).

In summary, CT optimal touch reduced pain ratings when controlling for distraction (shift of attention away from the heat pain) through temporally separated stimuli. Pain relief as indexed by AUC, peak pain ratings and time to pain onset was most pronounced when touch immediately preceded pain. Moreover, peak pain ratings were lower when pain was preceded by long (20 s) compared to short (8 s) duration of 234touch.

5. Experiment 3

5.1 Methods

Informed by the outcome of Experiment 2, we studied the analgesic effects of slow and fast skin stroking immediately preceding heat pain in a larger sample. Measures of state anxiety, state calmness, depression and alexithymia were collected to address sources of individual variability in pain reduction response to CT optimal touch.

5.1.1 Participants

Twenty-two healthy volunteers (median age 23, range 19–32 years, eight men) participated. The

higher number of subjects enabled investigation of sources of inter-individual variance in CT-related analgesia (correlation with results from questionnaires for mood state and psychiatric screening).

5.1.2 Experimental procedure

Each participant was tested with an individually determined temperature ranging from 46 to 49.5 °C (median 48 °C; procedure as explained above). We used a 1-s ISI, and the duration of the stroking stimulus was 12 s. The stroking distance was 18 cm. All other stimulus parameters were kept the same as in experiment 2. There were five stimulus conditions, delivered in a pseudo-randomized order (Fig. 1): heat pain only (seven repetitions), fast (30 cm/s) stroking preceding heat pain (seven repetitions), slow (3 cm/s) stroking preceding heat pain (seven repetitions), slow stroking only (three repetitions) and fast stroking only (three repetitions). The conditions with brush stroking only were added to the paradigm to avoid the subjects developing negative connotations by associating stroking stimuli with pain (i.e. conditioning). The heat pain only stimuli served as the baseline pain condition. The ITI was jittered from a minimum of 22 s to a maximum of 40 s, while keeping >40 s between subsequent heat pain stimuli to minimize sensitization.

5.1.3 Questionnaires

Following the stimulation paradigm, the participants were asked to respond to Becks Depression Inventory II (BDI-II), Toronto Alexithymia Scale (TAS-20), state questions from the State Trait Anxiety Inventory (STAI) and a post-task questionnaire with numeric ratings of touch pleasantness (anchors: very unpleasant–very pleasant, 0–10) and touch intensity (anchors: not intense–very intense, 0–10). Three participants chose not to respond to the questionnaires.

5.2 Results

5.2.1 Slow versus fast skin stroking preceding heat pain

A one-way ANOVA (repeated measures, three levels: pain only, pain preceded by slow brushing, pain preceded by fast brushing) was conducted to compare the AUC of moderate heat pain and heat pain preceded by stroking of the skin.

A significant difference between conditions was identified for the AUC measurements (Wilks'

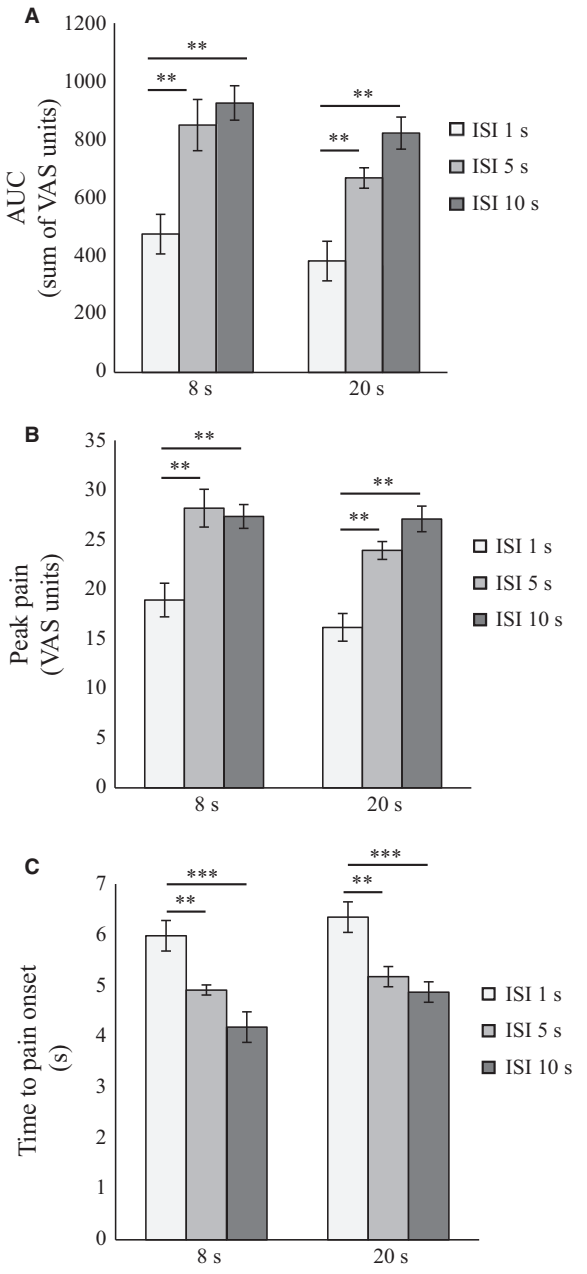

Figure 4 Experiment 2: Temporal spacing of CT optimal stroking and heat pain with two durations of stroking. Group mean data and within subject error bars, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. (A) The AUC was only significantly reduced for the 1-s compared to 5- ($p = 0.009$) and 10-s ($p = 0.003$) ISIs. There was no significant difference in AUC related to the duration of the stimulus. (B) Peak pain ratings were only significantly lower for the 1-s compared to the 5- ($p = 0.007$) and 10-s ($p = 0.003$) ISIs. The peak pain ratings were significantly lower when the heat pain was preceded by a 20-s compared to an 8-s CT optimal stroking stimulus ($p = 0.045$). There was no significant interaction effect of ISI and duration. (C) Time to pain onset was significantly longer for the 1-s ISI compared to the 5-s ($p = 0.001$) and 10-s ($p < 0.001$) ISIs. There was no significant difference in time to pain onset related to the duration of the stimulus.

Lambda = 0.71, $F(2, 20) = 4.8$, $p = 0.03$, Fig. 5A). AUC for pain preceded by slow stroking was significantly smaller than for pain preceded by fast stroking (slow = 857 ± 157, mean AUC ± SEM; fast = 970 ± 176; *t*-test, $p = 0.02$) and significantly smaller than that of heat pain only (1014 ± 186, $p = 0.04$). The AUC for pain preceded by fast stroking did not differ significantly from that for pain only ($p = 0.57$).

A repeated measures ANOVA was used to compare the peak pain ratings of the three experimental conditions and a significant difference was found for the peak pain ratings (Wilks' Lambda = 0.63, $F(2, 20) = 5.8$, $p = 0.01$, Fig. 5B). The ratings were significantly lower when the pain was preceded by slow than fast stroking (slow = 33 ± 5, mean peak pain ± SEM, fast = 37 ± 6, *t*-test, $p = 0.005$). The ratings of pain preceded by slow stroking were significantly lower than the ratings for heat pain only (36 ± 5, $p = 0.03$), whereas the ratings of pain preceded by fast stroking did not differ significantly from the ratings for heat pain only ($p = 0.32$). Slow stroking decreased peak pain ratings by 10% and fast stroking increased peak pain ratings by 1%. Fig. 5D shows the individual peak pain ratings across conditions. Given the wide range of ratings, we investigated if there was a correlation between the stimulus temperature and the degree of pain relief for CT-targeted touch but there was no significant correlation (Spearman's rho = 0.14, $p = 0.55$; Fig. 3C). We also investigated if the stimulus temperature correlated with the peak heat pain only ratings (Experiment 1 and 3 data collapsed) but there was no significant correlation (Spearman's rho = −0.26, $p = 0.13$; Fig. 3A). Similar to Experiment 1, we tested for any heat de/sensitization effect by comparing the second and the last peak pain ratings for each individual; however, no significant difference ($p = 0.1$) was found.

There was no significant difference between conditions on time to pain onset (heat only pain = 2.8 ± 0.3 mean s to pain onset ± SEM; slow = 3.0 ± 0.3; fast = 2.8 ± 0.3, repeated measures ANOVA: Wilks' Lambda = 0.85, $F(2, 20) = 1.8$, $p = 0.2$, Fig. 5C).

5.2.2 Ratings of slow versus fast skin stroking

Post-experiment ratings revealed that slow stroking was perceived as significantly more pleasant than fast stroking (slow = 5.8 ± 0.2, mean numeric rating ± SEM, fast = 4.3 ± 0.3, *t*-test, $t = 5.6$, $p < 0.001$). In contrast, fast stroking was perceived as significantly more intense than slow (slow = 2.7 ± 0.3, mean VAS ± SEM, fast = 3.3 ± 0.2, *t*-test, $t = 2.8$, $p = 0.01$).

5.2.3 State influences on CT optimal touch analgesia

In an exploratory analysis, state anxiety scores were significantly associated with individual differences in CT-targeted touch analgesia, such that low-state anxiety individuals showed the largest reduction in pain ratings. There was a significant negative correlation between STAI State Anxiety (Spearman's rho = −0.6, $p = 0.01$; Fig. 6A) and a positive correlation with STAI State Calmness (Spearman's rho = 0.5, $p = 0.03$; Fig. 6B) and the reduction in normalized AUC for slow compared to fast stroking. There were no significant correlations between AUC measurements and self-reports on depression (BDI) ($p = 0.1$) or alexithymia (TAS) scales ($p = 0.1$).

There was also a significant negative correlation between the reduction in normalized peak pain ratings for slow compared to fast stroking and STAI State Anxiety (Spearman's rho = −0.6, $p = 0.02$; Fig. 6C). There was a trend towards correlation between normalized peak pain ratings and STAI State Calmness ($p = 0.05$), but no significant correlations with BDI ($p = 0.1$) or TAS ($p = 0.1$).

Correlations were not investigated for time to pain rating onset as there was no difference across conditions for this measurement in experiment 3.

6. Discussion

In this experimental setting, slow, pleasant brushing was effective in reducing pain perception of a simultaneously or subsequently applied noxious heat stimulus. For subsequent stimulation, the pain reduction was more pronounced for a shorter time interval between brushing and pain. Participants with large reductions in pain ratings following slow brushing compared to fast brushing reported low anxiety and high calmness.

6.1 CT afferents

The differences in dynamic sensitivity of CT and Aβ afferents provide the means for 'preferentially' stimulating each afferent type (Löken et al., 2009; Gordon et al., 2011; Morrison et al., 2011; Ackerley et al., 2014; Bennett et al., 2014). CTs respond suboptimally to 50-Hz vibration (Wiklund Fernström et al., 2002) and preferentially to 1–10 cm/s stroking, whereas Aβs respond well to 50-Hz vibration (Mountcastle et al., 1967; Talbot et al., 1968) and respond better the faster the stroking velocity (Löken et al., 2009; Ackerley et al., 2014).

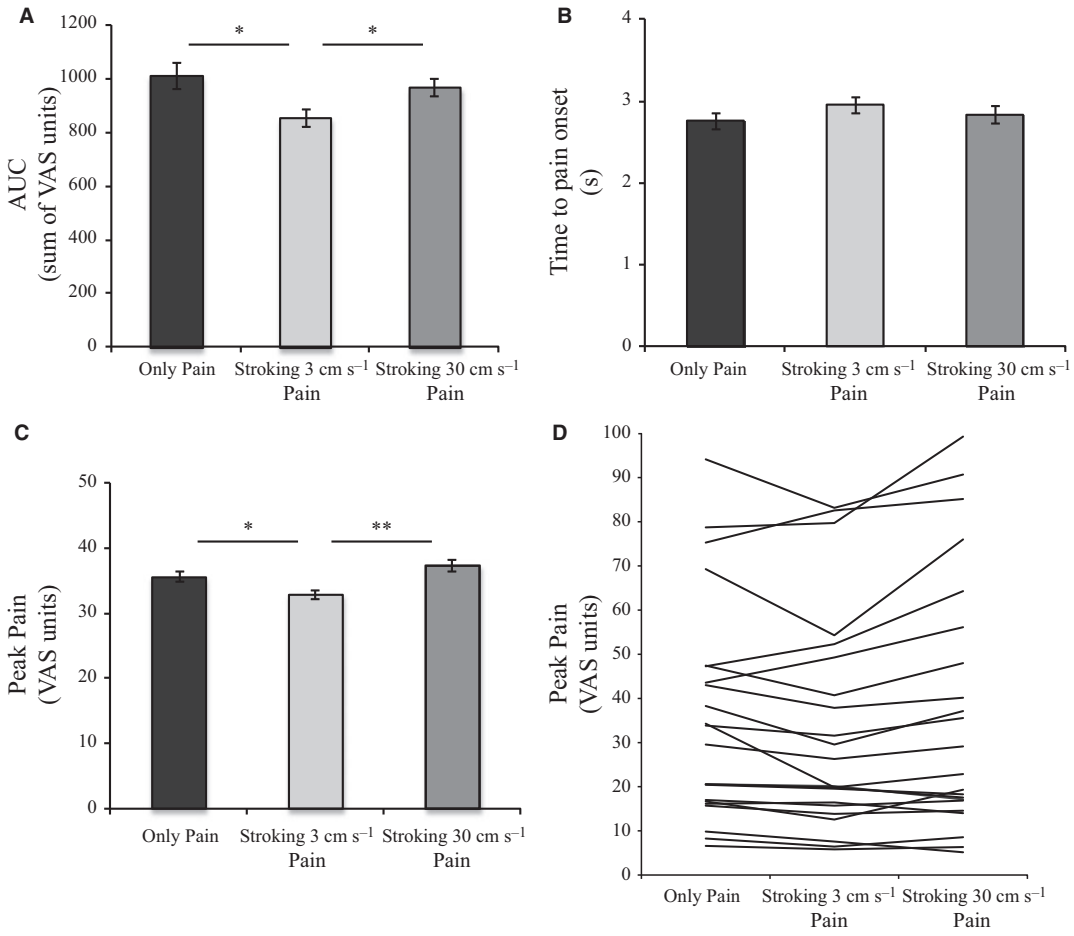

Figure 5 Experiment 3: Slow versus fast skin stroking preceding heat pain. Group mean data and within-subject error bars, $*p \leq 0.05$, $**p \leq 0.01$. (A) The AUC was significantly reduced by slow stroking preceding the pain compared to fast stroking and to heat pain only. (B) Peak pain ratings were significantly lower when pain was preceded by slow compared to fast stroking as well as only heat pain. (C) There was no significant effect of stroking on time to pain onset. (D) Mean peak pain ratings for each individual subject across conditions.

The spino-cortical projection pathways for human CT afferents are unknown. For C-LTMRs in rats, there is a similar projection as the nociceptive pathway, i.e. from the superficial laminae (I-II) and onward along the spinothalamic tract (Andrew, 2010). Functional brain imaging of CT stimuli suggests that the primary cortical receiving area for CTs is the posterior insular cortex, suggesting a spinothalamic projection for CTs in humans as well (Olausson et al., 2002; Björnsdotter et al., 2009; Morrison et al., 2011). In contrast, human Aβ afferents project from deeper laminae (III–IV) and along the dorsal column to the primary and secondary somatosensory cortices. However, recent evidence suggests integration of Aβ and C-LTMRs already at a dorsal horn level in mice (Li et al., 2011; Abraira and Ginty, 2013).

6.2 Pain reduction by touch

In experiment 1 when slow brushing was applied simultaneously with heat pain, we found a significant pain reduction. However, it might be argued that the brushing acted as a distracter from the heat pain, making attentional shift a possible explanatory factor. But, there are other observations suggesting a specific role for CTs. First, we did not observe significant analgesic effects of vibration. Second, we found a pain reduction not only when the CT optimal touch was applied simultaneously with the painful

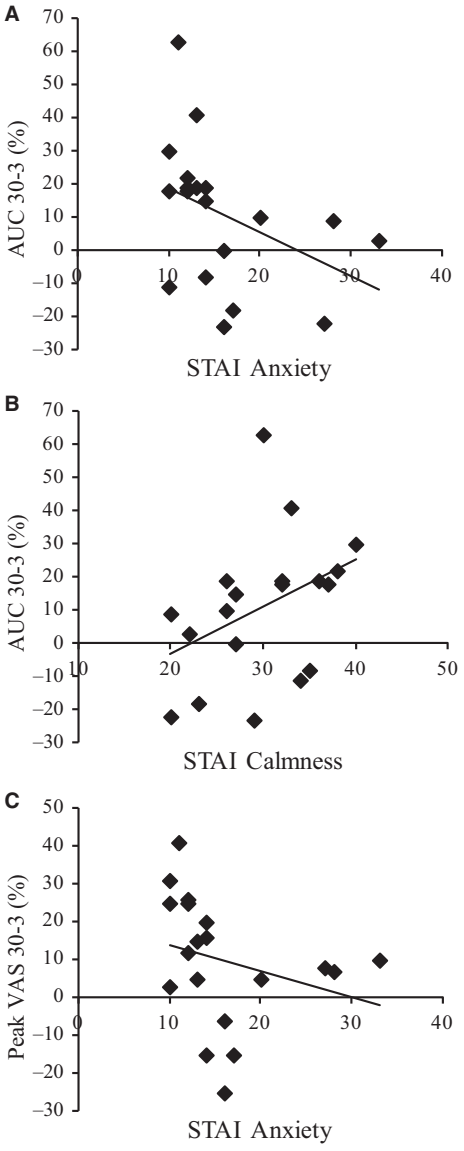

Figure 6 Questionnaire data Experiment 3: Slow versus fast skin stroking preceding heat pain. (A) There was a significant negative correlation ($r = -0.6$) between the normalized AUC reduction for slow compared to fast stroking and the questionnaire scores of STAI State Anxiety. (B) There was a significant positive correlation ($r = 0.5$) between the normalized AUC reduction for slow compared to fast stroking and the questionnaire scores of STAI State Calmness. (C) There was a significant negative correlation ($r = -0.6$) between the reduction in normalized peak pain ratings for slow compared to fast stroking and the questionnaire scores of STAI State Anxiety.

stimulation but also when the two stimuli were separated in time. Third, the reduction in pain ratings was more pronounced for a longer compared to a shorter lasting CT optimal touch. Therefore, it seems likely that, in addition to unspecific mechanisms such as shift of attention, there was an analgesic contribution from a CT-dependent mechanism. While the nociceptor de/sensitization (Fitzgerald and Lynn, 1977; Adriaensen et al., 1984) to repeated noxious heat stimulation cannot be discounted, the differential effect on pain *vis-à-vis* slow brushing (pain reduction) and fast brushing/vibration (no effect) makes this unlikely to account for our findings. Furthermore, the reduction in pain was elicited regardless of the duration of heating, but could be modulated by varying the parameters of slow brushing. Moreover, when we compared the first/second and last peak pain ratings for each individual, no significant differences were found.

The concept of touch inhibiting pain was proposed as the gate control theory almost 50 years ago (Melzack and Wall, 1965). The theory proposes that cells in lamina II of the spinal cord dorsal horn act as a gate to modulate the excitatory incoming afferent patterns before they penetrate further into the central nervous system. This inhibitory effect, exerted by the lamina II cells on the nociceptive fibres, is suggested to be driven by stimuli activating large diameter (Aβ) afferents (Melzack and Wall, 1965). While the focus of the current study was to examine the effect of CT optimal stimuli on heat pain, it is worthy of note that a similar effect (pain reduction) was not elicited by the Aβ preferential stimuli in our model (Nathan, 1976). However, fast brushing was perceived as less pleasant than slow brushing, consistent with earlier observations (Löken et al., 2009), and it may well be that pain modulation is determined, in part at least, by the affective valence of a stimulus. For instance, it was shown previously that stroking the skin with sandpaper, an unpleasant stimulus, resulted in amplification of the underlying muscle pain; conversely, velvet stroking, a pleasurable stimulus, elicited pain relief (Shaikh et al., 2015). While not systematically studied, it is likely that the 50-Hz vibratory stimulus used in the current study was devoid of any overt affective quality, which may explain, in part at least, the lack of pain-modulatory effect. However, regardless of the affective attributes, our test stimuli ought to have activated Aβ afferents and hence it is unclear why 'gating' of pain was not seen in our model. However, the interaction between touch and pain is highly complex as, for instance, indicated by the variable efficacy of transcutaneous electrical nerve stimulation – a technique based on the gate control theory, which uses brief electrical pulses to preferentially activate large diameter afferents. Although temporary pain relief has been observed, there have been

reports of exacerbation of pain during the course of neuro-stimulation (e.g. Richardson et al., 1981). Thus, any explanation of touch–pain interactions must include both inhibitory actions and convergence of tactile inputs with central pain pathways. Top-down contributions such as expectations, social context, attachment behaviour and motivation also play key roles in shaping the subjective experience of touch and pain (Ellingsen et al., 2015; Krahé et al., 2016), and likely interact with CT afferents in shaping real-life encounters involving touch/pain.

In the current study, vibration frequency and stroking velocity parameters were set based on previous electrophysiological data (Wessberg et al., 2003; Löken et al., 2009; Ackerley et al., 2014) on the responsiveness of CT afferents defining them as being optimal or sub-optimal. A recent study showed that skin indentation with monofilament contacts, a stimulus which activates both Aβ and CT afferents (Cole et al., 2006; Nagi et al., 2015), produces analgesia when delivered simultaneously with infrared laser heat (Mancini et al., 2014). Interestingly, other studies have argued an intensity-dependent effect of stroking, as it tapered off with an increase in laser strength from a barely detectable condition to pain threshold and above (Kakigi and Shibasaki, 1992; Nahra and Plaghki, 2003). Inhibition of both sub-cortical and cortical nociceptive processes has been proposed as the underlying mechanism of touch-mediated analgesia, albeit with a focus on the myelinated tactile system (Inui et al., 2006; Mancini et al., 2015).

At the time when the gate control theory was proposed, C-LTMRs had been found in animals but not in humans (Kumazawa and Perl, 1977). However, electrophysiological experiments identified a specific inhibitory pathway between lamina II dorsal horn neurons that received direct peripheral C-LTMR afferent projections and other lamina II cells receiving direct nociceptive input (Lu and Perl, 2003). This unmyelinated circuit represents a way for innocuous C-LTMR impulses to suppress nociceptive impulses (Lu and Perl, 2003).

The mechanisms for pain reduction following CT stimulation may not be limited to the spinal cord. Recently, it was shown in mice that VGLUT3 (vesicular glutamate transporter)-expressing C-LTMRs release TAFA4 protein when activated, and intrathecal administration of TAFA4 reverses hyperalgesia. This suggests a potent analgesic role of TAFA4, and thus for C-LTMRs (Delfini et al., 2013), which could theoretically exert its effects throughout the nervous system. Likewise, GINIP, a Gα1-interacting protein, expressed on nonpeptidergic C fibres (including C-LTMRs) has been shown to modulate the GABAergic inhibition of nociceptive transmission at the spinal level (Gaillard et al., 2014). Conversely, in mice, low-threshold T-type calcium channel, Cav3.2, is a selective marker for small diameter mechanoreceptors expressing VGLUT3/TAFA4/tyrosine hydroxylase and, more importantly, a conditional knockout of Cav3.2 on C-LTMRs resulted in the attenuation of mechanical and cold allodynia – an observation confirmed in humans by the use of a peripheral T-channel antagonist (Francois et al., 2015; Nagi et al., 2015; Samour et al., 2015). Thus, further exploration is warranted into the interplay between the excitatory and inhibitory actions of low-threshold C fibres in normal and altered conditions.

6.3 Pain reduction by hedonic stimuli

Since CT firing correlates significantly with the perceived pleasantness of the stimulus (Löken et al., 2009; Ackerley et al., 2014), CT stimuli may decrease pain perception in the same way as positive pictures (Kenntner-Mabiala and Pauli, 2005), beautiful music (Roy et al., 2008), pleasant odours (Villemure et al., 2003; Villemure and Bushnell, 2009), sweet tastes (Dum and Herz, 1984; Reboucas et al., 2005) and positive expectations (placebo) (Ellingsen et al., 2013). By providing a pleasant opponent sensation, pain processing can be modulated; a concept known as pleasure-related analgesia (Leknes and Tracey, 2008). In humans, psychological/emotional pain modulation is likely mediated by endogenous opioids activating on descending pain inhibitory pathways from the periaqueductal grey (PAG) and rostral ventral medulla (Fields, 2000) and also through direct opioid effects on cortical areas involved in pain processing such as the insula and cingulate cortices (Zubieta et al., 2001; Petrovic et al., 2002).

CT afferents are optimized to signal caress-like touch, which provides a sense of support and reassurance. Several previous studies have shown that social support relieves pain (Panksepp, 2005; Eisenberger et al., 2011; Dunbar et al., 2012), an effect that has been proposed to be opioid-mediated (Dunbar et al., 2012; Hsu et al., 2013). Presumably, touch-induced analgesia evolved primarily as a function of social affiliation (Loseth et al., 2014), hence it is tempting to suggest that the analgesic effect could be augmented by skin-to-skin stroking rather than being touched by a device; however, this

remains a matter for conjecture. Endogenous opioid activity is disrupted both during sad mood (Zubieta et al., 2003) and in chronic pain patients (Willoch et al., 2004). In humans, there is comorbidity between chronic pain and depression, often involving anhedonia (Marbach and Lund, 1981). In the current study, we found a negative correlation between CT-related analgesia and state anxiety ratings and conversely, a positive correlation between CT-related analgesia and state calmness ratings. In other words, a state of calmness and lack of anxiety was associated with the strongest pain relief after gentle skin stroking.

In summary, we found a reduction in pain following CT optimal touch suggesting that activation of the CT system modulates pain. Touch analgesia could be potentially relevant in the clinical domain as an add-on to conventional therapeutic strategies; however, further investigations are required for this to be ascertained. The precise mechanisms are as yet unknown but possible mechanisms include pain relief through inhibition of dorsal horn nociceptive projections and/or via cortical mechanisms.

Author contributions

J.L., D.M.E., H.H.K. and L.C.L. collected and analysed the data. All authors contributed to the design of the study, interpretation of the data and writing of the paper. The final version of the paper has been approved by all authors.

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