Thermo-nociceptive interaction: inter-channel pain modulation occurs before intra-channel convergence of warmth

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Contribution

AC and PH designed the experiment. AC collected the data. AC and ERF analyzed the data. All the authors wrote the manuscript.

Running Head

Warmth-pain interaction occurs prior to warmth summation

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Abstract

Non-noxious warmth reduces both perceived pain intensity, and the amplitude of EEG markers of pain. However, the spatial properties of thermo-nociceptive interaction, and the level of sensory processing at which it occurs remain unclear. Here, we investigated whether *inter-channel* warmth-pain interactions occur before or after *intra-channel* 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 pain perceived 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. They also rule out the possibility that warmth affects nociceptive processing after intra-channel warmth summation.

Keywords

Somatosensory interaction, spatial summation of warmth, pain inhibition, CO_2 laser evoked potentials, conditioned pain modulation

New & Noteworthy

We used spatial summation of warmth as a model to investigate thermo-nociceptive 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 or after spatial summation of warmth.

Introduction

Interactions between *nociception*, the neural processing of noxious stimuli, and other somatosensory sub-modalities 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, non-noxious 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).

Non-noxious *warm* signals can also modulate nociception: warm 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 non-noxious warmth receptors and nociceptors respond (Chéry-Croze 1983; Plaghki et al. 2010; Schepers and Ringkamp 2010). However, here we focus on the mild warmth intensity range, where non-nociceptive C-warm fibers are likely to predominate (LaMotte and Campbell 1978; Meyer and Campbell 1981). Importantly, while 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 sub-modalities (i.e. vibratory, tactile, cold, and warm stimuli) might influence pain. While vibrotactile inputs clearly diminished pain perception, and touch and cooling produced some pain relief, the effects of non-noxious warmth were not clear. Further, 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; Yarnitskya et al. 1997). While there is both electrophysiological (Tran et al. 2008) and behavioral (Casey et al. 1993)

evidence suggesting a spatially-specific attenuation of pain after inter-segmental 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 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) summation. Thus, perception of warmth does not only depend on the physical temperature of the stimulus, but also by 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 by how many non-contiguous 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 non-contiguous skin regions (Rózsa and Kenshalo 1977). Moreover, the spatial summation varies according to the properties of the skin: compared to 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 supra-spinal levels (Herget et al. 1941; Price et al. 1989; Stevens et al. 1974). Moreover, it remains unclear whether thermo-nociceptive interactions occur before or after summation of multiple thermal inputs.

Evidence indicates that thermo-nociceptive interactions are complex and multi-level. Here, we use a paired conditioning-test stimulus paradigm to investigate thermo-nociceptive interactions. In particular, we focused on whether these interactions are somatotopically

organized. We also investigated if inter-channel thermo-nociceptive interactions occur before or after intra-channel spatial summation of warmth. Painful CO_2 laser pulses were delivered to the middle finger, while the location and number of concurrent non-noxious warm stimuli to the fingers was systematically manipulated to achieve different degrees of spatial summation of warmth. We tested four specific hypotheses about warm-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 warm-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 warm on the finger that receives noxious stimulation. Fourth and finally, we investigated at which level of the somatosensory processing pathway, any thermo-nociceptive interaction occurs. If thermal-nociceptive 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 thermo-nociceptive interaction occurs *before* or independently of warm 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 here. 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 an 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 females, mean age ± SD: 25.9 ± 4.3 years). One participant was excluded because pain threshold could not be reliably established, leaving a final sample 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 hours prior the study, and abstention from caffeinated beverages for three hours 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, SIFEC, Belgium), controlled by a computer. The laser pulse (~100ms) was transmitted via an optical fiber and focused by lenses to a spot diameter of ~6mm. 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 Figure 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 a ~12mm 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.

Prior to the beginning of each experiment, participants were familiarized with the laser stimuli, through at least 3 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 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 13mm-diameter Peltier thermodes (Physitemp Instruments Inc, NTE-2A, New Jersey, USA). The mechanical contact between all three thermodes and the corresponding digits remained constant throughout. Non-noxious warm thermal stimulation could be delivered through any combination of the three thermodes (see Figure 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 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 2048 Hz using an Active Two 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. Electro-conductive gel was used to keep the impedance of all electrodes < 5kΩ throughout the experiment. An external electrode placed on the nose was used as reference. Electrooculographic signals (EOG) for eye movements and eye-blinks 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 250Hz, and then bandpass filtered between 1Hz and 30Hz. EEG epochs were extracted from the continuous data using a window analysis time of 3000ms (from -1000ms to 2000ms relative to the CO_2 laser pulse). The mean signal immediately preceding the laser stimulus (from -500ms to 0ms) was set as baseline and removed from each epoch. Artefacts originating from eye-blinks and ocular movements were identified and pruned by means of Independent Component Analysis (ICA) (Delorme and Makeig 2004; Jung et al. 2001; Makeig et al. 1997). For each participant, all the independent components representing artefacts or non-cortical processes, such as eye movements or facial muscle activity were manually selected and rejected. The criteria for the identification of muscular artefacts were based on each component's scalp topography, power spectrography, inter-trial coherency, and intra-trial time course.

Laser-evoked potentials (LEPs) data analysis were 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. Then, the main negative (N2 wave) and positive (P2 wave) vertex components associated with LEPs were 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 150ms and 500ms 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 LEPs amplitudes were tested in a series of planned comparisons involving eight different thermal conditions (see Figure 1). In condition 1, noxious CO_2 laser pulses were delivered to the middle finger in 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, and condition 4) and the number of thermally stimulated fingers (one: conditions 2 to 4; two: conditions 5 to 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, UK) and was kept between 28°C and 32°C (mean baseline temperature ± SD: 30°C ± 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' to the CO_2 laser stimulation reaction times (RTs) (Arendt-Nelsen and Bjerring 1988; Mancini et al. 2014a). A RT criterion of 650ms was used to discriminate between C (≥650ms) and Aδ fibers (<650ms) (Churyukanov et al. 2012; Jankovski et al. 2013). If RT to the preceding stimulus was ≥650ms, the laser intensity of the next stimulus was increased until the RT fell below 650ms, producing the first reversal. Conversely, if RT to a stimulus were shorter than 650ms, 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 <650ms) 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 Figure 1) were presented twice, in a fully randomized order, giving a total of 128 trials. To assure 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 (5-6s), a 100ms 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 3s, 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 that 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 inter-trial interval varied randomly between 12s and 27s, and the position of the laser beam on the finger was adjusted slightly between trials.

*** Please insert Figure 1 here ***

Statistical analysis

Behavioral and EEG data were analyzed using SPSS software (IBM SPSS Statistics for Windows, version 22.0. 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; Truini et al. 2007), we compared the no thermal stimulation condition (condition 1) to the warmth on the same finger condition (condition 3). 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 across index and ring finger stimulations. 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 condition 2, 3, and 4), two (average of condition 5, 6, and 7), or three fingers (condition 8) (Hays, 1994 ; Mancini et al. 2014b). As all our hypothesis 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 sample t-tests throughout. Statistical tests were considered significant if $p < 0.05$. Non-significant results

were further investigated through Bayesian one sample t-tests analyses, using JASP (version 0.8.0.1; JASP Team 2016, University of Amsterdam) to determine whether results supported the null hypothesis, or could alternatively reflect insufficient statistical power (Rouder et al. 2009; Wetzels and Wagenmakers 2012). EEG data were tested for normal distribution using Kolmogorov-Smirnov normality test (see Table S1 in the Supplementary Material: **https://doi.org/10.6084/m9.figshare.7808420.v2**). Out of the six Kolmogorov-Smirnov tests, only one showed significant non-normality, due to a single outlier. Because within-subjects ANOVA is relatively robust to violations of the normality assumption (Boneau 1960), we decided not to remove outliers or transform data.

*** Please insert Table 1 here ***

Results

Detailed LEP analysis is reported in the Supplementary Material (see Figure S1 https://doi.org/10.6084/m9.figshare.7808420.v2). Means and standard deviations of subjective ratings and LEPs are described in supplementary 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, mean ± SD: 2.82 ± 1.512) than during the no-warmth condition (condition 1, mean ± SD: 0.54 ± 0.608) (t_{13} = -6.158, $p < 0.001$; 95% CI: -∞, -1.625; Cohen's d = 2.148) (Figure 2A).

Second, to investigate the effect of warmth on co-located 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, mean ± SD: 3.2 ± 1.354) significantly decreased by 11.6% when a concomitant thermal stimulation was delivered on the same finger (condition 3, mean ± SD: 2.83 ± 1.007) (t_{13} = 2.106, p = 0.028; 95% CI: 0.061, +∞; Cohen's d = 0.314) (see Figure 2B). Concomitant warmth had a modulatory effect on the N2, but not on the P2 component (see Figure 2C and D). The peak amplitude of the N2 wave was significantly higher when pain was delivered in absence of warmth (condition 1, mean ± SD: -15.23 ± 7.282) than when a thermal stimulus was simultaneously presented on the same finger (condition 3, mean ± SD: -11.06 ± 4.137) (t_{13} = -2.13, p = 0.027; 95% CI: -∞, -0.723; Cohen's d = 0.730) (see Figure 2C). This reduction corresponded to a relative change of the 27.4%. The P2 wave did not show any significant modulation (t_{13} = 0.116, p = 0.455; 95% CI: -1.875, +∞; Cohen's d = 0.026). A Bayesian paired sample 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: -∞, -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, mean ± SD: 1.9 ± 0.909) than during the no-warmth condition (condition 1, mean ± SD: 0.54 ± 0.608) (see Figure 2A).

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, +∞; Cohen's d = 0.474). Baseline pain on the middle finger (mean ± SD: 3.2 ± 1.354) dropped by the 17.3% when a warm stimulus was delivered to either of the adjacent fingers (mean ± SD: 2.647 ± 0.983) (see Figure 2B). The subjective perception was supported by a decrease of the 22.2% in the amplitude of the N2 component (see Figure 2C). This effect did not formally reach the conventional boundaries for statistical significance (t_{13} = -1.769, p = 0.050; 95% CI: -∞, 0.016; Cohen's d = 0.629). However, a Bayesian paired-sample 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, +∞; 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 either onto 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 condition 2 and 4) conditions 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, +∞; Cohen's d = 0.754) (see Figure 3A), neither magnitude estimates of pain (t_{13} = 1.441, p = 0.087; 95% CI: -∞, 0.407; Cohen's d = 0.184), nor LEP amplitudes (N2: t_{13} = 0.967, p = 0.176; 95% CI: -0.654, +∞; Cohen's d = 0.209; P2: t_{13} = -0.13, p = 0.449; 95% CI: -∞, 1.102; Cohen's d = 0.019) were significantly different in the two thermal conditions (see Figure 2B, C, and D). While the Bayesian analysis on the behavioral data was inconclusive (BF_{01} = 0.881, error < 0.001%), those on the LEPs 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 condition 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, +∞; 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 Figure 3A).

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 condition 2, 3, and 4), two (average of condition 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: -∞, 0.104; Cohen's d = 0.653), nor LEPs (N2: t_{13} = -0.158, p = 0.438; 95% CI: -1.882, +∞; Cohen's d = 0.085; P2: t_{13} = -0.115, p = 0.455; 95% CI: -∞, 0.687; Cohen's d = 0.062). Increasing the number of simultaneous thermal stimuli did not affect subjective perception of pain (one finger: 2.708 ± 0.965; two fingers: 2.732 ± 0.949; three fingers: 2.509 ± 0.965) (see Figure 3B) nor the amplitude of N2 (one finger: -11.59 ± 3.404; two fingers: -11.66 ± 3.458; three fingers: -11.74 ± 5.458) or P2 (one finger: 10.02 ± 4.374; two fingers: 10.93 ± 4.777; three fingers: 9.97 ± 4.536) LEP components (see Figure 3C 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 3 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.

*** Please insert Figure 2 here ***

*** Please insert Figure 3 here ***

Discussion

Here 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 rule 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 while *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

458 that the modulation of nociception by warmth occurs either prior to, or independently of intra-
459 channel spatial summation of multiple thermal inputs.

460

461 ***Spatial organization of warmth-pain interaction***

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

474 One possible limitation of this study is that the effect of spatial summation was
475 investigated only across digits, rather than across more distant body parts. Previous studies
476 have shown that inhibitory interactions between multiple nociceptive stimuli occur across the
477 whole body (Le Bars 2002; Le Bars et al. 1979b, 1979a; Villanueva and Le 1995; Yarnitsky
478 2010; Yarnitsky et al. 2010). Additionally, we have only tested glabrous skin. We cannot
479 exclude different patterns of warm-nociceptive interaction in glabrous and hairy skin, due
480 either to differences in innervation density, or to factors such as skin thickness and heat
481 transfer. Therefore, further studies could address whether *thermo*-nociceptive interactions also
482 occur on a larger scale and on both glabrous and hairy skin. Given the different innervation
483 territories and segmental projections of the median and ulnar nerves, one may expect that
484 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, while 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 on interactions between simultaneous thermal or thermo-tactile stimuli. Finally, while 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 LEPs amplitudes. Thus, interaction between warmth and pain may involve a binary, rather than proportional, inhibitory mechanism. Inter-channel interaction between warmth and pain, then, must be mediated through a widely-distributed, non-somatotopic, all-or-nothing mechanism. This interaction mechanism would be independent from the intra-channel convergence and summation that characterizes purely thermal inputs. If warmth-pain interaction occurs subsequent to spatial summation, the stronger thermal signal that we observed for more numerous warm stimuli should produce a stronger suppression of nociceptive information.

Tran and colleagues (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 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 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 thermal referral condition compared with no thermal stimulation. This indicates that an illusory spread of perceived warmth across digits, also occurred in our paradigm.

Mechanisms underlying warmth-pain interaction

Different theories have been proposed to explain thermo-nociceptive interactions. Based on the finding that higher-intensity stimulation to one pathway produces a stronger inhibitory effect on the other, Truini and colleagues (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 and colleagues (2012), who postulated that Aδ fibers acts as local change detectors, rather than pure level detectors. The threshold for

538 Aδ fibers input would not depend only on the physical energy applied, but also on the
539 background input from C fibers innervating the skin surrounding the stimulated area.

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

554 Noticeably, our results do recall another well-known phenomenon, called *Diffuse*
555 *Noxious Inhibitory Control* (DNIC) in the animal literature (Le Bars et al. 1979a, 1979b;
556 Villanueva and Le 1995), and *Conditioned Pain Modulation* (CPM) in human studies (Davis
557 2013; Nir and Yarnitsky 2015; Yarnitsky 2010; Yarnitsky et al. 2010). CPM has been
558 described as a specific nociceptive mechanism where 'pain inhibits pain', and seems relevant
559 for our results in two key ways. First, it has been consistently shown that the inhibitory effect
560 of 'pain on pain' applies across the whole body, without apparent somatotopic spatial gradients
561 (Le Bars 2002; Le Bars et al. 1979b, 1979a; Villanueva and Le 1995; Yarnitsky 2010;
562 Yarnitsky et al. 2010). Second, Granot and colleagues (2008) also demonstrated that once the
563 analgesic effect on a test pain stimulus was evoked by a required degree of conditioning
564 painfulness, no further suppression occurred when the intensity of the conditioning stimulus
565 was increased. This led to the interpretation that the CPM is an all-or-nothing, 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 non-gradedness 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 brainstem (Granot et al. 2008).

*** Please insert Figure 4 here ***

Conclusion

Our study suggests four main results. First, behavioral and electrophysiological correlates of pain are attenuated by concomitant non-noxious 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 warm 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 produced 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 non-noxious thermal stimulation.

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Disclosure

The authors declare no conflict of interest.

References

Arendt-Nelsen L, **Bjerring P**. Reaction times to painless and painful CO2 and argon laser stimulation. *Eur J Appl Physiol* 58: 266–273, 1988.

Bini G, **Cruccu G**, **Hagbarth K-E**, **Schady W**, **Torebjörk E**. Analgesic effect of vibration and cooling on pain induced by intraneural electrical stimulation. *Pain* 18: 239–248, 1984.

Boneau CA. The effects of violations of assumptions underlying the t test. *Psychol Bull* 57: 49–64, 1960.

Bromm B, **Treede R-D**. Human cerebral potentials evoked by CO 2 laser stimuli causing pain. *Exp Brain Res* 67: 153–162, 1987.

Cain WS. Spatial discrimination of cutaneous warmth. *Am J Psychol* 169–181, 1973.

Casey KL, **Zumberg M**, **Heslep H**, **Morrow TJ**. Afferent modulation of warmth sensation and heat pain in the human hand. *Somatosens Mot Res* 10: 327–337, 1993.

Cataldo A, **Ferrè ER**, **Pellegrino G di**, **Haggard P**. Thermal referral: evidence for a thermoceptive uniformity illusion without touch. *Sci Rep* 6: 35286, 2016.

Chéry-Croze S. Painful sensation induced by a thermal cutaneous stimulus. *Pain* 17: 109–137, 1983.

Churyukanov M, **Plaghki L**, **Legrain V**, **Mouraux A**. Thermal detection thresholds of Aδ-and C-fibre afferents activated by brief CO2 laser pulses applied onto the human hairy skin. *PloS One* 7: e35817, 2012.

Cohen J. *Statistical power analysis for the behavioral sciences 2nd edn*. Erlbaum Associates, Hillsdale, 1988.

Darian-Smith I, **Johnson K**, **LaMotte C**, **Kenins P**, **Shigenaga Y**, **Ming V**. Coding of incremental changes in skin temperature by single warm fibers in the monkey. *J Neurophysiol* 42: 1316–1331, 1979a.

Darian-Smith I, **Johnson K**, **LaMotte C**, **Shigenaga Y**, **Kenins P**, **Champness P**. Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli. *J Neurophysiol* 42: 1297–1315, 1979b.

Davis MP. The clinical importance of conditioning pain modulation: a review and clinical implications. In: *Research and Development of Opioid-Related Ligands*. ACS Publications, 2013, p. 9–38.

Defrin R, **Petrini L**, **Arendt-Nielsen L**. Spatial summation of thermal sensations depends on skin type and skin sensitivity. *Exp Brain Res* 198: 29–36, 2009.

Defrin R, **Tsedek I**, **Lugasi I**, **Moriles I**, **Urca G**. The interactions between spatial summation and DNIC: effect of the distance between two painful stimuli and attentional factors on pain perception. *PAIN*® 151: 489–495, 2010.

Defrin R, **Urca G**. Spatial summation of heat pain: a reassessment. *Pain* 66: 23–29, 1996.

Delorme A, **Makeig S**. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* 134: 9–21, 2004.

Fardo F, **Finnerup NB**, **Haggard P**. Organization of the thermal grill illusion by spinal segments. *Ann Neurol* 84: 463–472, 2018.

Faul F, **Erdfelder E**, **Buchner A**, **Lang A-G**. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* 41: 1149–1160, 2009.

Garcia-Larrea L. *Somatosensory volleys and cortical evoked potentials:'first come, first served'?* LWW, 2004.

Granot M, **Weissman-Fogel I**, **Crispel Y**, **Pud D**, **Granovsky Y**, **Sprecher E**, **Yarnitsky D**. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: do conditioning stimulus painfulness, gender and personality variables matter? *Pain* 136: 142–149, 2008.

Green BG. Localization of thermal sensation: An illusion and synthetic heat. *Percept Psychophys* 22: 331–337, 1977.

Green BG. Referred thermal sensations: warmth versus cold. *Sens Processes* , 1978.

Greene LC, **Hardy JD**. Spatial summation of pain. *J Appl Physiol* 13: 457–464, 1958.

Hardy JD, **Oppel TW**. Studies in temperature sensation. III. The sensitivity of the body to heat and the spatial summation of the end organ responses. *J Clin Invest* 16: 533–540, 1937.

Hays W. Cengage Learning; Andover, UK: 1994. *Statistics*.

Herget C, **Granath L**, **Hardy J**. Warmth sense in relation to the area of skin stimulated. *Am J Physiol-Leg Content* 135: 20–26, 1941.

Ho H-N, **Watanabe J**, **Ando H**, **Kashino M**. Mechanisms Underlying Referral of Thermal Sensations to Sites of Tactile Stimulation. *J Neurosci* 31: 208–213, 2011.

Hu L, **Cai M**, **Xiao P**, **Luo F**, **Iannetti G**. Human brain responses to concomitant stimulation of Aδ and C nociceptors. *J Neurosci* 34: 11439–11451, 2014.

Iannetti G, **Leandri M**, **Truini A**, **Zambreanu L**, **Cruccu G**, **Tracey I**. Aδ nociceptor response to laser stimuli: selective effect of stimulus duration on skin temperature, brain potentials and pain perception. *Clin Neurophysiol* 115: 2629–2637, 2004.

Iannetti GD, **Hughes NP**, **Lee MC**, **Mouraux A**. Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? *J Neurophysiol* 100: 815–828, 2008.

Jankovski A, **Plaghki L**, **Mouraux A**. Reliable EEG responses to the selective activation of C-fibre afferents using a temperature-controlled infrared laser stimulator in conjunction with an adaptive staircase algorithm. *PAIN®* 154: 1578–1587, 2013.

Jung T-P, **Makeig S**, **Westerfield M**, **Townsend J**, **Courchesne E**, **Sejnowski TJ**. Analysis and visualization of single-trial event-related potentials. *Hum Brain Mapp* 14: 166–185, 2001.

Kakigi R, **Shibasaki H**. Mechanisms of pain relief by vibration and movement. *J Neurol Neurosurg Psychiatry* 55: 282–286, 1992.

Kakigi R, **Watanabe S**. Pain relief by various kinds of interference stimulation applied to the peripheral skin in humans: pain-related brain potentials following CO2 laser stimulation. *J Peripher Nerv Syst JPNS* 1: 189–198, 1996.

Kennedy DL, **Kemp HI**, **Ridout D**, **Yarnitsky D**, **Rice AS**. Reliability of conditioned pain modulation: a systematic review. *Pain* 157: 2410, 2016.

Kenshalo DR, **Decker T**, **Hamilton A**. Spatial summation on the forehead, forearm, and back produced by radiant and conducted heat. *J Comp Physiol Psychol* 63: 510, 1967.

Kleinböhl D, **Trojan J**, **Konrad C**, **Hölzl R**. Sensitization and habituation of AMH and C-fiber related percepts of repetitive radiant heat stimulation. *Clin Neurophysiol* 117: 118–130, 2006.

Kojo I, **Pertovaara A**. The effects of stimulus area and adaptation temperature on warm and heat pain thresholds in man. *Int J Neurosci* 32: 875–880, 1987.

Krahé C, **Paloyelis Y**, **Condon H**, **Jenkinson PM**, **Williams SC**, **Fotopoulou A**. Attachment style moderates partner presence effects on pain: a laser-evoked potentials study. *Soc Cogn Affect Neurosci* 10: 1030–1037, 2015.

LaMotte RH, **Campbell JN**. Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgments of thermal pain. *J Neurophysiol* 41: 509–528, 1978.

Le Bars D. The whole body receptive field of dorsal horn multireceptive neurones. *Brain Res Rev* 40: 29–44, 2002.

Le Bars D, **Dickenson AH**, **Besson J**. Diffuse noxious inhibitory controls (DNIC). II. Lack of effect on non-convergent neurones, supraspinal involvement and theoretical implications. *Pain* 6: 305–327, 1979a.

Le Bars D, **Dickenson AH**, **Besson J-M**. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain* 6: 283–304, 1979b.

Leandri M, **Saturno M**, **Spadavecchia L**, **Iannetti G**, **Cruccu G**, **Truini A**. Measurement of skin temperature after infrared laser stimulation. *Neurophysiol Clin Neurophysiol* 36: 207–218, 2006.

Machet-Pietropaoli H, **Chery-Croze S**. Spatial summation of thermal pain in human beings. *Sens Processes* , 1979.

Madden VJ, **Catley MJ**, **Grabherr L**, **Mazzola F**, **Shohag M**, **Moseley GL**. The effect of repeated laser stimuli to ink-marked skin on skin temperature—recommendations for a safe experimental protocol in humans. *PeerJ* 4: e1577, 2016.

Makeig S, **Jung T-P**, **Bell AJ**, **Ghahremani D**, **Sejnowski TJ**. Blind separation of auditory event-related brain responses into independent components. *Proc Natl Acad Sci* 94: 10979–10984, 1997.

Mancini F, **Bauleo A**, **Cole J**, **Lui F**, **Porro CA**, **Haggard P**, **Iannetti GD**. Whole-body mapping of spatial acuity for pain and touch. *Ann Neurol* 75: 917–924, 2014a.

Mancini F, **Nash T**, **Iannetti GD**, **Haggard P**. Pain relief by touch: A quantitative approach. *PAIN*® 155: 635–642, 2014b.

Marchand S, **Bushnell MC**, **Duncan GH**. Modulation of heat pain perception by high frequency transcutaneous electrical nerve stimulation (TENS). *Clin J Pain* 7: 122–129, 1991.

Marks LE. Spatial summation in the warmth sense. In: *Sensation and Measurement*. Springer, 1974, p. 369–378.

Marks LE, **Stevens JC**. Spatial summation of warmth: influence of duration and configuration of the stimulus. *Am J Psychol* 251–267, 1973.

Marotta A, **Ferrè ER**, **Haggard P**. Transforming the Thermal Grill Effect by Crossing the Fingers. *Curr Biol* 25: 1069–1073, 2015.

Melzack R, **Wall PD**. PAIN MECHANISMS: A NEW THEORY. *Surv Anesthesiol* 11: 89, 1967.

Meyer RA, **Campbell JN**. Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand. *Science* 213: 1527–1529, 1981.

Moayedi M, **Davis KD**. Theories of pain: from specificity to gate control. *J Neurophysiol* 109: 5–12, 2013.

Mouraux A, **Iannetti GD**. Across-trial averaging of event-related EEG responses and beyond. *Magn Reson Imaging* 26: 1041–1054, 2008.

Nahra H, **Plaghki L**. Modulation of perception and neurophysiological correlates of brief CO2 laser stimuli in humans using concurrent large fiber stimulation. *Somatosens Mot Res* 20: 139–147, 2003.

Nahra H, **Plaghki L**. Innocuous skin cooling modulates perception and neurophysiological correlates of brief CO2 laser stimuli in humans. *Eur J Pain* 9: 521–521, 2005.

Nathan P, **Rice R**. The localization of warm stimuli. *Neurology* 16: 533–533, 1966.

Nir R-R, **Yarnitsky D**. Conditioned pain modulation. *Curr Opin Support Palliat Care* 9: 131–137, 2015.

Plaghki L, **Decruynaere C**, **Van Dooren P**, **Le Bars D**. The fine tuning of pain thresholds: a sophisticated double alarm system. *PLoS One* 5: e10269, 2010.

Price DD, **McHaffie JG**. Effects of heterotopic conditioning stimuli on first and second pain: a psychophysical evaluation in humans. *Pain* 34: 245–252, 1988.

Price DD, **McHaffie JG**, **Larson MA**. Spatial summation of heat-induced pain: influence of stimulus area and spatial separation of stimuli on perceived pain sensation intensity and unpleasantness. *J Neurophysiol* 62: 1270–1279, 1989.

Quevedo AS, **Coghill RC**. Attentional modulation of spatial integration of pain: evidence for dynamic spatial tuning. *J Neurosci* 27: 11635–11640, 2007a.

Quevedo AS, **Coghill RC**. An illusion of proximal radiation of pain due to distally directed inhibition. *J Pain* 8: 280–286, 2007b.

Rouder JN, **Speckman PL**, **Sun D**, **Morey RD**, **Iverson G**. Bayesian t tests for accepting and rejecting the null hypothesis. *Psychon Bull Rev* 16: 225–237, 2009.

Rózsa AJ, **Kenshalo DR**. Bilateral spatial summation of cooling of symmetrical sites. *Percept Psychophys* 21: 455–462, 1977.

Schepers RJ, **Ringkamp M**. Thermoreceptors and thermosensitive afferents. *Neurosci Biobehav Rev* 34: 177–184, 2010.

Simmel ML, **Shapiro A**. The localization of non tactile thermal sensations. *Psychophysiology* 5: 415–425, 1969.

Stevens JC, **Marks LE**. Spatial summation and the dynamics of warmth sensation. *Percept Psychophys* 9: 391–398, 1971.

Stevens JC, **Marks LE**, **Simonson DC**. Regional sensitivity and spatial summation in the warmth sense. *Physiol Behav* 13: 825–836, 1974.

Tran TD, **Matre D**, **Casey KL**. An inhibitory interaction of human cortical responses to stimuli preferentially exciting Aδ or C fibers. *Neuroscience* 152: 798–808, 2008.

Truini A, **Galeotti F**, **Cruccu G**, **Garcia-Larrea L**. Inhibition of cortical responses to Aδ inputs by a preceding C-related response: Testing the “first come, first served” hypothesis of cortical laser evoked potentials. *Pain* 131: 341–347, 2007.

Van Ryckeghem DM, **Van Damme S**, **Crombez G**, **Eccleston C**, **Verhoeven K**, **Legrain V**. The role of spatial attention in attentional control over pain: an experimental investigation. *Exp Brain Res* 208: 269–275, 2011.

Villanueva L, **Le DB**. The activation of bulbo-spinal controls by peripheral nociceptive inputs: diffuse noxious inhibitory controls. *Biol Res* 28: 113–125, 1995.

Watanabe I, **Svensson P**, **Arendt-Nielsen L**. Influence of segmental and extra-segmental conditioning stimuli on cortical potentials evoked by painful electrical stimulation. *Somatosens Mot Res* 16: 243–250, 1999.

Wetzels R, **Wagenmakers E-J**. A default Bayesian hypothesis test for correlations and partial correlations. *Psychon Bull Rev* 19: 1057–1064, 2012.

Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. *Curr Opin Anesthesiol* 23: 611–615, 2010.

Yarnitsky D, **Arendt-Nielsen L**, **Bouhassira D**, **Edwards RR**, **Fillingim RB**, **Granot M**, **Hansson P**, **Lautenbacher S**, **Marchand S**, **Wilder-Smith O**. Recommendations on terminology and practice of psychophysical DNIC testing. *Eur J Pain* 14: 339–339, 2010.

Yarnitskya D, **Kunin M**, **Brik R**, **Sprecher E**. Vibration reduces thermal pain in adjacent dermatones. *Pain* 69: 75–77, 1997.

Zoppi M, **Voegelin MR**, **Signorini M**, **Zamponi A**. Pain threshold changes by skin vibratory stimulation in healthy subjects. *Acta Physiol Scand* 143: 439–444, 1991.

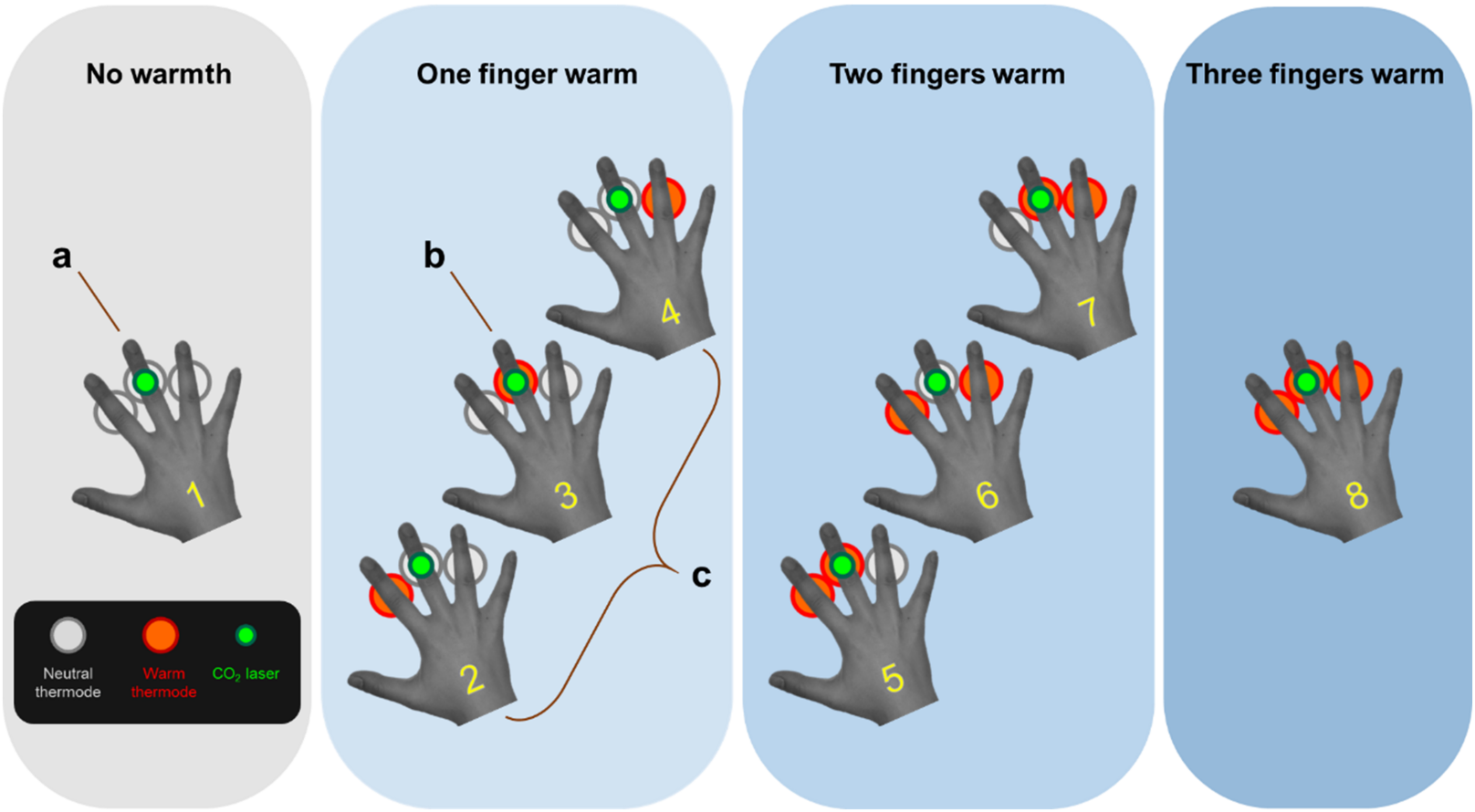
Figure Captions

Figure 1. Thermo-nociceptive 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 eight different conditions (see numbers). We then contrasted combinations of conditions in order to test four directional hypotheses regarding thermal-nociceptive interactions (see method). a. no warmth, laser only condition; b. warmth and laser pain on the middle finger; c. laser pain on the middle finger and warmth on the index or ring finger (i.e. adjacent fingers condition).

Figure 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 (avg. Ind, Rin). 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 condition. However, the amount of pain suppression was the same irrespectively of the site of stimulation. D. P2 wave. P2 component was not affected by neither of the thermal conditions. Error bars represent the standard error of the mean.
n.s., $p > 0.05$; +, $p = 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3. Effect of number of thermal stimuli on warmth perception (A), pain perception (B), and N2 (C) and P2 (D) LEP components.
A. Magnitude estimate of warmth. Increasing the number of fingers thermally stimulated induced a significant 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. Grey lines represent data from single participants. Colored lines represent the average across participants. Colored shading of black lines represents the standard error of the mean. ***, $p < 0.001$.

Figure 4. Schematic model of warmth-pain interaction.
Our results suggest that the inter-channel interaction between warmth and pain occurs before of, or independently from intra-channel convergence and summation of warmth.


No warmth
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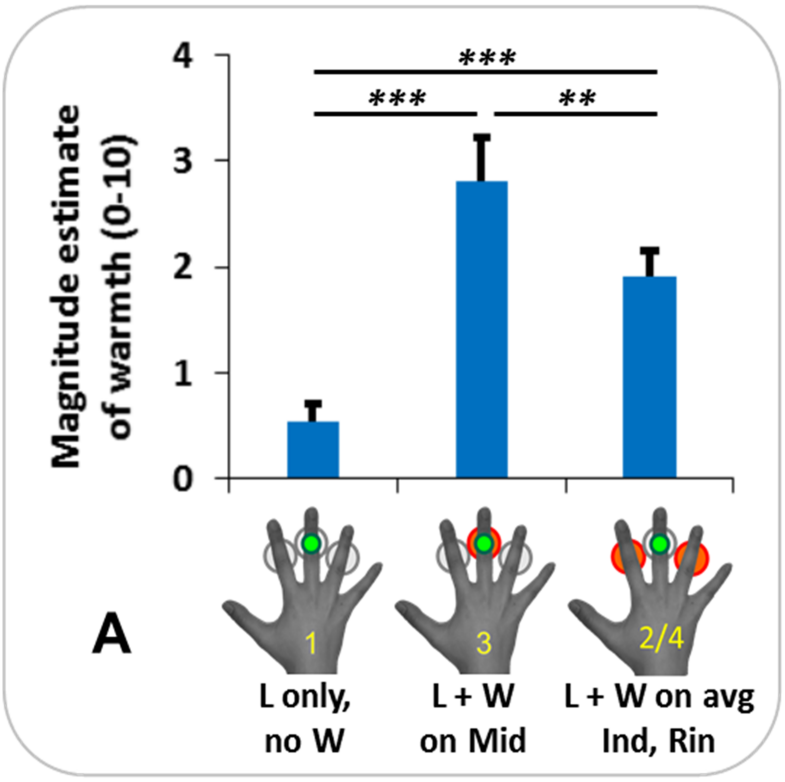
Warm thermode
CO_2 laser


Magnitude estimate of warmth (0-10)
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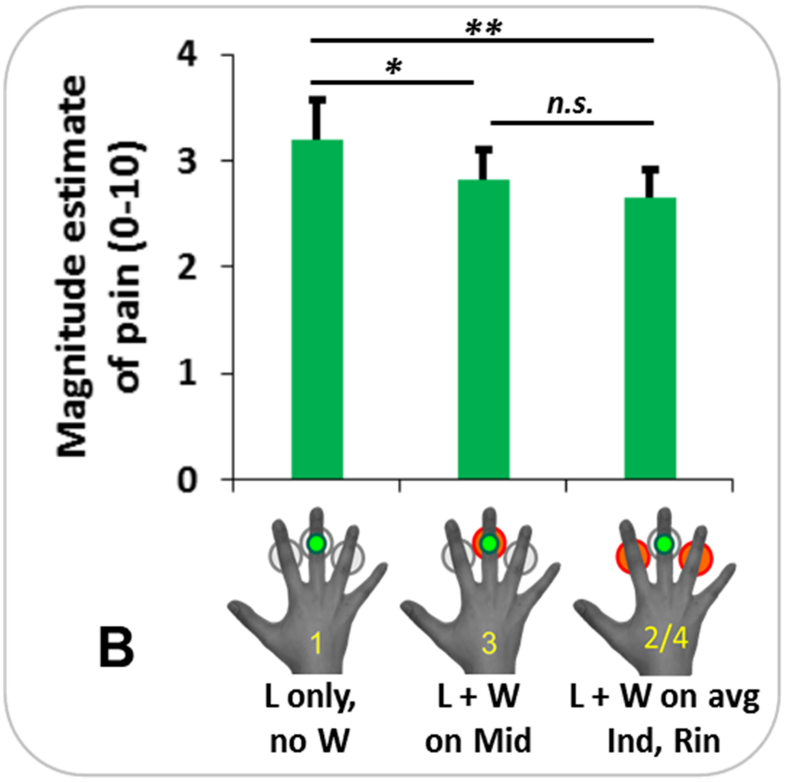
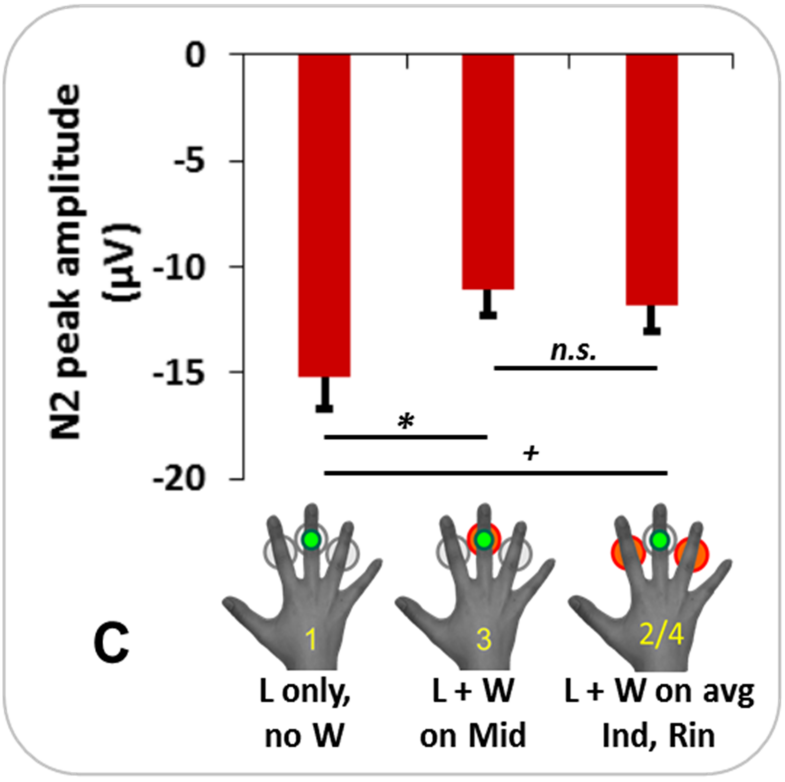
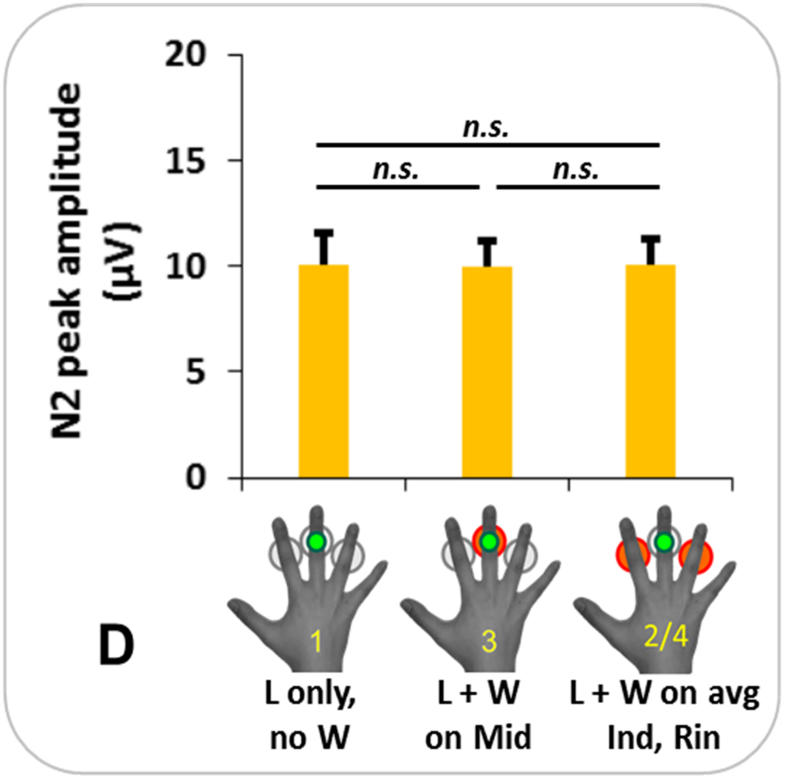
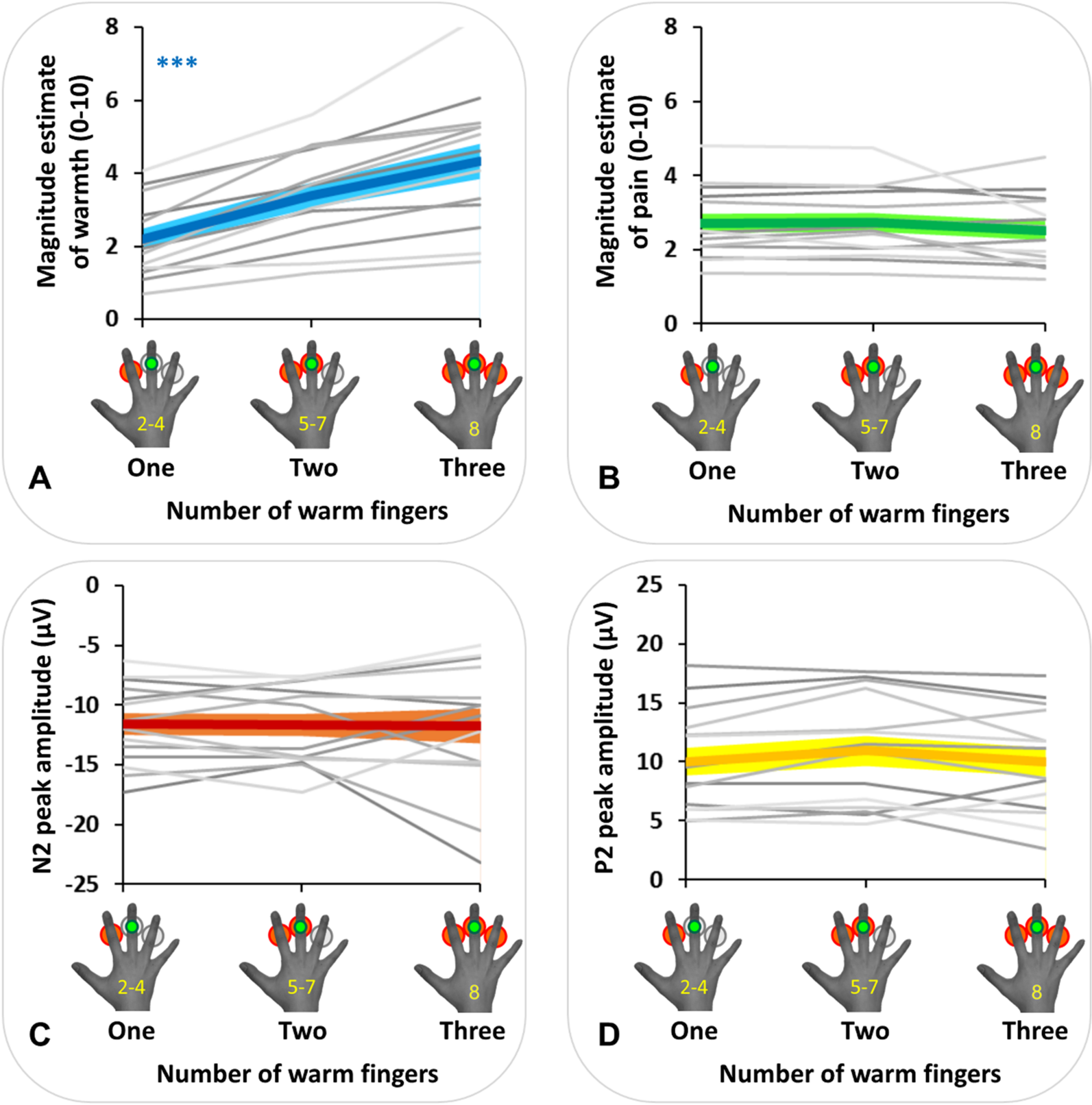
L + W on Mid
L + W on avg Ind, Rin


Magnitude estimate of pain (0-10)
4
3
2
1
0
**
*
n.s.
B
1
3
2/4
L only, no W
L + W on Mid
L + W on avg Ind, Rin


N2 peak amplitude (μV)
0
-5
-10
-15
-20
n.s.
*
+
C
1
3
2/4
L only, no W
L + W on Mid
L + W on avg Ind, Rin


N2 peak amplitude (μV)
20
15
10
5
0
n.s.
n.s.
n.s.
D
1
3
2/4
L only, no W
L + W on Mid
L + W on avg Ind, Rin


Magnitude estimate of warmth (0-10)
***
8
6
4
2
0
2-4
5-7
8
One
Two
Three
A
Number of warm fingers
Magnitude estimate of pain (0-10)
B
N2 peak amplitude (µV)
0
-5
-10
-15
-20
-25
C
P2 peak amplitude (µV)
25
20
15
10
5
0
D

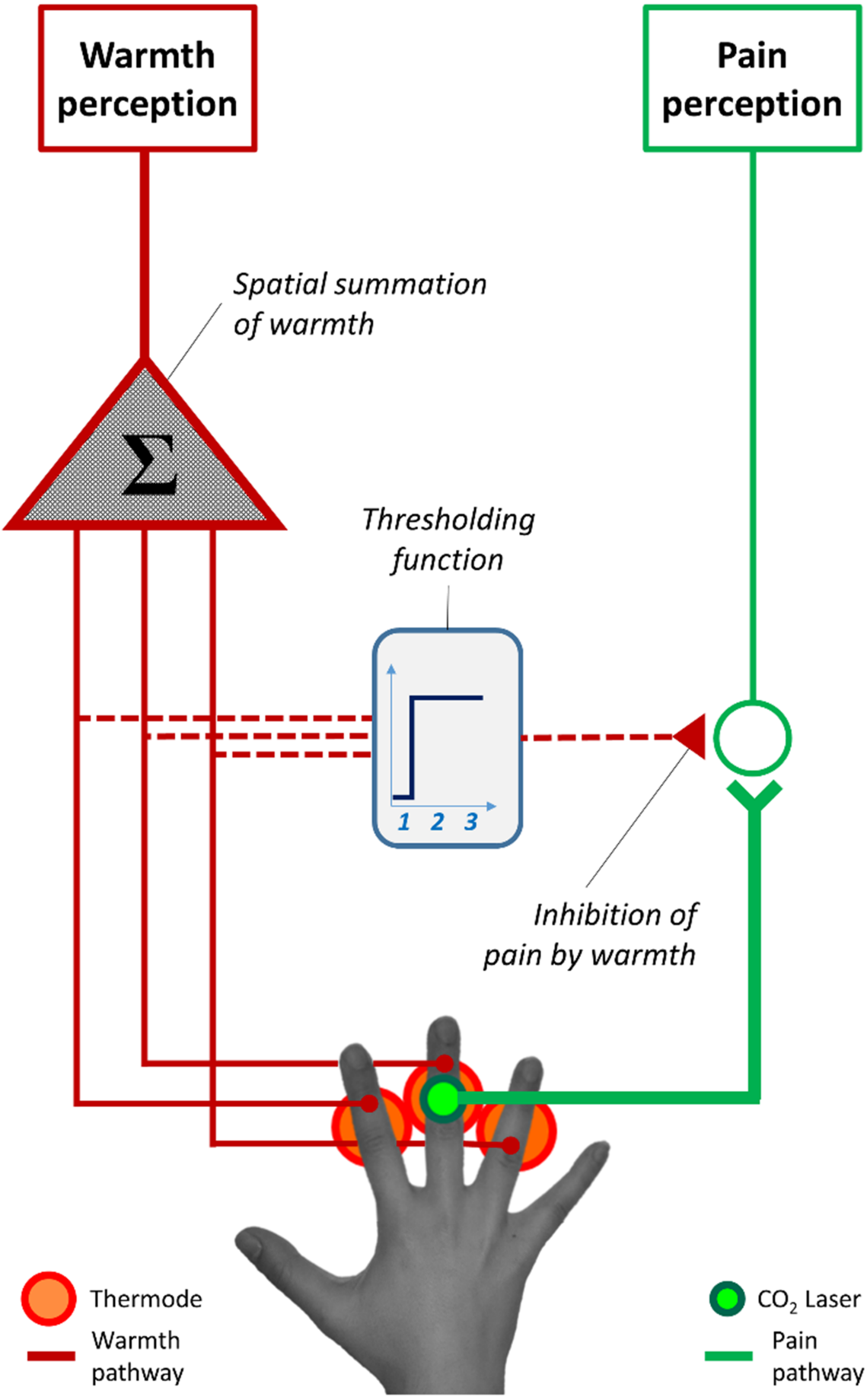

Warmth perception
Pain perception
Spatial summation of warmth
Σ
Thresholding function
1 2 3
Inhibition of pain by warmth
Thermode
Warmth pathway
CO2 Laser
Pain pathway

Table 1. Table of coefficients for the four research question

	Thermal conditions							
	No warmth	***Index warm***	***Middle warm***	***Ring warm***	***Ind+Mid warm***	***Ind+Rin warm***	***Mid+Rin 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**

Table 1 shows the coefficients used to test our four research questions. See text for explanation.