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RESEARCH ARTICLE | *Sensory Processing*

Thermonociceptive interaction: interchannel pain modulation occurs before intrachannel convergence of warmth

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Cataldo A, Ferrè ER, Haggard P. Thermonociceptive interaction: interchannel pain modulation occurs before intrachannel convergence of warmth. *J Neurophysiol* 121: 1798–1808, 2019. First published March 13, 2019; doi:10.1152/jn.00341.2018.—Nonnoxious warmth reduces both perceived pain intensity and the amplitude of EEG markers of pain. However, the spatial properties of thermonociceptive interaction, and the level of sensory processing at which it occurs, remain unclear. We investigated whether interchannel warmth-pain interactions occur before or after intrachannel spatial summation of warmth. Warm stimuli were applied to the fingers of the right hand. Their number and location were manipulated in different conditions. A concomitant noxious test pulse was delivered to the middle finger using a CO_2 laser. We replicated the classical suppressive effect of warmth on both perceived pain intensity and EEG markers. Importantly, inhibition of pain was not affected by the location and the number of thermal stimuli, even though they increased the perceived intensity of warmth. Our results therefore suggest that the inhibitory effect of warmth on pain is not somatotopically organized. The results also rule out the possibility that warmth affects nociceptive processing after intrachannel warmth summation.

NEW & NOTEWORTHY We used spatial summation of warmth as a model to investigate thermonociceptive interactions. Painful CO_2 laser pulses were delivered during different thermal conditions. We found that warmth inhibited pain regardless of its location. Crucially, spatial summation of multiple warm stimuli did not further inhibit pain. These findings suggest that warmth-pain interaction occurs independently of or after spatial summation of warmth.

CO_2 laser-evoked potentials; conditioned pain modulation; pain inhibition; somatosensory interaction; spatial summation of warmth

INTRODUCTION

Interactions between nociception, the neural processing of noxious stimuli, and other somatosensory submodalities have received increasing attention in the last decades probably due to their potential clinical relevance in the treatment and management of pain (Kennedy et al. 2016). For example, nonnoxious tactile signals have been shown to inhibit the transmission of nociceptive information, the well-known tactile gate control (Kakigi and Shibasaki 1992; Krahé et al. 2015; Mancini et al. 2014b; Marchand et al. 1991; Melzack and Wall 1967; Moayedi and Davis 2013; Watanabe et al. 1999; Zoppi et al. 1991).

Nonnoxious warm signals can also modulate nociception: warmth increases the tolerance for pain (Casey et al. 1993; Plaghki et al. 2010) and reduces the cortical responses evoked by noxious stimuli (Tran et al. 2008; Truini et al. 2007). Similarly, both cold (Bini et al. 1984; Nahra and Plaghki 2005) and noxious signals (Davis 2013; Nir and Yarnitsky 2015; Yarnitsky 2010; Yarnitsky et al. 2010) have been reported to affect pain perception. Moreover, there is overlap between the temperature ranges at which nonnoxious warmth receptors and nociceptors respond (Chéry-Croze 1983; Plaghki et al. 2010; Schepers and Ringkamp 2010). However, in the present study we focus on the mild warmth intensity range, where nonnociceptive warm C-fibers are likely to predominate (LaMotte and Campbell 1978; Meyer and Campbell 1981). Importantly, whereas the spatial features of touch-pain interactions have been widely investigated, spatial organization of warmth-pain interactions has received less attention and remains unclear. For instance, Bini et al. (1984) investigated whether other somatosensory submodalities (i.e., vibratory, tactile, cold, and warm stimuli) might influence pain. Whereas vibrotactile inputs clearly diminished pain perception, and touch and cooling produced some pain relief, the effects of nonnoxious warmth were not clear. Furthermore, touch-pain interactions show clear somatotopic organization: nociceptive processing is modulated when the tactile and pain inputs are both delivered within the same dermatome (Kakigi and Watanabe 1996; Mancini et al. 2014b; Nahra and Plaghki 2003; Watanabe et al. 1999; Yarnitsky et al. 1997). Whereas there is both electrophysiological (Tran et al. 2008) and behavioral (Casey et al. 1993) evidence suggesting a spatially specific attenuation of pain after intersegmental and contralateral presentation of thermal stimuli, no spatially specific modulation of pain seems to occur when thermal stimulation is delivered on more distant skin regions (Price and McHaffie 1988). In fact, some authors have questioned whether thermal-nociceptive reactions have any spatial organization at all and have instead attributed spatially specific effects to general, amodal mechanisms such as distraction or

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shifts in spatial attention (Defrin et al. 2010; Quevedo and Coghill 2007a, 2007b; Van Ryckeghem et al. 2011).

On the other hand, spatial effects within the thermoceptive system alone have been extensively studied. Thermoception is strongly affected by spatial summation (Hardy and Oppel 1937; Kenshalo et al. 1967; Marks 1974; Marks and Stevens 1973; Stevens and Marks 1971). Thus perception of warmth depends not only on the physical temperature of the stimulus but also on where the thermal stimuli are applied (Defrin and Urca 1996; Hardy and Oppel 1937; Kojo and Pertovaara 1987; Machet-Pietropaoli and Chery-Croze 1979) and how many noncontiguous thermal stimuli are delivered (Hardy and Oppel 1937; Kenshalo et al. 1967; Price et al. 1989; Rózsa and Kenshalo 1977). Warmth spatial summation occurs locally when multiple nearby fibers are simultaneously activated by the warm stimulus (Greene and Hardy 1958) or even across noncontiguous skin regions (Rózsa and Kenshalo 1977). Moreover, the spatial summation varies according to the properties of the skin: compared with hairy skin, glabrous skin shows much larger magnitude of spatial summation (Defrin et al. 2009).

The level at which spatial summation of warmth occurs is not certain. Most authors suggest that warm spatial summation reflects integration of thermal information at second- and third-order neurons in the spinal cord and/or supraspinal levels (Herget et al. 1941; Price et al. 1989; Stevens et al. 1974). Moreover, it remains unclear whether thermonociceptive interactions occur before or after summation of multiple thermal inputs.

Evidence indicates that thermonociceptive interactions are complex and multilevel. In the present study, we use a paired conditioning-test stimulus paradigm to investigate thermonociceptive interactions. In particular, we focused on whether these interactions are somatotopically organized. We also investigated if interchannel thermonociceptive interactions occur before or after intrachannel spatial summation of warmth. Painful CO_2 laser pulses were delivered to the middle finger while the location and number of concurrent nonnoxious warm stimuli to the fingers were systematically manipulated to achieve different degrees of spatial summation of warmth. We tested four specific hypotheses about warmth-pain interaction, using planned comparisons motivated by established neurophysiological theories about both thermal and nociceptive channels. First, we tested the prediction of a warmth gating of pain (Casey et al. 1993; Plaghki et al. 2010; Tran et al. 2008; Truini et al. 2007), where warm stimulation on the middle finger attenuates perceived pain and nociceptive processing for a noxious laser pulse delivered to the same middle finger. A directional prediction is justified, since the literature agrees that warmth inhibits pain, and, to our knowledge, it has never been reported that innocuous warm stimulation increases pain and nociceptive processes. Second, we investigated whether the warm-inhibits-pain effect remained when the warm stimulus was delivered on the adjacent index and ring fingers while noxious stimulation was applied to the middle finger. An affirmative result would show some degree of spatial spread in warmth-pain interactions. Indeed, given the low spatial resolution (Cain 1973; Nathan and Rice 1966; Simmel and Shapiro 1969) and high spatial summation (Hardy and Oppel 1937; Marks and Stevens 1973; Stevens and Marks 1971) of the thermoceptive system, we expect a "perceptual spread of warmth" to the thermally neutral middle finger (Cataldo et al. 2016; Green 1977, 1978; Ho et al. 2011). Accordingly, Green (1978) demonstrated referred warmth on a thermally neutral finger when a thermal stimulation was applied to the adjacent finger: importantly, the neutral middle finger felt on average 54.5% less warm than the stimulated adjacent finger. Third, we tested whether the warmth gates pain in a spatially tuned fashion by contrasting pain attenuation when warmth was delivered on the same finger as noxious laser stimulation versus the situation where warmth is delivered on fingers adjacent to the noxious stimulation. Previous studies suggest that the spatial spread of warmth is partial rather than complete. For example, measures of thermal referral found that 30–60% of the warmth delivered to one finger is perceptually referred to an adjacent finger (Green 1978). Thus we hypothesized that warmth on adjacent fingers would produce less pain inhibition than warmth on the finger that receives noxious stimulation. Fourth and finally, we investigated at which level of the somatosensory processing pathway any thermonociceptive interaction occurs. If thermonociceptive interaction occurs after summation of warmth, then progressively increasing the number of fingers that are simultaneously warmed (i.e., increasing the area of thermal stimulation) while maintaining the same physical temperature on the middle finger would produce a stronger suppression of pain. Conversely, if thermonociceptive interaction occurs before or independently of warmth spatial summation, progressively increasing the number/area of warm stimulations would not affect pain processing. We therefore constructed a systematic set of stimulation conditions to test these four directional predictions.

METHODS

Participants

The sample size was calculated a priori by means of a statistical power analysis for sample size estimation based on the results of a previous EEG pilot study ($n = 10$) testing the same eight thermal conditions studied presently. The effect size for comparing the electrophysiological correlate of a painful CO_2 laser pulse during no thermal stimulation, warmth on the same finger, and warmth on the adjacent fingers in the pilot study was $\eta^2 = 0.380$, considered to be very large using Cohen's (1988) criteria. With alpha = 0.05 and power = 0.80, the projected sample size indicated for this effect is 11 participants (G*Power 3.1.9.2 software) (Faul et al. 2009). We tested 15 healthy right-handed volunteers (10 women, age 25.9 ± 4.3 yr, mean ± SD). One participant was excluded because pain threshold could not be reliably established, leaving a final sample size of 14. This gave sufficient power for the main objectives of this study. Inclusion criteria for the study were the absence of any history of previous traumatic hand injury, absence of sensitive skin or skin conditions, abstention from analgesic medication for 24 h prior to the study, and abstention from caffeinated beverages for 3 h prior to the study.

The experimental protocol was approved by the research ethics committee of University College London. Recruitment of participants and experimental procedures were conducted in accordance with the Declaration of Helsinki. All participants provided their written informed consent at the beginning of each experiment, after receiving written and verbal explanation of the purpose of the study.

Apparatus

CO_2 laser stimulation. Nociceptive stimulation was delivered on the dorsum of participants' right middle finger by a CO_2 laser

stimulator [*Laser Stimulation Device* (LSD); *SIFEC*, Ferrières, *Belgium*], controlled by a computer. The laser pulse (~100 ms) was transmitted via an optical fiber and focused by lenses to a spot diameter of ~6 mm. A radiometer collinear with the laser beam detected the skin temperature at the site of stimulation, providing safe and reproducible noxious thermal radiant stimuli at a ramping rate of ~350°C/s (Churyukanov et al. 2012; Jankovski et al. 2013).

Participants rested their right hand pronated on a molded support. Vision of the hand was blocked with a screen. The laser head was positioned above the hand, with the laser beam pointing on the dorsal aspect of the middle finger's intermediate phalanx (see Fig. 1). A visible helium-neon laser spot was used to point the CO_2 laser to the target location. To ensure a consistent stimulus location across the experiment, the target area was delimited by an ~12-mm-diameter circle drawn on the dorsum of the middle finger. Extra care was taken during the testing to prevent any laser stimulation on the skin blackened by the ink, which could affect absorption of radiant heat (Leandri et al. 2006; Madden et al. 2016). Participants wore protective goggles and were asked to maintain their gaze on a fixation cross centrally located in front of them. Intensity, duration, and timing of the CO_2 laser stimuli were controlled by computer software.

Before the beginning of each experiment, participants were familiarized with the laser stimuli, through at least three stimulations delivered at 46°C (i.e., the standard threshold for thermal pain) (Darian-Smith et al. 1979a, 1979b; LaMotte and Campbell 1978). Participants were asked to press a button with their left hand as soon as they felt any stimulation on the dorsum of the right middle finger and to verbally rate the intensity of the stimulus on a scale from 0 to 10 where 0 meant "no pain," 1 "slight pinprick," and 10 "the worst pain imaginable" (Tran et al. 2008). Participants were informed that they were not restricted to use of integers. The reports from the familiarization phase were not further analyzed.

Thermal stimulation. Thermal stimuli were applied to the volar intermediate phalanges of the right index, middle, and ring fingers by means of three 13-mm-diameter Peltier thermodes (NTE-2A; Physitemp Instruments, Clifton, NJ). The mechanical contact between all three thermodes and the corresponding digits remained constant throughout. Nonnoxious warm thermal stimulation could be delivered through any combination of the three thermodes (see Fig. 1). The thermode temperature for neutral baseline was set at 32°C. The temperature of warm stimulation was always 40°C, based on a pilot study ($n = 10$) in which we ensured that this intensity was not perceived as painful.

Before the beginning of the experiment, participants were familiarized with the warm stimuli, which were randomly applied by the thermodes on one or more fingers. Participants were asked to verbally rate the thermal sensation felt from the middle finger thermode only, on a scale from 0 to 10 where 0 meant "no warmth," 1 "barely warm," and 10 "very hot" (Tran et al. 2008). Participants were informed that they were not restricted to use of integers. The reports from this familiarization phase served to encourage participants to attend to the warmth sensation and were not further analyzed. Participants were asked to report throughout the experiment if the sensation on the fingertips was ever painful or slightly uncomfortable. No participants reported painful sensation from the thermal stimulation.

EEG recording and LEP analysis. EEG laser-evoked potentials (LEPs) are considered an objective measurement of nociception (Bromm and Treede 1987), which consists of several transient responses that are time-locked and phase-locked to the onset of painful laser stimuli (Mouraux and Iannetti 2008). EEG data were acquired from the scalp at a sampling rate of 2,048 Hz using an ActiveTwo BioSemi EEG amplifier and ActiView software (Biosemi, Amsterdam, The Netherlands). Sixteen Ag-AgCl active electrodes were positioned on the scalp according to the 10-20 International System.

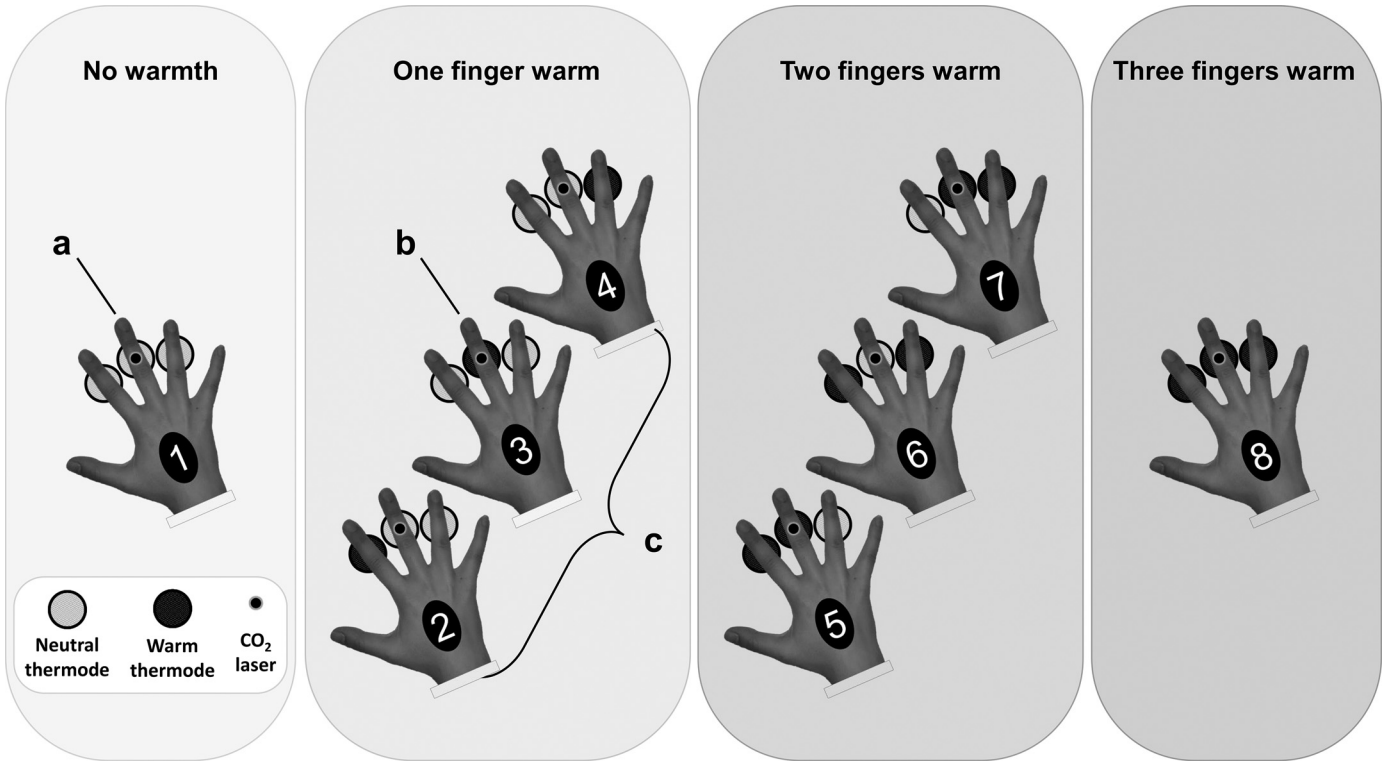

Fig. 1. Thermonociceptive conditions. Painful stimuli were delivered to the dorsum of participants' right middle finger through a CO_2 laser pulse. Thermal stimuli were delivered by three 13-mm-diameter Peltier-based thermodes applied at the level of the intermediate phalanges of right index, middle, and ring fingers. Warm stimulation was given in 8 different conditions (*conditions 1–8* indicated by numbers). We then contrasted combinations of conditions to test 4 directional hypotheses regarding thermonociceptive interactions (see METHODS): *a*, no warmth, laser only condition; *b*, warmth and laser pain on the middle finger; and *c*, laser pain on the middle finger and warmth on the index or ring finger (i.e., adjacent fingers condition).

Electroconductive gel was used to keep the impedance of all electrodes <5 kΩ throughout the experiment. An external electrode placed on the nose was used as reference. Electrooculographic signals for eye movements and eye-blink monitoring were simultaneously recorded.

EEG data were processed using EEGlab (Delorme and Makeig 2004) running on MATLAB. Continuous raw data for each participant in each block were recorded and stored on ActiView and successively imported on EEGlab for off-line analysis. Data were resampled to 250 Hz and then bandpass filtered between 1 and 30 Hz. EEG epochs were extracted from the continuous data using a window analysis time of 3,000 ms (from −1,000 to 2,000 ms relative to the CO_2 laser pulse). The mean signal immediately preceding the laser stimulus (from −500 to 0 ms) was set as baseline and removed from each epoch. Artifacts originating from eye blinks and ocular movements were identified and pruned by means of independent component analysis (Delorme and Makeig 2004; Jung et al. 2001; Makeig et al. 1997). For each participant, all the independent components representing artifacts or noncortical processes, such as eye movements or facial muscle activity, were manually selected and rejected. The criteria for the identification of muscular artifacts were based on each component's scalp topography, power spectrography, intertrial coherency, and intratrial time course.

LEP data analysis was computed on the signal recorded at the vertex (electrode Cz) referenced to the nose. Epochs from each specific experimental condition were averaged within participants and time-locked to the onset of the CO_2 laser pulse. The main negative (N2 wave) and positive (P2 wave) vertex components associated with LEPs were then identified and selected on the basis of their latency and polarity. N2 and P2 components were defined as the most negative and positive biphasic deflections between 150 and 500 ms after stimulus onset (Hu et al. 2014; Iannetti et al. 2008). The peak amplitude of these components was used for statistical analysis.

Experimental design and procedure. We designed a within-subject paradigm where participants' magnitude estimates of pain and LEP amplitudes were tested in a series of planned comparisons involving eight different thermal conditions (see Fig. 1). In *condition 1*, noxious CO_2 laser pulses were delivered to the middle finger in the absence of any thermal stimulation, providing a baseline measure of pain perception. In the remaining conditions, the site of thermal stimulation (index, middle, or ring finger: *condition 2*, *condition 3*, or *condition 4*, respectively) and the number of thermally stimulated fingers (one: *conditions 2–4*; two: *conditions 5–7*; or three: *condition 8*) were systematically manipulated to produce different levels of spatial summation of warmth.

The experiment took place in a temperature-controlled room at 23°C. The superficial skin temperature of the hand dorsum was systematically measured at several points during the experiment by means of an infrared thermometer (Precision Gold N85FR; Maplin, London, UK) and was kept between 28°C and 32°C (mean baseline temperature: 30 ± 1.4°C). First, laser-induced pain thresholds were established through an adaptive psychophysical staircase procedure: the first stimulus of the staircase was set at 40°C, and the intensity of the following stimuli was adaptively changed according to participants' reaction times (RTs) to the CO_2 laser stimulation (Arendt-Nielsen and Bjerring 1988; Mancini et al. 2014a). An RT criterion of 650 ms was used to discriminate between C-fibers (≥650 ms) and Aδ-fibers (<650 ms) (Churyukanov et al. 2012; Jankovski et al. 2013). If the RT to the preceding stimulus was ≥650 ms, the laser intensity of the next stimulus was increased until the RT fell below 650 ms, producing the first reversal. Conversely, if the RT to a stimulus were <650 ms, the laser intensity of the upcoming stimulus was decreased. The step size of the staircase was progressively reduced after each reversal, from 4°C, to 2°C, and finally 1°C. After the third reversal, any intensity producing an Aδ-like response (RT < 650 ms) was repeated three times. The pain threshold was defined as the lowest laser intensity inducing two out of three consecutive Aδ-like responses.

After pain thresholds were established, the EEG cap was mounted and the experiment began. Participants completed eight blocks of 16 trials each. In each block, the eight different thermal conditions described above (see Fig. 1) were presented twice, in a fully randomized order, giving a total of 128 trials. To ensure attention to the stimuli, a beep signaled the beginning of each trial. Before and after the trial, the temperature of the thermodes was set at 32°C. After the beep, the thermal stimulation on the designated finger/s ramped up to 40°C at a rate of ~2°C/s and remained steady for the entire duration of the trial. After a random delay from the beginning of the thermal stimulation (5-6 s), a 100-ms CO_2 laser pulse was delivered to the dorsum of the right middle finger. The intensity of the laser stimulation for each participant was set at the individual pain threshold +6°C and remained fixed throughout the entire experiment. Participants were asked to maintain gaze on a central fixation cross placed in front of them and to attend to the thermal and laser stimuli. After 3 s, a further beep occurred, and participants verbally rated the intensity first of warmth and then of pain, providing a number from 0 to 10 for each sensation based on the initial training with these scales (see above). For example, if the subject said "3, 5," that meant their rating was 3 for the perceived warmth on the middle finger and 5 for laser pain on the same finger (Tran et al. 2008). To prevent any possible effect of sensitization or habituation of the thermoreceptors/nociceptors at the site of stimulation (Iannetti et al. 2004; Kleinböhl et al. 2006), the intertrial interval varied randomly between 12 and 27 s, and the position of the laser beam on the finger was adjusted slightly between trials.

Statistical Analysis

Behavioral and EEG data were analyzed using SPSS software (SPSS Statistics for Windows, version 22.0; IBM, Armonk, NY). Our experimental design aimed to address four independent research questions to investigate the spatial and summative properties of warmth-nociceptive interaction (see Table 1). We therefore used a priori planned comparisons between specific experimental conditions, as follows. First, to test whether warmth inhibits pain delivered at the same skin site (Casey et al. 1993; Plaghki et al. 2010; Tran et al. 2008;

Table 1. *Coefficients for the four research questions*

	Thermal Conditions							
Question	No warmth	Index warm	Middle warm	Ring warm	Index+Middle warm	Index+Ring warm	Middle+Ring warm	All warm
1) Does warmth on the same finger inhibit pain?	−1	0	1	0	0	0	0	0
2) Does warmth on the adjacent fingers inhibit pain?	1	−1/2	0	−1/2	0	0	0	0
3) Is the effect of warmth on pain spatially specific?	0	−1/2	1	−1/2	0	0	0	0
4) Does warmth summation cause graded inhibition?	n/a	−1/3	−1/3	−1/3	0	0	0	1

Data are the coefficients used to test the four research questions used to investigate the spatial and summative properties of warmth-nociceptive interaction (see METHODS, *Statistical Analysis*, for explanation).

Truini et al. 2007), we compared *condition 1* (no thermal stimulation) with *condition 3* (warmth on the same finger). Second, to test whether warmth on adjacent fingers (Cataldo et al. 2016; Green 1977, 1978; Ho et al. 2011) could similarly inhibit pain, we compared *condition 1* (no thermal stimulation) with the average of *conditions 2* and *4* (warmth on adjacent index/ring fingers). We found no statistical evidence for perceptual differences between these fingers when stimulated alone ($P > 0.200$ for all variables studied), vindicating our a priori decision to average over stimulations across index and ring fingers. Third, to test whether the warmth-pain interaction is spatially specific, we compared pain inhibition in *condition 3* (warmth on the same finger) with the average of *conditions 2* and *4* (warmth on index/ring finger; i.e., adjacent fingers) (see *question 3* in Table 1 for the coefficient used for the comparison). Finally, to test the effect of progressive spatial summation of multiple simultaneous thermal stimuli, we performed a linear trend analysis, with weights −1, 0, and 1 for the conditions where warmth was applied on one (average *of conditions 2*, *3*, and *4*), two (average of *conditions 5*, *6*, and *7*), or three fingers (*condition 8*) (Hays 1994; Mancini et al. 2014b). Because all our hypotheses are unidirectional and supported by previous evidence (Cataldo et al. 2016; Green 1977, 1978; Ho et al. 2011; Plaghki et al. 2010; Tran et al. 2008; Truini et al. 2007), we used one-tailed paired-samples *t*-tests throughout. Statistical tests were considered significant if $P < 0.05$. Nonsignificant results were further investigated through Bayesian one-sample *t*-test analyses, using JASP (version 0.8.0.1; JASP Team 2016, University of Amsterdam) to determine whether results supported the null hypothesis or, alternatively, could reflect insufficient statistical power (Rouder et al. 2009; Wetzels and Wagenmakers 2012). EEG data were tested for normal distribution using the Kolmogorov-Smirnov normality test (see Supplemental Table S1 at https://doi.org/10.6084/m9.figshare.7808420.v2). Of the six Kolmogorov-Smirnov tests, only one showed significant nonnormality, due to a single outlier. Because within-subject ANOVA is relatively robust to violations of the normality assumption (Boneau 1960), we decided not to remove outliers or transform data.

RESULTS

Detailed LEP analysis is reported in Supplemental Fig. S1 (https://doi.org/10.6084/m9.figshare.7808420.v2). Means and SD of subjective ratings and LEPs are described in Supplemental Table S2 (https://doi.org/10.6084/m9.figshare.7808420.v2).

Planned Comparison 1: Does Warmth Inhibit Pain Delivered to the Same Finger?

We first compared warmth magnitude estimates between *condition 1* (no thermal stimulation) and *condition 3* (warmth on the middle finger). As predicted, ratings of warmth were significantly higher when the thermal stimulus was presented on the middle finger (*condition 3*, 2.82 ± 1.512) than during the no-warmth condition (*condition 1*, 0.54 ± 0.608) [$t_{13} = -6.158$, $P < 0.001$; 95% confidence interval (CI): $-\infty$, −1.625; Cohen's $d = 2.148$; Fig. 2*A*].

Second, to investigate the effect of warmth on colocated pain, we performed planned comparisons on both perceptual and electrophysiological responses to pain. A planned comparison on the magnitude estimates of pain showed that participants' pain rating during the no-warmth condition (*condition 1*, 3.2 ± 1.354) significantly decreased by 11.6% when a concomitant thermal stimulation was delivered on the same finger (*condition 3*, 2.83 ± 1.007) ($t_{13} = 2.106$, $P = 0.028$; 95% CI: 0.061, +∞; Cohen's $d = 0.314$; see Fig. 2*B*). Concomitant warmth had a modulatory effect on the N2, but not on the P2

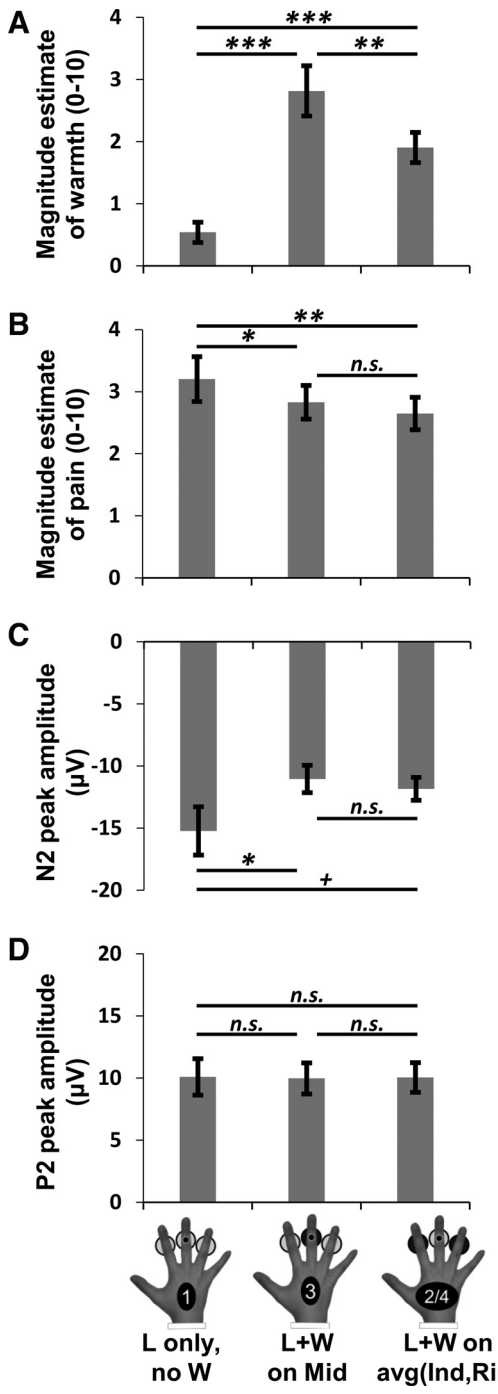

Fig. 2. Effect of location of thermal stimulation on warmth (W) and laser pain (L) processing. *A*: magnitude estimate of warmth. Compared with the laser-only (no warmth) condition, participants perceived higher intensities of warmth in both thermal conditions (same/adjacent finger). Crucially, perceived warmth on the middle finger was significantly higher when the thermal stimulus was delivered on the middle finger itself (Mid), rather than on an adjacent finger [average of index (Ind) and ring (Rin) fingers]. *B*: magnitude estimate of pain. Pain perception was significantly reduced in both thermal conditions (same/adjacent finger/s) compared with no thermal stimulation. However, same and adjacent finger conditions were not statistically different. *C*: N2 wave. Peak amplitude of N2 component was significantly reduced in both thermal conditions compared with no thermal stimulation. However, the amount of pain suppression was the same irrespective of the site of stimulation. *D*: P2 wave. P2 component was not affected by either of the thermal conditions. Values are means; error bars are SE. Numbers indicate *conditions 1–8* (see METHODS). n.s., $P > 0.05$; $+P = 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

component (see Fig. 2, *C* and *D*). The peak amplitude of the N2 wave was significantly higher when pain was delivered in the absence of warmth (*condition 1*, -15.23 ± 7.282) than when a thermal stimulus was simultaneously presented on the same finger (*condition 3*, -11.06 ± 4.137) ($t_{13} = -2.13$, $P = 0.027$; 95% CI: $-\infty$, -0.723; Cohen's $d = 0.730$; see Fig. 2*C*). This reduction corresponded to a relative change of 27.4%. The P2 wave did not show any significant modulation ($t_{13} = 0.116$, $P = 0.455$; 95% CI: -1.875, $+\infty$; Cohen's $d = 0.026$). A Bayesian paired-samples *t*-test supported the null result ($BF_{01} = 4.026$, error $< 0.001\%$), suggesting that this result was not due to a lack of statistical power (Rouder et al. 2009; Wetzels and Wagenmakers 2012). Dissociations between N2 and P2 components have been previously reported (Tran et al. 2008). Thus both behavioral and electrophysiological correlates of pain were attenuated by a concomitant warm stimulus delivered to the same finger.

Planned Comparison 2: Does Warmth Inhibit Pain Delivered on an Adjacent Finger?

A direct comparison between ratings of warmth in *condition 1* (no thermal stimulation) and the average of *conditions 2* and *4* (warmth on the adjacent fingers) was significant ($t_{13} = -8.476$, $P < 0.001$; 95% CI: $-\infty$, -1.080; Cohen's $d = 1.797$) with participants rating warmth on the middle finger as significantly higher when the thermal stimulus was presented on the adjacent fingers (average of *conditions 2* and *4*, 1.9 ± 0.909) than during the no-warmth condition (*condition 1*, 0.54 ± 0.608) (see Fig. 2*A*).

The planned comparison between participants' pain ratings during no-warmth (*condition 1*) and warmth on the adjacent fingers (average of *conditions 2* and *4*) was statistically significant ($t_{13} = 4.184$, $P = 0.001$; 95% CI: 0.321, $+\infty$; Cohen's $d = 0.474$). Baseline pain on the middle finger (3.2 ± 1.354) dropped by 17.3% when a warm stimulus was delivered to either of the adjacent fingers (2.647 ± 0.983) (see Fig. 2*B*). The subjective perception was supported by a decrease of 22.2% in the amplitude of the N2 component (see Fig. 2*C*). This effect did not formally reach the conventional boundaries for statistical significance ($t_{13} = -1.769$, $P = 0.050$; 95% CI: $-\infty$, 0.016; Cohen's $d = 0.629$). However, a Bayesian paired-samples *t*-test showed that it is very unlikely that this result could be explained by the null hypothesis ($BF_{01} = 0.572$, error $< 0.001\%$). The amplitude of the P2 component was not modulated by warmth ($t_{13} = 0.043$, $P = 0.483$; 95% CI: -1.767, $+\infty$; Cohen's $d = 0.009$; $BF_{01} = 3.822$, error $< 0.001\%$). Warmth delivered on an adjacent finger had a significant suppressive effect on pain perception and LEPs.

Planned Comparison 3: Is the Suppressive Effect of Warmth on Pain Spatially Graded?

The previous results showed that a warm stimulus delivered onto either the same or an adjacent finger was able to reduce both the subjective perception of pain and the amplitude of the N2 LEP component associated to it. We conducted a further planned comparison on the same (*condition 3*) and adjacent fingers (average of *conditions 2* and *4*) to investigate whether this inhibitory effect of warmth on pain was spatially graded.

Importantly, although perceived warmth between same and adjacent fingers was significant ($t_{13} = 3.267$, $P = 0.003$; 95% CI: 0.420, $+\infty$; Cohen's $d = 0.754$; see Fig. 3*A*), neither magnitude estimates of pain ($t_{13} = 1.441$, $P = 0.087$; 95% CI: $-\infty$, 0.407; Cohen's $d = 0.184$) nor LEP amplitudes (N2: $t_{13} = 0.967$, $P = 0.176$; 95% CI: -0.654, $+\infty$; Cohen's $d =$

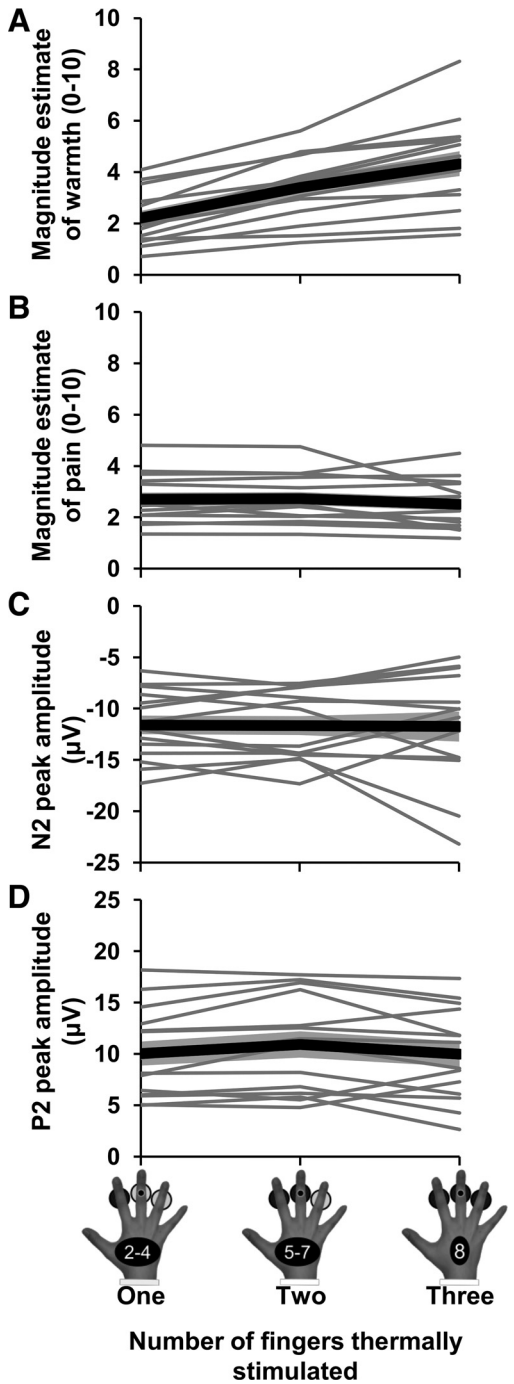

Fig. 3. Effect of number of thermal stimuli on warmth perception (*A*), pain perception (*B*), and N2 (*C*) and P2 (*D*) laser-evoked potential (LEP) components. *A*: magnitude estimate of warmth. Increasing the number of fingers thermally stimulated induced a significant ($P < 0.001$) monotonic increase in the apparent intensity of warmth on the middle finger. However, neither perceptual (*B*) nor electrophysiological (*C* and *D*) correlates of pain were affected by the number of simultaneous thermal stimulations. Gray lines represent data from single participants. Solid black lines represent the average across participants, and gray shading represents SE. Numbers indicate *conditions 1–8* (see METHODS).

0.209; P2: $t_{13} = -0.13$, $P = 0.449$; 95% CI: $-\infty$, 1.102; Cohen's $d = 0.019$) were significantly different in the two thermal conditions (see Fig. 2, *B–D*). Although the Bayesian analysis of the behavioral data was inconclusive ($BF_{01} = 0.881$, error $< 0.001\%$), analyses of the LEP data strongly favored the null hypothesis (N2: $BF_{01} = 6.521$, error $< 0.001\%$; P2: $BF_{01} = 3.358$, error $< 0.001\%$). Therefore, perceptual and electrophysiological correlates of pain were not statistically different when a warm stimulus was delivered to the same finger or to the adjacent fingers.

Planned Comparison 4: Does Warmth Summation Cause Graded Inhibition?

To test whether spatial summation increases with number of thermal stimuli, we performed a linear trend analysis on warmth intensity ratings during single (average of *conditions 2*, *3*, and *4*), double (average of *conditions 5*, *6*, and *7*), and triple finger stimulation (*condition 8*). As expected, warmth perception on the middle finger parametrically increased along with the number of stimulated fingers ($t_{13} = 7.728$, $P < 0.001$; 95% CI: 1.465, $+\infty$; Cohen's $d = 4.129$). Thermal stimulation on the middle finger was rated lower when one finger was stimulated (2.21 ± 1.034) and linearly increased when two fingers (3.4 ± 1.304) and three fingers (4.33 ± 1.801) were simultaneously stimulated (see Fig. 3*A*).

To test whether spatial summation of multiple simultaneous thermal stimuli had a graded inhibitory effect on pain processing, we conducted a linear trend analysis with weights -1, 0, and 1 on the conditions where warmth was applied on one (average of *conditions 2*, *3*, and *4*), two (average of *conditions 5*, *6*, and *7*), or three fingers (*condition 8*). The analyses showed no effect of spatial summation of warmth on either pain perception ($t_{13} = -1.22$, $P = 0.141$; 95% CI: $-\infty$, 0.104; Cohen's $d = 0.653$) or LEPs (N2: $t_{13} = -0.158$, $P = 0.438$; 95% CI: -1.882, $+\infty$; Cohen's $d = 0.085$; P2: $t_{13} = -0.115$, $P = 0.455$; 95% CI: $-\infty$, 0.687; Cohen's $d = 0.062$). Increasing the number of simultaneous thermal stimuli did not affect subjective perception of pain (1 finger: 2.708 ± 0.965; 2 fingers: 2.732 ± 0.949; 3 fingers: 2.509 ± 0.965; see Fig. 3*B*) or the amplitude of N2 (1 finger: -11.59 ± 3.404; 2 fingers: -11.66 ± 3.458; 3 fingers: -11.74 ± 5.458) or P2 (1 finger: 10.02 ± 4.374; 2 fingers: 10.93 ± 4.777; 3 fingers: 9.97 ± 4.536) LEP components (see Fig. 3, *C* and *D*).

We then performed a Bayesian analysis to determine whether the data supported the null hypothesis or could be due to a lack of statistical power. We found that the null hypothesis was always more than three times more likely than the alternative hypothesis (magnitude estimates of pain: $BF_{01} = 7.208$, error $< 0.001\%$; N2: $BF_{01} = 3.284$, error $< 0.001\%$; P2: $BF_{01} = 4.021$, error $< 0.001\%$), suggesting that the absence of a linear trend among conditions with increasing number of thermal stimuli was not simply due to a lack of statistical power. Therefore, perception and EEG markers of pain were not affected by different amounts of spatial summation of warmth.

DISCUSSION

In the present study we investigated the spatial properties of warmth-pain interaction and the level of somatosensory processing at which this sensory interaction takes place. We exploited, seemingly for the first time, the properties of spatial summation of warmth to modulate perception of warmth without modifying skin temperature at a given target location. We manipulated the number/area and the location of warm thermal stimuli during concomitant noxious laser stimulation. Our results replicated the well-known suppressive effect of warmth on pain processing observed in previous studies (Casey et al. 1993; Plaghki et al. 2010; Tran et al. 2008; Truini et al. 2007). Specifically, ongoing thermal stimulation induced a significant attenuation of both subjective (magnitude estimates) and objective (LEPs) correlates of laser-induced pain. Warmth had similar inhibitory effects on pain not only when the two stimuli were delivered to the same finger but also when they were located on adjacent fingers. Thus thermal inhibition of pain did not require strict spatial coincidence. This suggests that effect of warmth on nociceptive pathways and pain perception does not follow a strongly somatotopic gradient.

Moreover, we found no evidence that the number/area of warm stimuli influenced either pain ratings or LEP amplitudes. Thus delivering warmth to one, two, or three digits did not linearly modulate pain sensation evoked by laser stimulation. This results thus rules out a model in which warm inputs first undergo spatial summation, followed by a subsequent suppressive effect of the total warm signal on nociception. That model would predict a linear decreasing trend in pain ratings and LEP amplitudes as the number/area of warm stimuli increased, since this would have produced a stronger, summated warm signal that might potentially inhibit nociceptive signaling. Our linear trend analysis clearly showed that although thermal perception was strongly affected by the number of simultaneous stimuli presented, neither perceptual nor electrophysiological correlates of pain delivered during thermal stimulation followed this trend. In fact, using Bayesian methods, we found statistical evidence that no such trend existed. Summation of warmth did not influence the degree of pain suppression. We therefore conclude that the modulation of nociception by warmth occurs either before or independently of intrachannel spatial summation of multiple thermal inputs.

Spatial Organization of Warmth-Pain Interaction

Previous works have investigated the spatial gradient of thermonociceptive interaction (Casey et al. 1993; Price and McHaffie 1988; Tran et al. 2008). These studies suggested that warmth-pain interaction is nonsomatotopic. Tran et al. (2008) systematically manipulated the site of thermal stimuli presented during painful electrical pain stimulation. Their data showed that the cortical response associated with pain-related Aδ-fibers was equally affected by warmth C-fiber conditioning at intrasegmental, intersegmental, and even contralateral stimulation sites (Tran et al. 2008), suggesting a diffuse, rather than spatially dependent, interaction mechanism. Although their study used intraepidermal nociceptive stimulation, in contrast to the laser stimulation used in our study, we also did not observe any difference in the modulation of pain when the thermal and noxious stimuli were presented on different fingers. As a consequence, a strictly somatotopic account of warmth-pain interaction can be ruled out.

One possible limitation of this study is that the effect of spatial summation was investigated only across digits, rather than across more distant body parts. Previous studies have

shown that inhibitory interactions between multiple nociceptive stimuli occur across the whole body (Le Bars 2002; Le Bars et al. 1979b, 1979a; Villanueva and Le Bars 1995; Yarnitsky 2010; Yarnitsky et al. 2010). Additionally, we have only tested glabrous skin. We cannot exclude different patterns of warm-nociceptive interaction in glabrous and hairy skin, due either to differences in innervation density or to factors such as skin thickness and heat transfer. Therefore, further studies could address whether thermonociceptive interactions also occur on a larger scale and on both glabrous and hairy skin. Given the different innervation territories and segmental projections of the median and ulnar nerves, one may expect that warmth delivered on the index vs. the ring finger might show different interactions with pain delivered on the middle finger (Fardo et al. 2018). However, although this hypothesis would predict a significant difference in pain ratings and/or LEPs between our *condition 2* (warmth on the index finger) and *condition 4* (warmth on the ring finger), we found no evidence for any difference in sensory ratings or LEPs ($P > 0.200$ in all cases). This is in line with previous studies (Green 1978; Marotta et al. 2015) showing that the differing segmental projections of medial and ulnar nerves have little to no effect on interactions between simultaneous thermal or thermotactile stimuli. Finally, although we assume that warmth-induced pain relief reflects a central interaction, we cannot entirely exclude a contribution of some unknown peripheral interactions (e.g., through vascular effects). However, the fact that we delivered warm stimuli on the fingertips and laser pain on the middle finger dorsum makes explanations based on local peripheral changes unlikely.

Spatial Summation of Warmth During Warmth-Pain Interaction

Magnitude estimate of warmth delivered to the middle finger was heavily dependent on the number of warm stimuli presented at the same time on adjacent fingers, supporting evidence for a spatial summation of warmth (Hardy and Oppel 1937; Kenshalo et al. 1967; Marks 1974; Marks and Stevens 1973; Stevens and Marks 1971). However, this increase in the perceived intensity of warmth did not produce a linear decrease in the perceived pain as well as in LEP amplitudes. Thus interaction between warmth and pain may involve a binary, rather than proportional, inhibitory mechanism. Interchannel interaction between warmth and pain, then, must be mediated through a widely distributed, nonsomatotopic, all-or-nothing mechanism. This interaction mechanism would be independent from the intrachannel convergence and summation that characterizes purely thermal inputs. If warmth-pain interaction occurs subsequently to spatial summation, the stronger thermal signal that we observed for more numerous warm stimuli should produce a stronger suppression of nociceptive information.

Tran et al. (2008) showed that the physical intensity of a thermal stimulus affects nociceptive processing in a graded manner: the Aδ-mediated cortical responses induced by electrical epidermal stimulation were much more attenuated by a 50°C than by a 37°C C-fiber conditioning stimulus. This suggests that spatial summation-induced increases in perceived warmth might produce a similar monotonic, progressive reduction of pain and nociceptive cortical responses. Conversely, our findings clearly show that warmth-pain interaction is an all-or-nothing phenomenon. Neither pain ratings nor LEPs showed progressive modulation by increasing levels of perceived warmth.

When warm stimulation is applied on the index and/or ring finger of one hand, an illusory perception of warmth occurs on the thermally neutral middle finger (Cataldo et al. 2016; Green 1977, 1978; Ho et al. 2011). This phenomenon, known as thermal referral, has been linked to spatial summation mechanisms occurring within the thermoceptive system (Cataldo et al. 2016). In the present study, when a single adjacent (index or ring) finger was thermally stimulated, ratings of warmth on the middle finger were significantly higher than in the no-warmth condition. Although the thermal state of the middle finger was in fact neutral in each of these conditions, all participants reported higher perception of warmth during the thermal referral condition compared with no thermal stimulation. This indicates that an illusory spread of perceived warmth across digits also occurred in our paradigm.

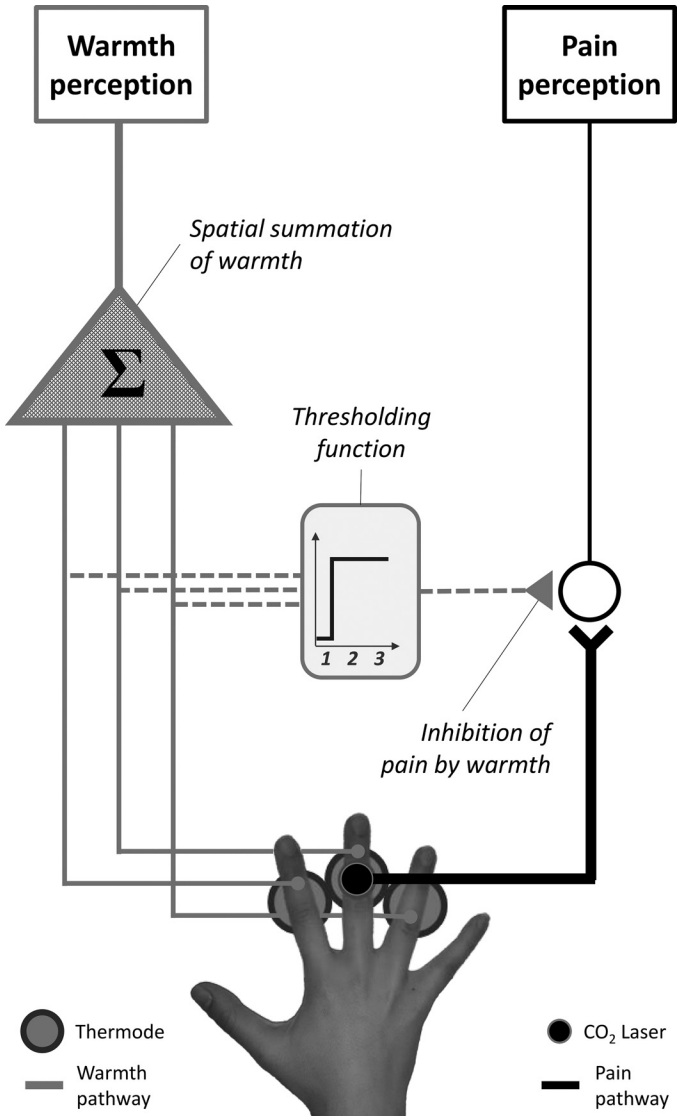

Fig. 4. Schematic model of warmth-pain interaction. Our results suggest that the interchannel interaction between warmth and pain occurs before or independently of intrachannel convergence and summation of warmth.

Mechanisms Underlying Warmth-Pain Interaction

Different theories have been proposed to explain thermonociceptive interactions. Based on the finding that higher intensity stimulation to one pathway produces a stronger inhibitory effect on the other, Truini et al. (2007) proposed that the Aδ-C interaction is based on a first come, first served principle, where only the earliest signals can induce cerebral responses. LEPs would then reflect the output of a network detecting rapid temporal changes in firing relative to a preceding state (Garcia-Larrea 2004; Truini et al. 2007). A similar conclusion in the spatial domain has been proposed by Churyukanov et al. (2012), who postulated that Aδ-fibers act as local change detectors, rather than pure level detectors. The threshold for Aδ-fiber input would depend not only on the physical energy applied but also on the background input from C-fibers innervating the skin surrounding the stimulated area.

Our findings that behavioral and electrophysiological correlates of pain are not affected by spatial summation of warmth do not contradict, but rather extend, the previous models, by showing that the temporal contrast mechanism described by Truini et al. (2007) takes place at early stages of thermonociceptive processing. That is, pain modulation occurs before multiple warmth sources are spatially summated into an illusory percept of increased apparent warmth (see Fig. 4). In contrast, a model based on strictly peripheral spatial change detection cannot readily explain our results. This model would predict the strongest Aδ response (i.e., higher pain levels) when C-fiber firing from the same immediate area is lowest. In our design, this would imply lower pain ratings when warmth was delivered on the same finger as pain, and higher pain ratings when warmth was delivered on the adjacent fingers. Yet, we observed a strong pain suppression for the middle finger also when the index and ring fingers received warmth. Therefore, sensory mechanisms located at higher levels than those detecting the relative firing rate between digit-specific Aδ and C afferent fibers must underlie the suppression of pain by warmth.

Noticeably, our results do recall another well-known phenomenon, called diffuse noxious inhibitory control (DNIC) in the animal literature (Le Bars et al. 1979a, 1979b; Villanueva and Le Bars 1995) and conditioned pain modulation (CPM) in human studies (Davis 2013; Nir and Yarnitsky 2015; Yarnitsky 2010; Yarnitsky et al. 2010). CPM has been described as a specific nociceptive mechanism where "pain inhibits pain" and seems relevant for our results in two key ways. First, it has been consistently shown that the inhibitory effect of "pain on pain" applies across the whole body, without apparent somatotopic spatial gradients (Le Bars 2002; Le Bars et al. 1979b, 1979a; Villanueva and Le Bars 1995; Yarnitsky 2010; Yarnitsky et al. 2010). Second, Granot et al. (2008) also demonstrated that once the analgesic effect on a test pain stimulus was evoked by a required degree of conditioning painfulness, no further suppression occurred when the intensity of the conditioning stimulus was increased. This led to the interpretation that the CPM is an all-or-nothing mechanism, rather than a graded phenomenon, where the ascending activity in the spinal pain tracts is sufficient to activate a descending modulatory response regardless of whether the final cortical experience induced by that barrage is painful or not (Granot et al. 2008). Our results suggest that these key properties of CPM, namely, nongradedness and lack of spatial specificity, also apply to the "warmth inhibits pain" interaction. Similarly to CPM, warmth-related thermoceptive channels may interact with nociceptive pathways through an endogenous descending modulatory system, possibly originating in the brain stem (Granot et al. 2008).

Conclusion

Our study suggests four main results. First, behavioral and electrophysiological correlates of pain are attenuated by concomitant nonnoxious warm stimulation delivered to the same finger. Second, pain is also inhibited when warmth is delivered to an adjacent finger, suggesting that interaction between warmth and pain occurs through a mechanism that is not strictly somatotopic. Third, warmth on adjacent fingers produces as much pain inhibition as warmth on the finger that receives noxious stimulation, suggesting that the warmth-pain interaction is not spatially graded. Fourth, the analgesic effect of warmth does not have a direct proportional relationship with the magnitude of perceived warmth. In particular, increases in perceived warmth induced by spatial summation do not produce additional inhibition of pain levels evoked by noxious laser stimulation, nor of cortical responses to the noxious laser stimulus. Therefore, the interaction between warmth and nociceptive modalities is independent from the convergence and summation taking place within the warm channel. This might have important clinical implications, providing a novel approach for the treatment and management of pain involving nonnoxious thermal stimulation.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

A.C. and P.H. conceived and designed research; A.C. performed experiments; A.C. and E.R.F. analyzed data; A.C., E.R.F., and P.H. interpreted results of experiments; A.C. prepared figures; A.C. drafted manuscript; A.C., E.R.F., and P.H. edited and revised manuscript; A.C., E.R.F., and P.H. approved final version of manuscript.

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