

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

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12 **Contribution**

13 AC and PH designed the experiment. AC collected the data. AC and ERF analyzed the data.

14 All the authors wrote the manuscript.

15 **Running Head**

16 Warmth-pain interaction occurs prior to warmth summation

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21 **Abstract**

22 Non-noxious warmth reduces both perceived pain intensity, and the amplitude of EEG markers  
23 of pain. However, the spatial properties of thermo-nociceptive interaction, and the level of  
24 sensory processing at which it occurs remain unclear. Here, we investigated whether *inter-*  
25 *channel* warmth-pain interactions occur before or after *intra-channel* spatial summation of  
26 warmth. Warm stimuli were applied to the fingers of the right hand. Their number and  
27 location were manipulated in different conditions. A concomitant noxious test pulse was  
28 delivered to the middle finger using a CO<sub>2</sub> laser. We replicated the classical suppressive effect  
29 of warmth on both pain perceived intensity and EEG markers. Importantly, inhibition of pain  
30 was not affected by the location and the number of thermal stimuli, even though they increased  
31 the perceived intensity of warmth. Our results therefore suggest that the inhibitory effect of  
32 warmth on pain is not somatotopically organized. They also rule out the possibility that  
33 warmth affects nociceptive processing after intra-channel warmth summation.

34 **Keywords**

35 Somatosensory interaction, spatial summation of warmth, pain inhibition, CO<sub>2</sub> laser evoked  
36 potentials, conditioned pain modulation

37 **New & Noteworthy**

38 We used spatial summation of warmth as a model to investigate thermo-nociceptive  
39 interactions. Painful CO<sub>2</sub> laser pulses were delivered during different thermal conditions. We  
40 found that warmth inhibited pain regardless of its location. Crucially, spatial summation of  
41 multiple warm stimuli did not further inhibit pain. These findings suggest that warmth-pain  
42 interaction occurs independently or after spatial summation of warmth.

## 43 **Introduction**

44 Interactions between *nociception*, the neural processing of noxious stimuli, and other  
45 somatosensory sub-modalities have received increasing attention in the last decades probably  
46 due to their potential clinical relevance in the treatment and management of pain (Kennedy et  
47 al. 2016). For example, non-noxious tactile signals have been shown to inhibit the transmission  
48 of nociceptive information – the well-known Tactile Gate Control (Kakigi and Shibasaki 1992;  
49 Krahé et al. 2015; Mancini et al. 2014b; Marchand et al. 1991; Melzack and Wall 1967;  
50 Moayedi and Davis 2013; Watanabe et al. 1999; Zoppi et al. 1991).

51 Non-noxious *warm* signals can also modulate nociception: warm increases the  
52 tolerance for pain (Casey et al. 1993; Plaghki et al. 2010) and reduces the cortical responses  
53 evoked by noxious stimuli (Tran et al. 2008; Truini et al. 2007). Similarly, both cold (Bini et  
54 al. 1984; Nahra and Plaghki 2005) and noxious signals (Davis 2013; Nir and Yarnitsky 2015;  
55 Yarnitsky 2010; Yarnitsky et al. 2010) have been reported to affect pain perception. Moreover,  
56 there is overlap between the temperature ranges at which non-noxious warmth receptors and  
57 nociceptors respond (Chéry-Croze 1983; Plaghki et al. 2010; Schepers and Ringkamp 2010).  
58 However, here we focus on the mild warmth intensity range, where non-nociceptive C-warm  
59 fibers are likely to predominate (LaMotte and Campbell 1978; Meyer and Campbell 1981).  
60 Importantly, while the spatial features of touch-pain interactions have been widely investigated,  
61 spatial organization of warmth-pain interactions has received less attention and remains  
62 unclear. For instance, Bini et al. (1984) investigated whether other somatosensory sub-  
63 modalities (i.e. vibratory, tactile, cold, and warm stimuli) might influence pain. While  
64 vibrotactile inputs clearly diminished pain perception, and touch and cooling produced some  
65 pain relief, the effects of non-noxious warmth were not clear. Further, touch-pain interactions  
66 show clear somatotopic organization: nociceptive processing is modulated when the tactile and  
67 pain inputs are both delivered within the same dermatome (Kakigi and Watanabe 1996;  
68 Mancini et al. 2014b; Nahra and Plaghki 2003; Watanabe et al. 1999; Yarnitsky et al. 1997).  
69 While there is both electrophysiological (Tran et al. 2008) and behavioral (Casey et al. 1993)

70 evidence suggesting a spatially-specific attenuation of pain after inter-segmental and  
71 contralateral presentation of thermal stimuli, no spatially-specific modulation of pain seems to  
72 occur when thermal stimulation is delivered on more distant skin regions (Price and McHaffie  
73 1988). In fact, some authors have questioned whether thermal-nociceptive reactions have any  
74 spatial organization at all, and have instead attributed spatially-specific effects to general,  
75 amodal mechanisms such as distraction or shifts in spatial attention (Defrin et al. 2010;  
76 Quevedo and Coghill 2007a, 2007b; Van Ryckegehem et al. 2011).

77 On the other hand, spatial effects within the thermoceptive system alone have been  
78 extensively studied. Thermoception is strongly affected by spatial summation (Hardy and  
79 Opiel 1937; Kenshalo et al. 1967; Marks 1974; Marks and Stevens 1973; Stevens and Marks  
80 1971) summation. Thus, perception of warmth does not only depend on the physical  
81 temperature of the stimulus, but also by where the thermal stimuli are applied (Defrin and Urca  
82 1996; Hardy and Opiel 1937; Kojo and Pertovaara 1987; Machet-Pietropaoli and Chery-Croze  
83 1979), and by how many non-contiguous thermal stimuli are delivered (Hardy and Opiel 1937;  
84 Kenshalo et al. 1967; Price et al. 1989; Rózsa and Kenshalo 1977). Warmth spatial summation  
85 occurs locally when multiple nearby fibers are simultaneously activated by the warm stimulus  
86 (Greene and Hardy 1958) or even across non-contiguous skin regions (Rózsa and Kenshalo  
87 1977). Moreover, the spatial summation varies according to the properties of the skin:  
88 compared to hairy skin, glabrous skin shows much larger magnitude of spatial summation  
89 (Defrin et al. 2009).

90 The level at which spatial summation of warmth occurs is not certain. Most authors suggest  
91 that warm spatial summation reflects integration of thermal information at second- and third-  
92 order neurons in the spinal cord, and/or supra-spinal levels (Herget et al. 1941; Price et al.  
93 1989; Stevens et al. 1974). Moreover, it remains unclear whether thermo-nociceptive  
94 interactions occur before or after summation of multiple thermal inputs.

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

98 organized. We also investigated if inter-channel thermo-nociceptive interactions occur before  
99 or after intra-channel spatial summation of warmth. Painful CO<sub>2</sub> laser pulses were delivered to  
100 the middle finger, while the location and number of concurrent non-noxious warm stimuli to the  
101 fingers was systematically manipulated to achieve different degrees of spatial summation of  
102 warmth. We tested four specific hypotheses about warm-pain interaction, using planned  
103 comparisons motivated by established neurophysiological theories about both thermal and  
104 nociceptive channels. First, we tested the prediction of a *warmth gating of pain* (Casey et al.  
105 1993; Plaghki et al. 2010; Tran et al. 2008; Truini et al. 2007), where warm stimulation on the  
106 middle finger attenuates perceived pain and nociceptive processing for a noxious laser pulse  
107 delivered to the *same* middle finger. A directional prediction is justified, since the literature  
108 agrees that warmth inhibits pain, and, to our knowledge, it has never been reported that  
109 innocuous warm stimulation increases pain and nociceptive processes. Second, we investigated  
110 whether the warm-inhibits-pain effect remained when the warm stimulus was delivered on the  
111 adjacent index and ring fingers, while noxious stimulation was applied to the middle finger. An  
112 affirmative result would show some degree of spatial spread in warm-pain interactions. Indeed,  
113 given the low spatial resolution (Cain 1973; Nathan and Rice 1966; Simmel and Shapiro 1969)  
114 and high spatial summation (Hardy and Opiel 1937; Marks and Stevens 1973; Stevens and  
115 Marks 1971) of the thermoceptive system, we expect a “perceptual spread of warmth” to the  
116 thermally neutral middle finger (Cataldo et al. 2016; Green 1977, 1978; Ho et al. 2011).  
117 Accordingly, Green (1978) demonstrated referred warmth on a thermally neutral finger when a  
118 thermal stimulation was applied to the adjacent finger: importantly, the neutral middle finger  
119 felt on average 54.5% less warm than the stimulated adjacent finger. Third, we tested whether  
120 the warmth gates pain in a spatially tuned fashion by contrasting pain attenuation when warmth  
121 was delivered on the same finger as noxious laser stimulation, versus the situation where  
122 warmth is delivered on fingers adjacent to the noxious stimulation. Previous studies suggest  
123 that the spatial spread of warmth is partial rather than complete. For example measures of  
124 thermal referral found that 30% - 60% of the warmth delivered to one finger is perceptually  
125 referred to an adjacent finger (Green 1978). Thus, we hypothesized that warmth on adjacent

126 fingers would produce less pain inhibition than warm on the finger that receives noxious  
127 stimulation. Fourth and finally, we investigated at which level of the somatosensory processing  
128 pathway, any thermo-nociceptive interaction occurs. If thermal-nociceptive interaction occurs  
129 *after* summation of warmth, then progressively increasing the number of fingers that are  
130 simultaneously warmed (i.e., increasing the area of thermal stimulation) while maintaining the  
131 same physical temperature on the middle finger, would produce a *stronger* suppression of pain.  
132 Conversely, if thermo-nociceptive interaction occurs *before* or independently of warm spatial  
133 summation, progressively increasing the number/area of warm stimulations would not affect  
134 pain processing. We therefore constructed a systematic set of stimulation conditions to test  
135 these four directional predictions.

## 136 **Methods**

### 137 ***Participants***

138 The sample size was calculated a priori by means of a statistical power analysis for sample size  
139 estimation based on the results of a previous EEG pilot study (n = 10) testing the same eight  
140 thermal conditions studied here. The effect size for comparing the electrophysiological  
141 correlate of a painful CO<sub>2</sub> laser pulse during no thermal stimulation, warmth on the same  
142 finger, and warmth on the adjacent fingers in the pilot study was  $\eta^2 = 0.380$ , considered to be  
143 very large using Cohen's (1988) criteria. With an alpha = 0.05 and power = 0.80, the projected  
144 sample size indicated for this effect is 11 participants (G\*Power 3.1.9.2 software) (Faul et al.  
145 2009). We tested 15 healthy right-handed volunteers (10 females, mean age  $\pm$  SD: 25.9  $\pm$  4.3  
146 years). One participant was excluded because pain threshold could not be reliably established,  
147 leaving a final sample of 14. This gave sufficient power for the main objectives of this study.  
148 Inclusion criteria for the study were the absence of any history of previous traumatic hand  
149 injury, absence of sensitive skin or skin conditions, abstention from analgesic medication for 24  
150 hours prior the study, and abstention from caffeinated beverages for three hours prior to the  
151 study.

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

157

## 158 ***Apparatus***

### 159 *CO<sub>2</sub> Laser stimulation*

160 Nociceptive stimulation was delivered on the dorsum of participants' right middle finger by a  
161 CO<sub>2</sub> laser stimulator (Laser Stimulation Device, SIFEC, Belgium), controlled by a computer.  
162 The laser pulse (~100ms) was transmitted via an optical fiber and focused by lenses to a spot  
163 diameter of ~6mm. A radiometer collinear with the laser beam detected the skin temperature at  
164 the site of stimulation, providing safe and reproducible noxious thermal radiant stimuli at a  
165 ramping rate of ~350°C/s (Churyukanov et al. 2012; Jankovski et al. 2013).

166           Participants rested their right hand pronated on a molded support. Vision of the hand  
167 was blocked with a screen. The laser head was positioned above the hand, with the laser beam  
168 pointing on the dorsal aspect of the middle finger's intermediate phalanx (see Figure 1). A  
169 visible helium-neon laser spot was used to point the CO<sub>2</sub> laser to the target location. To ensure  
170 a consistent stimulus location across the experiment, the target area was delimited by a ~12mm  
171 diameter circle drawn on the dorsum of the middle finger. Extra care was taken during the  
172 testing to prevent any laser stimulation on the skin blackened by the ink, which could affect  
173 absorption of radiant heat (Leandri et al. 2006; Madden et al. 2016). Participants wore  
174 protective goggles and were asked to maintain their gaze on a fixation cross centrally located in  
175 front of them. Intensity, duration, and timing of the CO<sub>2</sub> laser stimuli were controlled by  
176 computer software.

177 Prior to the beginning of each experiment, participants were familiarized with the laser  
178 stimuli, through at least 3 stimulations delivered at 46°C (i.e., the standard threshold for  
179 thermal pain (Darian-Smith et al. 1979a, 1979b; LaMotte and Campbell 1978). Participants  
180 were asked to press a button with their left hand as soon as they felt any stimulation on the  
181 dorsum of the right middle finger and to verbally rate the intensity of the stimulus on a scale  
182 from 0 to 10 where 0 meant “no pain”, 1 “slight pinprick”, and 10 “the worst pain imaginable”  
183 (Tran et al. 2008). Participants were informed that they were not restricted to use integers. The  
184 reports from the familiarization phase were not further analyzed.

### 185 *Thermal stimulation*

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

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



203 *EEG recording and LEP analysis*

204 EEG Laser Evoked Potentials (LEPs) are considered an objective measurement of nociception  
205 (Bromm and Treede 1987), which consists of several transient responses that are time locked  
206 and phase locked to the onset of painful laser stimuli (Mouraux and Iannetti 2008). EEG data  
207 were acquired from the scalp at a sampling rate of 2048 Hz using an Active Two BioSemi EEG  
208 amplifier and ActiView software (Biosemi, Amsterdam, The Netherlands). Sixteen Ag-AgCl  
209 active electrodes were positioned on the scalp according to the 10-20 International System.  
210 Electro-conductive gel was used to keep the impedance of all electrodes  $< 5k\Omega$  throughout the  
211 experiment. An external electrode placed on the nose was used as reference.  
212 Electrooculographic signals (EOG) for eye movements and eye-blinks monitoring were  
213 simultaneously recorded.

214 EEG data were processed using EEGLab (Delorme and Makeig 2004) running on  
215 MATLAB. Continuous raw data for each participant in each block were recorded and stored on  
216 ActiView, and successively imported on EEGLab for off-line analysis. Data were resampled to  
217 250Hz, and then bandpass filtered between 1Hz and 30Hz. EEG epochs were extracted from  
218 the continuous data using a window analysis time of 3000ms (from -1000ms to 2000ms relative  
219 to the CO<sub>2</sub> laser pulse). The mean signal immediately preceding the laser stimulus (from -  
220 500ms to 0ms) was set as baseline and removed from each epoch. Artefacts originating from  
221 eye-blinks and ocular movements were identified and pruned by means of Independent  
222 Component Analysis (ICA) (Delorme and Makeig 2004; Jung et al. 2001; Makeig et al. 1997).  
223 For each participant, all the independent components representing artefacts or non-cortical  
224 processes, such as eye movements or facial muscle activity were manually selected and  
225 rejected. The criteria for the identification of muscular artefacts were based on each  
226 component's scalp topography, power spectrography, inter-trial coherency, and intra-trial time  
227 course.

228 Laser-evoked potentials (LEPs) data analysis were computed on the signal recorded at  
229 the vertex (electrode Cz) referenced to the nose. Epochs from each specific experimental

230 condition were averaged within participants and time-locked to the onset of the CO<sub>2</sub> laser pulse.  
231 Then, the main negative (N2 wave) and positive (P2 wave) vertex components associated with  
232 LEPs were identified and selected on the basis of their latency and polarity. N2 and P2  
233 components were defined as the most negative and positive biphasic deflections between 150ms  
234 and 500ms after stimulus onset (Hu et al. 2014; Iannetti et al. 2008). The peak amplitude of  
235 these components was used for statistical analysis.

### 236 *Experimental design and procedure*

237 We designed a within-subject paradigm where participants' magnitude estimates of pain, and  
238 LEPs amplitudes were tested in a series of planned comparisons involving eight different  
239 thermal conditions (see Figure 1). In condition 1, noxious CO<sub>2</sub> laser pulses were delivered to  
240 the middle finger in absence of any thermal stimulation, providing a baseline measure of pain  
241 perception. In the remaining conditions, the site of thermal stimulation (index, middle, or ring  
242 finger; condition 2, condition 3, and condition 4) and the number of thermally stimulated  
243 fingers (one: conditions 2 to 4; two: conditions 5 to 7; or three: condition 8) were  
244 systematically manipulated to produce different levels of spatial summation of warmth.

245 The experiment took place in a temperature-controlled room at 23°C. The superficial  
246 skin temperature of the hand dorsum was systematically measured at several points during the  
247 experiment by means of an infrared thermometer (Precision Gold, N85FR Maplin, UK) and was  
248 kept between 28°C and 32°C (mean baseline temperature  $\pm$  SD: 30°C  $\pm$  1.4°C). First, laser-  
249 induced pain thresholds were established through an adaptive psychophysical staircase  
250 procedure: the first stimulus of the staircase was set at 40°C, and the intensity of the following  
251 stimuli was adaptively changed according to participants' to the CO<sub>2</sub> laser stimulation reaction  
252 times (RTs) (Arendt-Nelsen and Bjerring 1988; Mancini et al. 2014a). A RT criterion of 650ms  
253 was used to discriminate between C ( $\geq$ 650ms) and A $\delta$  fibers (<650ms) (Churyukanov et al.  
254 2012; Jankovski et al. 2013). If RT to the preceding stimulus was  $\geq$ 650ms, the laser intensity  
255 of the next stimulus was increased until the RT fell below 650ms, producing the first reversal.  
256 Conversely, if RT to a stimulus were shorter than 650ms, the laser intensity of the upcoming

257 stimulus was decreased. The step size of the staircase was progressively reduced after each  
258 reversal, from 4°C, to 2°C, and finally 1°C. After the third reversal, any intensity producing an  
259 A $\delta$ -like response (RT <650ms) was repeated three times. The pain threshold was defined as the  
260 lowest laser intensity inducing two out of three consecutive A $\delta$ -like responses.

261 After pain thresholds were established, the EEG cap was mounted, and the experiment  
262 began. Participants completed eight blocks of 16 trials each. In each block, the eight different  
263 thermal conditions described above (see Figure 1) were presented twice, in a fully randomized  
264 order, giving a total of 128 trials. To assure attention to the stimuli, a beep signaled the  
265 beginning of each trial. Before and after the trial, the temperature of the thermodes was set at  
266 32°C. After the beep, the thermal stimulation on the designated finger/s ramped up to 40°C at a  
267 rate of ~2°C/s and remained steady for the entire duration of the trial. After a random delay  
268 from the beginning of the thermal stimulation (5-6s), a 100ms CO<sub>2</sub> laser pulse was delivered to  
269 the dorsum of the right middle finger. The intensity of the laser stimulation for each participant  
270 was set at the individual pain threshold +6°C and remained fixed throughout the entire  
271 experiment. Participants were asked to maintain gaze on a central fixation cross placed in front  
272 of them, and to attend to the thermal and laser stimuli. After 3s, a further beep occurred, and  
273 participants verbally rated the intensity first of warmth, and then of pain providing a number  
274 from 0 to 10 for each sensation based on the initial training with these scales (see above). For  
275 example, if the subject said “3, 5” that meant that their rating was 3 for the perceived warmth  
276 on the middle finger and 5 for laser pain on the same finger (Tran et al. 2008). To prevent any  
277 possible effect of sensitization or habituation of the thermoreceptors/nociceptors at the site of  
278 stimulation (Iannetti et al. 2004; Kleinböhl et al. 2006), the inter-trial interval varied randomly  
279 between 12s and 27s, and the position of the laser beam on the finger was adjusted slightly  
280 between trials.

281

282

283

\*\*\* Please insert Figure 1 here \*\*\*

284

285

286 ***Statistical analysis***

287 Behavioral and EEG data were analyzed using SPSS software (IBM SPSS Statistics for  
288 Windows, version 22.0. Armonk, NY).

289 Our experimental design aimed to address four independent research questions to  
290 investigate the spatial and summative properties of warmth-nociceptive interaction (see Table  
291 1). We therefore used a priori planned comparisons between specific experimental conditions,  
292 as follows. First, to test whether warmth inhibits pain delivered at the same skin site (Casey et  
293 al. 1993; Plaghki et al. 2010; Tran et al. 2008; Truini et al. 2007), we compared the no thermal  
294 stimulation condition (condition 1) to the warmth on the same finger condition (condition 3).  
295 Second, to test whether warmth on adjacent fingers (Cataldo et al. 2016; Green 1977, 1978; Ho  
296 et al. 2011) could similarly inhibit pain, we compared condition 1 (no thermal stimulation) with  
297 the average of conditions 2 and 4 (warmth on adjacent index/ring fingers). We found no  
298 statistical evidence for perceptual differences between these fingers when stimulated alone ( $p >$   
299  $0.200$  for all variables studied), vindicating our *a priori* decision to average over across index  
300 and ring finger stimulations. Third, to test whether the warmth-pain interaction is spatially  
301 specific, we compared pain inhibition in condition 3 (warmth on the same finger) with the  
302 average of conditions 2 and 4 (warmth on index/ring finger; i.e. adjacent fingers) (see question  
303 3 in Table 1 for the coefficient used for the comparison). Finally, to test the effect of  
304 progressive spatial summation of multiple simultaneous thermal stimuli, we performed a linear  
305 trend analysis, with weights  $-1$ ,  $0$ , and  $1$  for the conditions where warmth was applied on one  
306 (average of condition 2, 3, and 4), two (average of condition 5, 6, and 7), or three fingers  
307 (condition 8) (Hays, 1994 ; Mancini et al. 2014b). As all our hypothesis are unidirectional and  
308 supported by previous evidence (Cataldo et al. 2016; Green 1977, 1978; Ho et al. 2011; Plaghki  
309 et al. 2010; Tran et al. 2008; Truini et al. 2007), we used one-tailed paired sample t-tests  
310 throughout. Statistical tests were considered significant if  $p < 0.05$ . Non-significant results

311 were further investigated through Bayesian one sample t-tests analyses, using JASP (version  
312 0.8.0.1; JASP Team 2016, University of Amsterdam) to determine whether results supported  
313 the null hypothesis, or could alternatively reflect insufficient statistical power (Rouder et al.  
314 2009; Wetzels and Wagenmakers 2012). EEG data were tested for normal distribution using  
315 Kolmogorov-Smirnov normality test (see Table S1 in the Supplementary Material:  
316 <https://doi.org/10.6084/m9.figshare.7808420.v2>). Out of the six Kolmogorov-Smirnov  
317 tests, only one showed significant non-normality, due to a single outlier. Because within-  
318 subjects ANOVA is relatively robust to violations of the normality assumption (Boneau 1960),  
319 we decided not to remove outliers or transform data.

320

321

322

\*\*\* Please insert Table 1 here \*\*\*

323

324

325 **Results**

326 Detailed LEP analysis is reported in the Supplementary Material (see Figure S1  
327 <https://doi.org/10.6084/m9.figshare.7808420.v2>). Means and standard deviations of  
328 subjective ratings and LEPs are described in supplementary Table S2  
329 (<https://doi.org/10.6084/m9.figshare.7808420.v2>).

330

331 ***Planned comparison 1: Does warmth inhibit pain delivered to the same finger?***

332 We first compared *warmth* magnitude estimates between condition 1 (no thermal stimulation)  
333 and condition 3 (warmth on the middle finger). As predicted, ratings of warmth were  
334 significantly higher when the thermal stimulus was presented on the middle finger (condition 3,  
335 mean  $\pm$  SD:  $2.82 \pm 1.512$ ) than during the no-warmth condition (condition 1, mean  $\pm$  SD:  $0.54$   
336  $\pm 0.608$ ) ( $t_{13} = -6.158, p < 0.001$ ; 95% CI:  $-\infty, -1.625$ ; Cohen's  $d = 2.148$ ) (Figure 2A).

337 Second, to investigate the effect of warmth on co-located pain, we performed planned  
338 comparisons on both perceptual and electrophysiological responses to pain. A planned  
339 comparison on the magnitude estimates of pain showed that participants' pain rating during the  
340 no-warmth condition (condition 1, mean  $\pm$  SD:  $3.2 \pm 1.354$ ) significantly decreased by 11.6%  
341 when a concomitant thermal stimulation was delivered on the same finger (condition 3, mean  $\pm$   
342 SD:  $2.83 \pm 1.007$ ) ( $t_{13} = 2.106, p = 0.028$ ; 95% CI:  $0.061, +\infty$ ; Cohen's  $d = 0.314$ ) (see Figure  
343 2B). Concomitant warmth had a modulatory effect on the N2, but not on the P2 component  
344 (see Figure 2C and D). The peak amplitude of the N2 wave was significantly higher when pain  
345 was delivered in absence of warmth (condition 1, mean  $\pm$  SD:  $-15.23 \pm 7.282$ ) than when a  
346 thermal stimulus was simultaneously presented on the same finger (condition 3, mean  $\pm$  SD:  $-$   
347  $11.06 \pm 4.137$ ) ( $t_{13} = -2.13, p = 0.027$ ; 95% CI:  $-\infty, -0.723$ ; Cohen's  $d = 0.730$ ) (see Figure 2C).  
348 This reduction corresponded to a relative change of the 27.4%. The P2 wave did not show any  
349 significant modulation ( $t_{13} = 0.116, p = 0.455$ ; 95% CI:  $-1.875, +\infty$ ; Cohen's  $d = 0.026$ ). A  
350 Bayesian paired sample t-test supported the null result ( $BF_{01} = 4.026, \text{error} < 0.001\%$ ),  
351 suggesting that this result was not due to a lack of statistical power (Rouder et al. 2009;

352 Wetzels and Wagenmakers 2012). Dissociations between N2 and P2 components have been  
353 previously reported (Tran et al. 2008). Thus, both behavioral and electrophysiological  
354 correlates of pain were attenuated by a concomitant warm stimulus delivered to the same  
355 finger.

356 ***Planned comparison 2: Does warmth inhibit pain delivered on an adjacent***  
357 ***finger?***

358 A direct comparison between ratings of warmth in condition 1 (no thermal stimulation) and the  
359 average of conditions 2 and 4 (warmth on the adjacent fingers) was significant ( $t_{13} = -8.476$ ,  $p <$   
360  $0.001$ ; 95% CI:  $-\infty$ ,  $-1.080$ ; Cohen's  $d = 1.797$ ) with participants rating warmth on the middle  
361 finger as significantly higher when the thermal stimulus was presented on the adjacent fingers  
362 (average of conditions 2 and 4, mean  $\pm$  SD:  $1.9 \pm 0.909$ ) than during the no-warmth condition  
363 (condition 1, mean  $\pm$  SD:  $0.54 \pm 0.608$ ) (see Figure 2A).

364 The planned comparison between participants' pain ratings during no-warmth (condition 1) and  
365 warmth on the adjacent fingers (average of conditions 2 and 4) was statistically significant ( $t_{13}$   
366  $= 4.184$ ,  $p = 0.001$ ; 95% CI:  $0.321$ ,  $+\infty$ ; Cohen's  $d = 0.474$ ). Baseline pain on the middle finger  
367 (mean  $\pm$  SD:  $3.2 \pm 1.354$ ) dropped by the 17.3% when a warm stimulus was delivered to either  
368 of the adjacent fingers (mean  $\pm$  SD:  $2.647 \pm 0.983$ ) (see Figure 2B). The subjective perception  
369 was supported by a decrease of the 22.2% in the amplitude of the N2 component (see Figure  
370 2C). This effect did not formally reach the conventional boundaries for statistical significance  
371 ( $t_{13} = -1.769$ ,  $p = 0.050$ ; 95% CI:  $-\infty$ ,  $0.016$ ; Cohen's  $d = 0.629$ ). However, a Bayesian paired-  
372 sample t-test showed that it is very unlikely that this result could be explained by the null  
373 hypothesis ( $BF_{01} = 0.572$ , error  $< 0.001\%$ ). The amplitude of the P2 component was not  
374 modulated by warmth ( $t_{13} = 0.043$ ,  $p = 0.483$ ; 95% CI:  $-1.767$ ,  $+\infty$ ; Cohen's  $d = 0.009$ ;  $BF_{01} =$   
375  $3.822$ , error  $< 0.001\%$ ). Warmth delivered on an adjacent finger had a significant suppressive  
376 effect on pain perception and LEPs.

377 ***Planned comparison 3: Is the suppressive effect of warmth on pain spatially***  
378 ***graded?***

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

384 Importantly, although perceived warmth between same and adjacent fingers was  
385 significant ( $t_{13} = 3.267$ ,  $p = 0.003$ ; 95% CI: 0.420,  $+\infty$ ; Cohen's  $d = 0.754$ ) (see Figure 3A),  
386 neither magnitude estimates of pain ( $t_{13} = 1.441$ ,  $p = 0.087$ ; 95% CI:  $-\infty$ , 0.407; Cohen's  $d =$   
387 0.184), nor LEP amplitudes (N2:  $t_{13} = 0.967$ ,  $p = 0.176$ ; 95% CI: -0.654,  $+\infty$ ; Cohen's  $d =$   
388 0.209; P2:  $t_{13} = -0.13$ ,  $p = 0.449$ ; 95% CI:  $-\infty$ , 1.102; Cohen's  $d = 0.019$ ) were significantly  
389 different in the two thermal conditions (see Figure 2B, C, and D). While the Bayesian analysis  
390 on the behavioral data was inconclusive ( $BF_{01} = 0.881$ , error  $< 0.001\%$ ), those on the LEPs data  
391 strongly favored the null hypothesis (N2:  $BF_{01} = 6.521$ , error  $< 0.001\%$ ; P2:  $BF_{01} = 3.358$ , error  
392  $< 0.001\%$ ). Therefore, perceptual and electrophysiological correlates of pain were not  
393 statistically different when a warm stimulus was delivered to the same finger or to the adjacent  
394 fingers.

395 ***Planned comparison 4: Does warmth summation cause graded inhibition?***

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



404 To test whether spatial summation of multiple simultaneous thermal stimuli had a  
405 graded inhibitory effect on pain processing, we conducted a linear trend analysis with weights  
406 -1, 0, and 1 on the conditions where warmth was applied on one (average of condition 2, 3, and  
407 4), two (average of condition 5, 6, and 7), or three fingers (condition 8). The analyses showed  
408 no effect of spatial summation of warmth on either pain perception ( $t_{13} = -1.22$ ,  $p = 0.141$ ; 95%  
409 CI:  $-\infty$ , 0.104; Cohen's  $d = 0.653$ ), nor LEPs (N2:  $t_{13} = -0.158$ ,  $p = 0.438$ ; 95% CI:  $-\infty$ ,  $+\infty$ ;  
410 Cohen's  $d = 0.085$ ; P2:  $t_{13} = -0.115$ ,  $p = 0.455$ ; 95% CI:  $-\infty$ , 0.687; Cohen's  $d = 0.062$ ).  
411 Increasing the number of simultaneous thermal stimuli did not affect subjective perception of  
412 pain (one finger:  $2.708 \pm 0.965$ ; two fingers:  $2.732 \pm 0.949$ ; three fingers:  $2.509 \pm 0.965$ ) (see  
413 Figure 3B) nor the amplitude of N2 (one finger:  $-11.59 \pm 3.404$ ; two fingers:  $-11.66 \pm 3.458$ ;  
414 three fingers:  $-11.74 \pm 5.458$ ) or P2 (one finger:  $10.02 \pm 4.374$ ; two fingers:  $10.93 \pm 4.777$ ;  
415 three fingers:  $9.97 \pm 4.536$ ) LEP components (see Figure 3C and D).

416 We then performed a Bayesian analysis to determine whether the data supported the  
417 null hypothesis or could be due to a lack of statistical power. We found that the null hypothesis  
418 was always more than 3 times more likely than the alternative hypothesis (magnitude estimates  
419 of pain:  $BF_{01} = 7.208$ , error < 0.001%; N2:  $BF_{01} = 3.284$ , error < 0.001%; P2:  $BF_{01} = 4.021$ ,  
420 error < 0.001%), suggesting that the absence of a linear trend among conditions with increasing  
421 number of thermal stimuli was not simply due to a lack of statistical power. Therefore,  
422 perception and EEG markers of pain were not affected by different amounts of spatial  
423 summation of warmth.

424

425

426 \*\*\* Please insert Figure 2 here \*\*\*

427

428

429 \*\*\* Please insert Figure 3 here \*\*\*

430

## 431 **Discussion**

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

446         Moreover, we found no evidence that the number/area of warm stimuli influenced either  
447 pain ratings or LEP amplitudes. Thus, delivering warmth to one, two or three digits did not  
448 linearly modulate pain sensation evoked by laser stimulation. This results thus rule out a model  
449 in which warm inputs first undergo spatial summation, followed by a subsequent suppressive  
450 effect of the *total* warm signal on nociception. That model would predict a linear decreasing  
451 trend in pain ratings and LEP amplitudes as the number/area of warm stimuli increased – since  
452 this would have produced a stronger, summated warm signal that might potentially inhibit  
453 nociceptive signaling. Our linear trend analysis clearly showed that while *thermal* perception  
454 was strongly affected by the number of simultaneous stimuli presented, neither perceptual nor  
455 electrophysiological correlates of pain delivered during thermal stimulation followed this trend.  
456 In fact, using Bayesian methods, we found statistical evidence that no such trend existed.  
457 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 A $\delta$  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

485 delivered on the middle finger (Fardo et al. 2018). However, while this hypothesis would  
486 predict a significant difference in pain ratings and/or LEPs between our condition 2 (warmth on  
487 the index finger) and condition 4 (warmth on the ring finger), we found no evidence for any  
488 difference in sensory ratings or LEPs ( $p > 0.200$  in all cases). This is in line with previous  
489 studies (Green 1978; Marotta et al. 2015) showing that the differing segmental projections of  
490 medial and ulnar nerves have little to no on interactions between simultaneous thermal or  
491 thermo-tactile stimuli. Finally, while we assume that warmth-induced pain relief reflects a  
492 central interaction, we cannot entirely exclude a contribution of some unknown peripheral  
493 interactions, e.g. through vascular effects. However, the fact that we delivered warm stimuli on  
494 the fingertips, and laser pain on the middle finger dorsum, makes explanations based on local  
495 peripheral changes unlikely.

496

#### 497 ***Spatial summation of warmth during warmth-pain interaction***

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

510 Tran and colleagues (2008) showed that the physical intensity of a thermal stimulus  
511 affects nociceptive processing in a graded manner: the A $\delta$ -mediated cortical responses induced

512 by electrical epidermal stimulation were much more attenuated by a 50°C, than a 37°C, C fiber  
513 conditioning stimulus. This suggests that spatial-summation-induced increases in *perceived*  
514 warmth, might produce a similar monotonic, progressive reduction of pain and nociceptive  
515 cortical responses. Conversely, our findings clearly show that warmth-pain interaction is an  
516 all-or-nothing phenomenon. Neither pain ratings nor LEPs showed progressive modulation by  
517 increasing levels of perceived warmth.

518         When warm stimulation is applied on the index and/or ring finger of one hand, an  
519 illusory perception of warmth occurs on the thermally neutral middle finger (Cataldo et al.  
520 2016; Green 1977, 1978; Ho et al. 2011). This phenomenon, known as Thermal Referral, has  
521 been linked to spatial summation mechanisms occurring within the thermoceptive system  
522 (Cataldo et al. 2016). In the present study, when a single adjacent (index or ring) finger was  
523 thermally stimulated, ratings of warmth on the middle finger were significantly higher than the  
524 no warmth condition. Although the thermal state of the middle finger was in fact neutral in  
525 each of these conditions, all participants reported higher perception of warmth during thermal  
526 referral condition compared with no thermal stimulation. This indicates that an illusory spread  
527 of perceived warmth across digits, also occurred in our paradigm.

528

### 529 ***Mechanisms underlying warmth-pain interaction***

530 Different theories have been proposed to explain thermo-nociceptive interactions. Based on the  
531 finding that higher-intensity stimulation to one pathway produces a stronger inhibitory effect on  
532 the other, Truini and colleagues (2007) proposed that the A $\delta$ -C interaction is based on a *first*  
533 *come, first served* principle, where only the earliest signals can induce cerebral responses.  
534 LEPs would then reflect the output of a network detecting rapid *temporal* changes in firing  
535 relative to a preceding state (Garcia-Larrea 2004; Truini et al. 2007). A similar conclusion in  
536 the *spatial* domain has been proposed by Churyukanov and colleagues (2012), who postulated  
537 that A $\delta$  fibers acts as local change detectors, rather than pure level detectors. The threshold for

538 A $\delta$  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 A $\delta$   
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 A $\delta$  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

566 graded phenomenon, where the ascending activity in the spinal pain tracts is sufficient to  
567 activate a descending modulatory response, regardless of whether the final cortical experience  
568 induced by that barrage is painful or not (Granot et al. 2008). Our results suggest that these key  
569 properties of CPM, namely non-gradedness and lack of spatial specificity, also apply to the  
570 ‘warmth inhibits pain’ interaction. Similarly to CPM, warmth-related thermoceptive channels  
571 may interact with nociceptive pathways through an endogenous descending modulatory system,  
572 possibly originating in the brainstem (Granot et al. 2008).

573

574 \*\*\* Please insert Figure 4 here \*\*\*

575

## 576 **Conclusion**

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

591

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601 **Disclosure**

602 The authors declare no conflict of interest.



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- 779

780 **Figure Captions**

781 **Figure 1. Thermo-nociceptive conditions.**

782 Painful stimuli were delivered to the dorsum of participants' right middle finger through a CO<sub>2</sub> laser  
783 pulse. Thermal stimuli were delivered by three 13 mm diameter Peltier-based thermodes applied at the  
784 level of the intermediate phalanges of right index, middle, and ring fingers. Warm stimulation was  
785 given in eight different conditions (see numbers). We then contrasted combinations of conditions in  
786 order to test four directional hypotheses regarding thermal-nociceptive interactions (see method). a. no  
787 warmth, laser only condition; b. warmth and laser pain on the middle finger; c. laser pain on the middle  
788 finger and warmth on the index or ring finger (i.e. adjacent fingers condition).

789

790 **Figure 2. Effect of location of thermal stimulation on warmth (W) and laser pain (L) processing.**

791 A. Magnitude estimate of warmth. Compared with the laser only (no warmth) condition, participants  
792 perceived higher intensities of warmth in both thermal conditions (same/adjacent finger). Crucially,  
793 perceived warmth on the middle finger was significantly higher when the thermal stimulus was delivered  
794 on the middle finger itself (Mid), rather than on an adjacent finger (avg. Ind, Rin). B. Magnitude  
795 estimate of pain. Pain perception was significantly reduced in both thermal conditions (same/adjacent  
796 finger/s), compared with no thermal stimulation. However, same and adjacent finger conditions were  
797 not statistically different. C. N2 wave. Peak amplitude of N2 component was significantly reduced in  
798 both thermal conditions compared with no thermal stimulation condition. However, the amount of pain  
799 suppression was the same irrespectively of the site of stimulation. D. P2 wave. P2 component was not  
800 affected by neither of the thermal conditions. Error bars represent the standard error of the mean.

801 n.s.,  $p > 0.05$ ; +,  $p = 0.05$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

802

803 **Figure 3. Effect of number of thermal stimuli on warmth perception (A), pain perception (B), and**  
804 **N2 (C) and P2 (D) LEP components.**

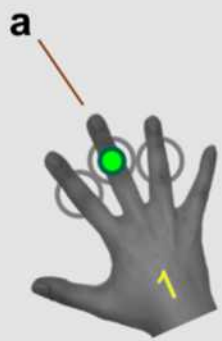
805 A. Magnitude estimate of warmth. Increasing the number of fingers thermally stimulated induced a  
806 significant monotonic increase in the apparent intensity of warmth on the middle finger. However,  
807 neither perceptual (B) nor electrophysiological (C and D) correlates of pain were affected by the number  
808 of simultaneous thermal stimulations. Grey lines represent data from single participants. Colored lines  
809 represent the average across participants. Colored shading of black lines represents the standard error of  
810 the mean. \*\*\*,  $p < 0.001$ .

811

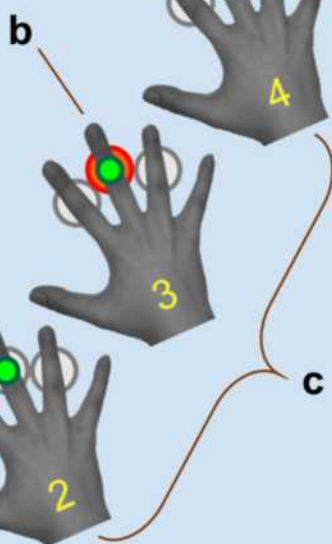
812 **Figure 4. Schematic model of warmth-pain interaction.**

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

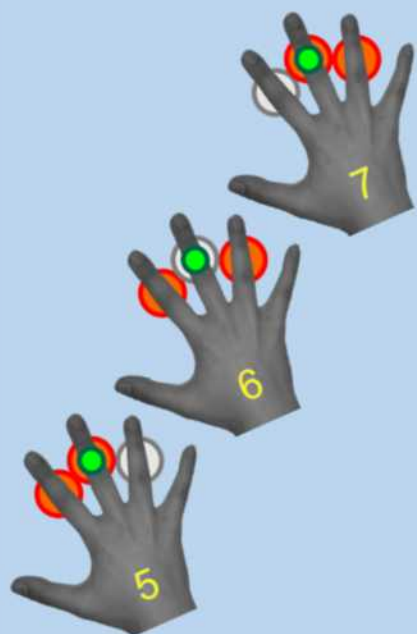
No warmth



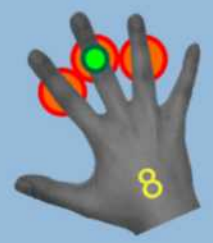
One finger warm

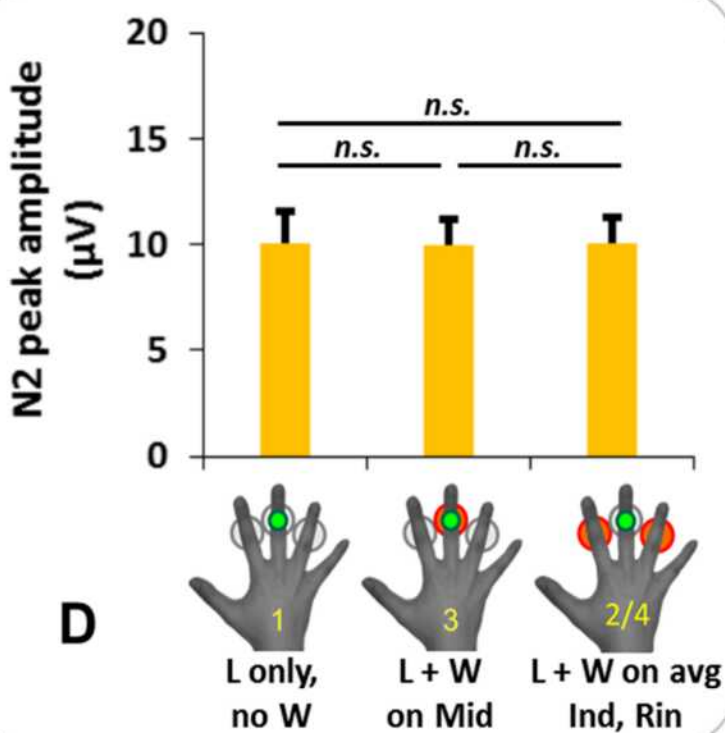
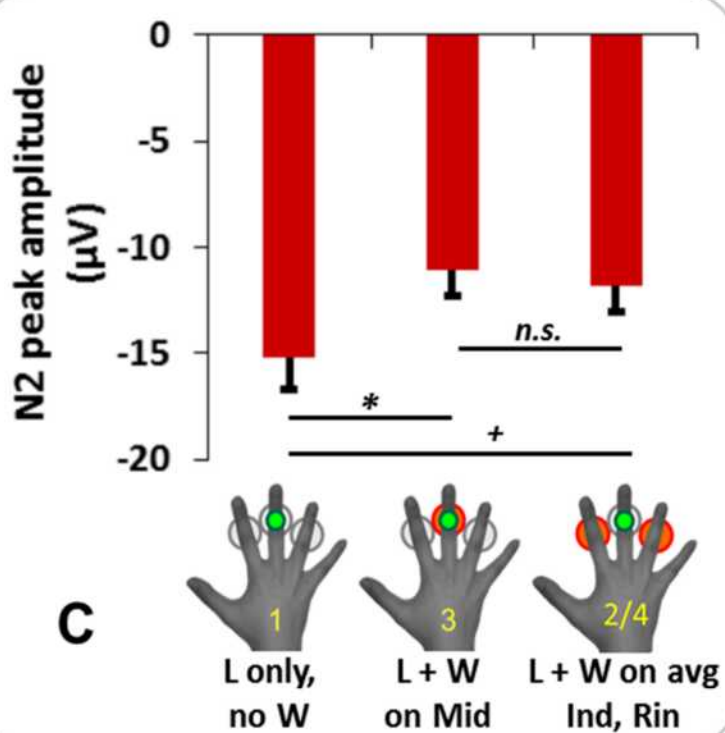
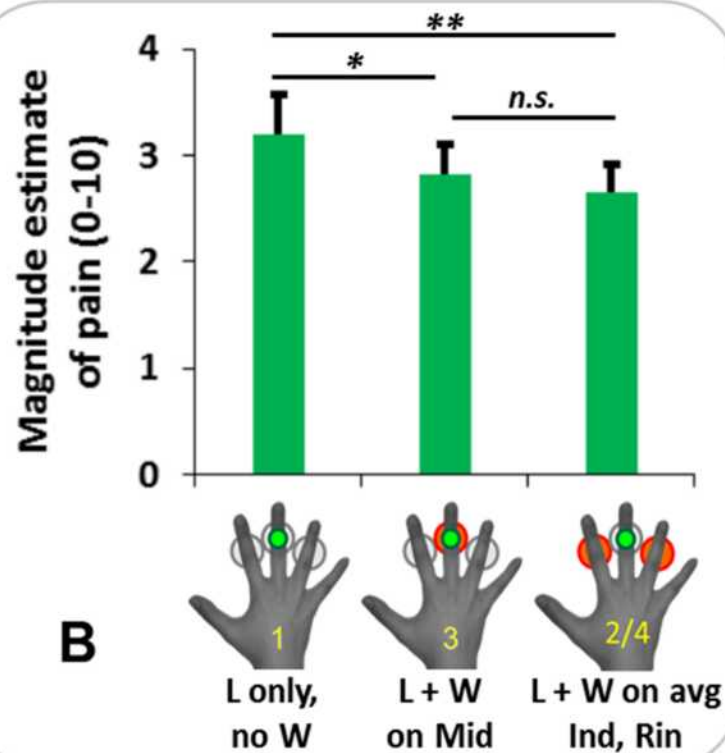
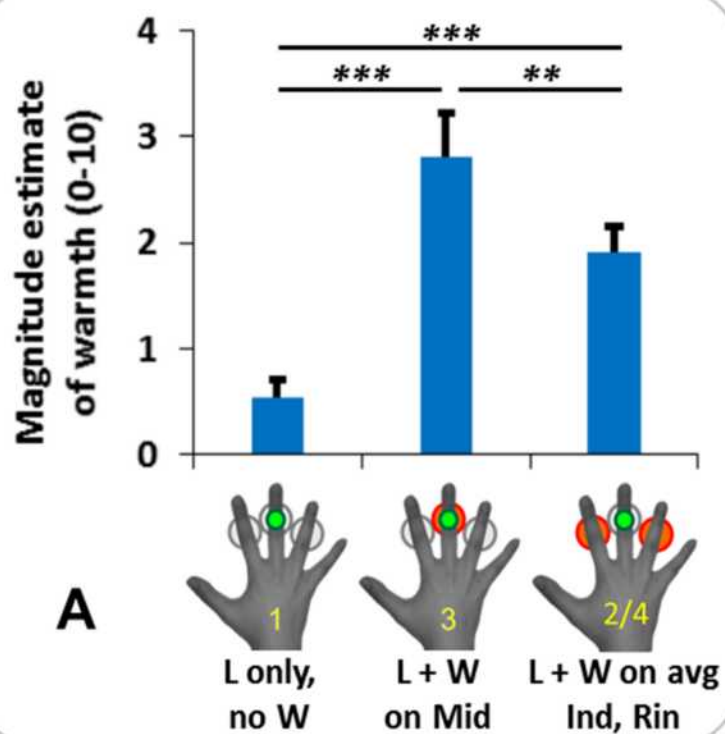


Two fingers warm

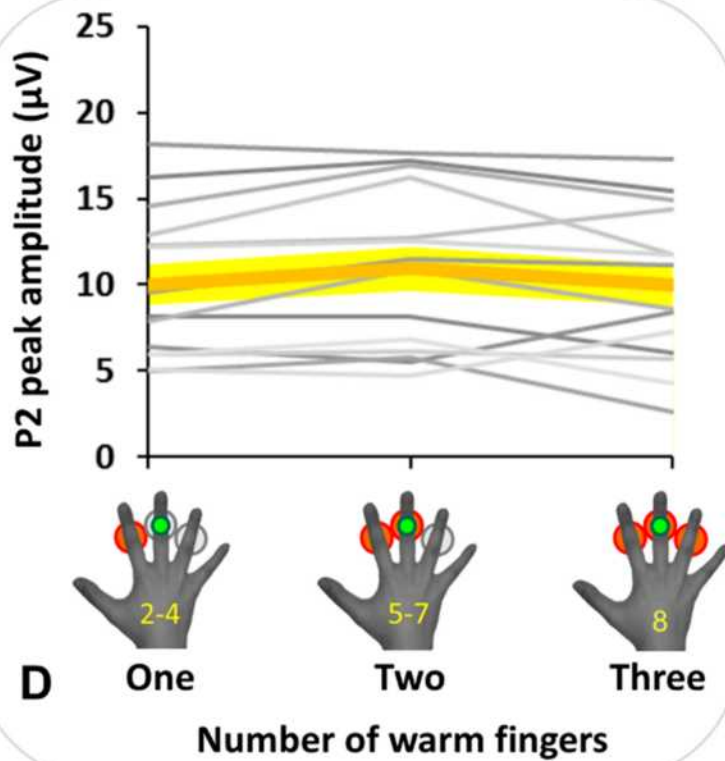
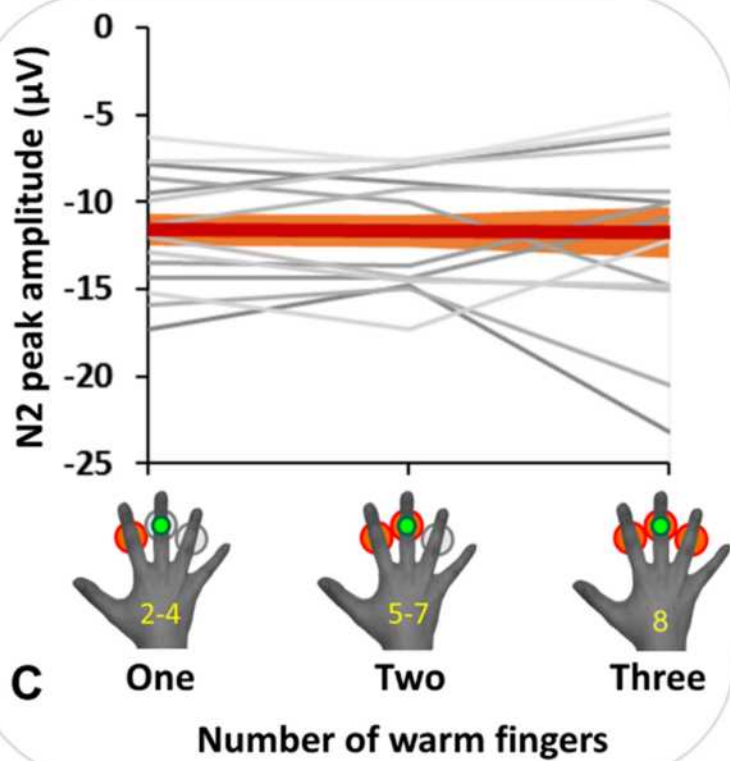
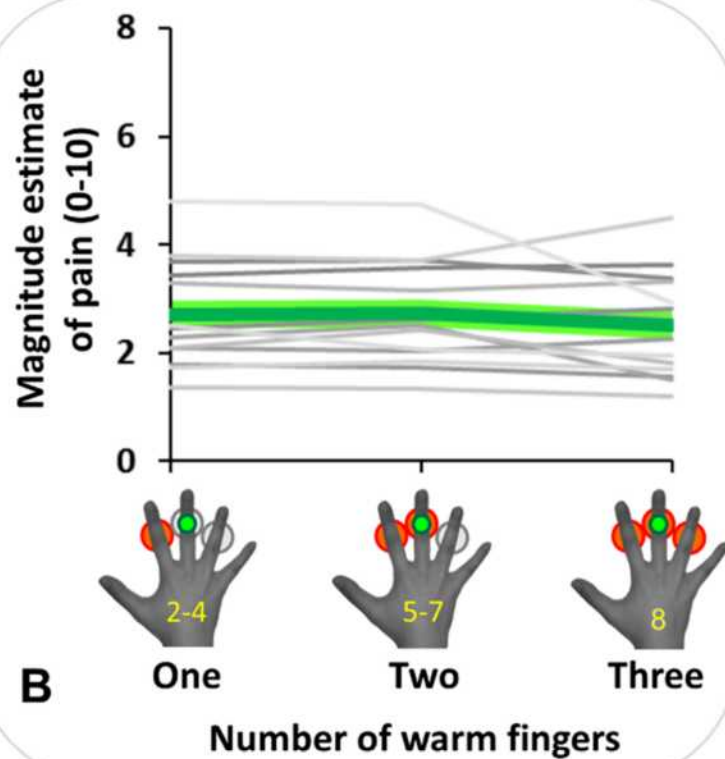
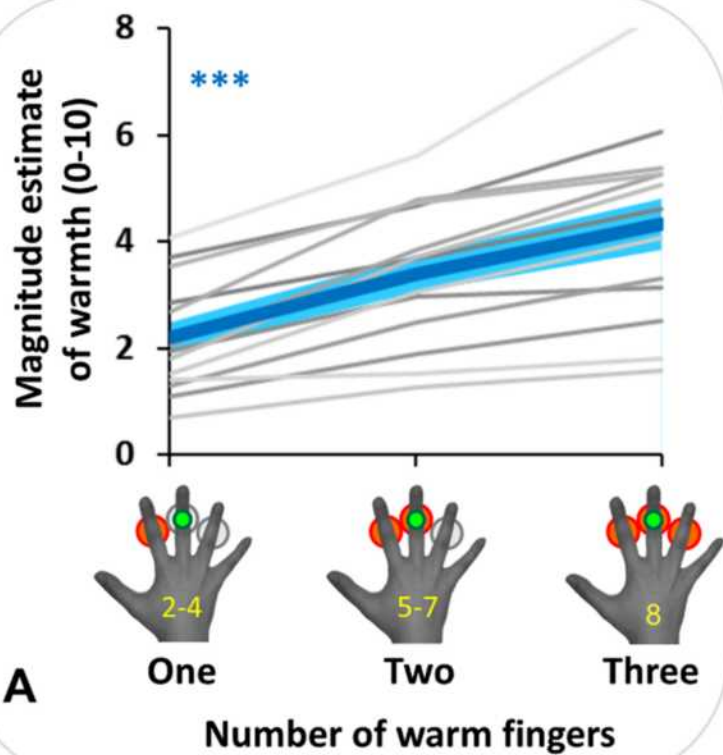


Three fingers warm









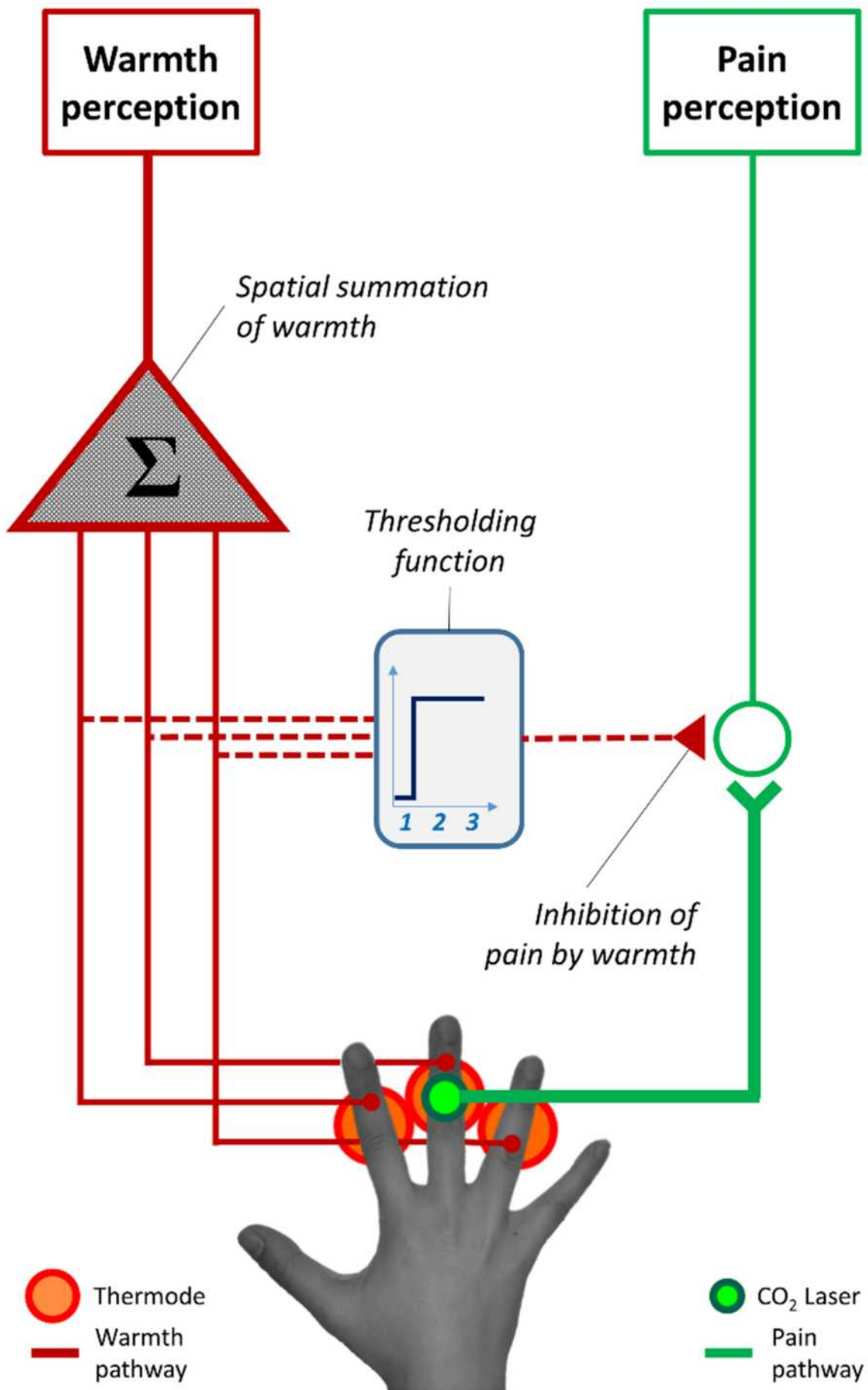


Table 1. Table of coefficients for the four research question

	<i>Thermal conditions</i>							
	<i>No warmth</i>	<i>Index warm</i>	<i>Middle warm</i>	<i>Ring warm</i>	<i>Ind+Mid warm</i>	<i>Ind+Rin warm</i>	<i>Mid+Rin warm</i>	<i>All warm</i>
<i>1. Does warmth on the same finger inhibit pain?</i>	<b>-1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<i>2. Does warmth on the adjacent fingers inhibit pain?</i>	<b>1</b>	<b>-1/2</b>	<b>0</b>	<b>-1/2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<i>3. Is the effect of warmth on pain spatially specific?</i>	<b>0</b>	<b>-1/2</b>	<b>1</b>	<b>-1/2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<i>4. Does warmth summation cause graded inhibition?</i>	n/a	<b>-1/3</b>	<b>-1/3</b>	<b>-1/3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>

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