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Published in:
Clinical Neurophysiology

DOI (link to publication from Publisher):
[10.1016/j.clinph.2022.11.008](https://doi.org/10.1016/j.clinph.2022.11.008)

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Publication date:
2023

Document Version
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Lejeune, N., Petrossova, E., Frahm, K. S., & Mouraux, A. (2023). High-speed heating of the skin using a contact thermode elicits brain responses comparable to CO₂ laser-evoked potentials. *Clinical Neurophysiology*, 146, 1-9. Advance online publication. <https://doi.org/10.1016/j.clinph.2022.11.008>

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1 **High-speed heating of the skin using a contact thermode elicits comparable**
2 **brain responses to those elicited by a CO₂-laser device**

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33
34 Number of words: 4265 words

35 Number of tables and figures: 2 tables and 8 figures

36 Number of words in structured abstract : 196 words

1 **ABSTRACT**

2 **OBJECTIVE**

3 To compare nociceptive event-related brain potentials elicited by a high-speed contact-
4 thermode vs. an infrared CO₂ laser stimulator.

5 **METHODS**

6 Contact heat-evoked potentials (CHEPs) and CO₂ laser-evoked potentials (LEPs) were
7 recorded in healthy volunteers using a high-speed contact-thermode (>200°C/s) and a
8 temperature-controlled CO₂ laser. In separate experiments, stimuli were matched in terms of
9 target surface temperature (55°C) and intensity of perception. A finite-element model (FEM)
10 of skin heat transfer was used to explain observed differences.

11 **RESULTS**

12 For 55°C stimuli, CHEPs were reduced in amplitude and delayed in latency as compared to
13 LEPs. For perceptually matched stimuli (CHEPs: 62°C; LEPs: 55°C), amplitudes were similar,
14 but CHEPs latencies remained delayed. These differences could be explained by skin thermal
15 inertia producing differences in the heating profile of contact vs radiant heat at the dermo-
16 epidermal junction (DEJ).

17 **CONCLUSION**

18 Provided that steep heating ramps are used, and that target temperature is matched at the DEJ,
19 contact and radiant laser heat stimulation elicit responses of similar magnitude. CHEPs are
20 delayed compared to LEPs.

21 **SIGNIFICANCE**

22 CHEPs could be used as an alternative to LEPs for the diagnosis of neuropathic pain. Dedicated
23 normative values must be used to account for differences in skin thermal transfer.

24 **KEYWORDS**

25 Nociception; Pain; Thermal stimulation; Electroencephalography

26

27 **HIGHLIGHTS**

- 28 • Contact heat- (CHEPs) and laser- (LEPs) evoked potentials have a similar amplitude
29 provided that target temperature is matched at the level of the dermo-epidermal junction.
- 30 • CHEPs are delayed as compared to LEPs, due to differences in the nature of the heating
31 mechanism and thermal inertia of the skin.
- 32 • CHEPs could be used to assess spinothalamic function in patients, provided that specific
33 normative values are used.

34

35 **INTRODUCTION**

36 The synchronous and phasic activation of skin nociceptors elicits event-related brain
37 potentials (ERPs) that can be recorded using scalp electroencephalography (EEG)
38 (Baumgärtner et al., 2005; Mouraux and Iannetti, 2018). To generate such synchronous
39 activation of peripheral nociceptors, infrared laser devices have been used extensively both in
40 research and in clinical practice (Cruccu et al., 2008; Nahra and Plaghki, 2003; R.D. Treede et
41 al., 2003). Laser devices can produce very rapid increases in skin temperature by thermal
42 radiation, allowing a preferential and phasic activation of heat-sensitive A δ - and C- fiber free
43 nerve endings without co-activating large-diameter non-nociceptive mechano-sensitive
44 A β -fibers (Plaghki and Mouraux, 2003). The so-called laser-evoked brain potentials (LEPs)
45 usually display a good signal-to-noise ratio (SNR) because the steep heating ramps generate a
46 very synchronized afferent volley within quickly-responding heat-sensitive A-fiber nociceptors
47 (Treede et al., 1995). However, laser devices remain seldom used in clinical centers, probably
48 because their use requires strict safety regulations to avoid eye injury, and because most
49 available devices offer no control over target temperature. Nevertheless, the recording of LEPs
50 is currently the recommended diagnostic technique to assess the function of spino-thalamic
51 pathways in patients (Cruccu et al., 2008). Alternative methods have been proposed, such as
52 intra-epidermal electrical stimulation (IES) to selectively activate superficial free nerve endings
53 (Inui et al., 2002), and contact heat stimulation using thermodes (Greffrath et al., 2007).
54 Unfortunately, IES selectively activates nociceptors only if very low intensities are used, and
55 the elicited responses have a low SNR (Mouraux et al., 2010). Similarly, previous recordings
56 of contact heat-evoked potentials (CHEPs) have used thermal probes producing relatively slow
57 heating slopes (less than 70°C/second), resulting in a poor temporal recruitment of the afferent
58 volley, and low SNR responses (Atherton et al., 2007). Very recently, a novel contact thermode
59 based on micro Peltier elements, the Thermal Cutaneous Stimulator, has been developed and

60 made commercially available (TCSII, QST.Lab, Strasbourg, France). The device can generate
61 very steep cooling and heating ramps of up to 300 °C/s. Furthermore, the device is light-weight
62 and easy to manipulate even at patient bedside. Previous studies showed that the very steep
63 cooling ramps produced by the TCSII allows the recording of cool-evoked brain potentials
64 having a high SNR (De Keyser et al., 2018; Leone et al., 2019). Therefore, contact heat
65 stimulation with very steep heating ramps similar to those produced by infrared laser
66 stimulation could constitute a compelling alternative to the recording of LEPs.

67 The aim of the present study was to compare CHEPs elicited by very steep heating ramps
68 of 300°C/s to LEPs elicited by infrared CO₂-laser stimulation, and to assess whether reliable
69 responses can be obtained at single-subject level, as required for clinical diagnosis. **Stimuli**
70 **were delivered to the volar forearm.** The study consisted in four parts. In Experiment 1, we
71 compared the EEG responses elicited by radiant heat and contact heat stimuli matched in terms
72 of target skin surface temperature, stimulated surface area and stimulus duration. In Experiment
73 2, the target temperatures used for radiant heat and contact heat stimulation were adjusted to
74 match their intensity of perception. In Experiment 3, we compared LEPs and CHEPs elicited
75 by such perception-matched stimuli. Finally, in Experiment 4, we utilized a computational
76 model of radiant heat and contact heat stimulation of the skin to compare the temperature **time**
77 **course** at the dermal-epidermal junction (DEJ), where the majority of heat-sensitive free nerve
78 endings are located, to investigate if the observed differences between LEPs and CHEPs can be
79 explained by differences in heat transfer within the skin.

80

81 **METHODS**

82 Participants

83 **The three first experiments of the study were conducted in healthy subjects, while Experiment**
84 **4 used the data obtained in Experiments 1 and 3 .** Three different groups of healthy volunteers

85 took part in Experiment 1 (12 participants; 7 females and 5 males, aged 20-34, all right-handed),
86 in Experiment 2 (10 participants; 4 females and 6 males, aged 22-33, all right-handed) and in
87 Experiment 3 (12 participants; 7 females and 5 males, aged 20-28, 10 right-handed),
88 respectively. Participants did not suffer from any neurological disorder. The study was
89 approved by the local ethics committee and conformed to the latest version of the declaration
90 of Helsinki. All participants gave written informed consent.

91 Stimulation devices

92 **Temperature-controlled CO₂ laser stimulation.** In all three experiments, radiant heat stimuli
93 were generated using a temperature-controlled CO₂ laser stimulator (Laser Stimulation Device
94 [LSD]; SIFEC, Belgium). The control of temperature uses a thermal sensor that continuously
95 records temperature at the skin target surface with a sampling rate of 10000 Hz. The measured
96 skin surface temperature is sent to a controller that regulates the laser power output to maintain
97 skin temperature as close as possible to the target temperature throughout the duration of the
98 stimulus. The heat source is a 25 W radiofrequency-excited CO₂ laser (Synrad 48-2; Synrad,
99 WA). The skin surface temperature measured by the thermal sensor also drives the voltage of
100 an analog output (0 V = 20°C and 10 V = 70°C) which can be used to record the time course of
101 each thermal stimulus. Power control is achieved by pulse width modulation at a 5-kHz clock
102 frequency. The stimuli are delivered through a 6 meters optical fiber. By vibrating this fiber at
103 some distance of the source, a quasi-uniform spatial distribution of radiative power within the
104 stimulated area is obtained. At the end of the fiber, optics are used to collimate the beam. Beam
105 diameter at target was 12 mm, resulting in a 113 mm² stimulus surface area

106 **Contact heat stimulation.** The TCSII (QST Lab, Strasbourg, France) is a micro Peltier
107 elements-based contact thermode able to generate very steep heating ramps of up to 300 °C/s.
108 The stimulation probe consists, on its extremity, of a flat 30-mm diameter surface containing
109 15 micro-Peltier elements of 7.7 mm² each. The baseline temperature can be set to the neutral

110 skin temperature of each participant. Feedback on the temperature is obtained via five
111 thermocouples, evenly distributed on the surface of the probe, that measure skin temperature
112 with a sampling rate of 100-200 Hz and drive the micro-Peltier elements to target temperature
113 (De Keyser et al., 2018; Mulders et al., 2020). After each stimulation, these temperature time
114 courses can be downloaded from the device for offline analyses.

115 *Experiment 1: Brain responses to laser- and contact heat-stimuli matched in terms of target*
116 *skin surface temperature*

117 In Experiment 1, we compared LEPs and CHEPs elicited by transient nociceptive heat stimuli
118 applied onto the skin of the volar forearm using either the LSD or the TCSII. The stimuli were
119 matched in terms of skin surface target temperature, stimulus duration and stimulated surface
120 area. The target temperature was set to 55°C for both devices. Stimulus duration was set to 200
121 ms to ensure that both devices would easily reach the target temperature within the stimulus
122 duration. Stimulation surface was set to 113 mm² for the LSD (12 mm diameter lens) and 115.50
123 mm² for the TCSII (15 micro-Peltier elements of 7.7 mm² each). The time courses of skin
124 temperature measured by both devices from -0.5 s to +1.0 s relative to stimulation onset were
125 recorded for offline analysis (LSD: analog voltage output sampled at 1 kHz using a National
126 Instruments NI USB-6343 analog-to-digital converter; TCSII: digitized measure sampled at 50
127 Hz by the device and downloaded after each stimulus). The maximal heating slopes of the
128 devices were of 1041 ±122 °C/s for the LSD and 388 ±15 °C/s for the TCII, respectively.
129 Maximal heating slopes were achieved 17 ±2 ms and 30 ±1 ms after stimulation onset for the
130 LSD and the TCSII, respectively. The maximum temperature measured by the LSD during laser
131 stimulation was 58 ±1°C. The maximum temperature measured by the TCSII during contact
132 heat stimulation 55 ±1°C. The temperature time course generated by the LSD and the TCSII
133 were different after the end of the 200 ms stimulus, whereas the TCSII actively cooled the skin

134 to return it to the baseline skin temperature, the LSD device cannot actively cool the skin,
135 resulting in a slower cooling phase (Figure 1).

136 **Procedure.** Before the start of the EEG recording, participants were familiarized to the heat
137 sensations produced by the LSD and the TCSII using two test stimuli for each device. Then,
138 stimuli were delivered in four blocks of 20 stimuli delivered using either the LSD or the TCSII
139 to the left or right volar forearm, resulting in 40 stimuli for each condition. Stimuli were
140 delivered to both forearms to reduce the total number of heat stimuli delivered at each forearm
141 and thereby lessen possible effects of stimulus repetition due to fatigue or sensitization of
142 nociceptors, and central habituation or sensitization. The order of the blocks was
143 counterbalanced across subjects. The first stimulated forearm was randomized between
144 participants and the stimulated forearm was interchanged between each block. The subjects
145 were given a five-minute break after each stimulation block. The interstimulus interval (ISI)
146 was self-paced by the experimenter (with a minimum of seven seconds) and the stimulated spot
147 of the laser or the probe of the TCSII was slightly shifted between each stimulus (Cruccu et al.,
148 2008). This long ISI also allowed placing the probe of the TCSII on the skin for some time
149 before delivery of the thermal stimulus to avoid interference from concomitant activation of
150 low-threshold mechanoreceptors when the probe is applied on the skin. After each stimulation
151 block, the subjects were asked to rate the average intensity of the stimuli across the block, using
152 a numerical rating scale (NRS). They were asked to rate the intensity of the stimulation from 0
153 to 100, with 0 meaning that the stimulus was not perceived, 50 being the pain threshold and
154 100 being the most painful percept imaginable. This adaptation of the numerical pain rating
155 scale allows the subject to rate, under 50, the intensity of a stimulus eliciting a percept without
156 evoking a painful experience.

157 **EEG recording, preprocessing and analysis.** The EEG was recorded using 64 Ag-AgCl
158 electrodes placed on the scalp according to the international 10-10 system (WaveGuard 64-

159 channel cap; Advanced Neuro Technologies). Signals were amplified and digitized at 1000 Hz
160 (ASA 64; Advanced Neuro Technologies). Impedances were kept below 10 k Ω , The EEG
161 recordings were analyzed offline using Matlab R2017a (The MathWorks) and the Letswave 6
162 toolbox for EEG data analysis (<http://letswave.org>) (Mouraux & Iannetti, 2008). The
163 continuous EEG recordings were filtered using a 0.5-30 Hz bandpass 4th degree Butterworth
164 filter. The EEG was then segmented in epochs of 1.5 second, starting 0.5 second before stimulus
165 onset. An Independent Component Analysis using the FastICA algorithm was used to remove
166 eye movement and eye blink artifacts. Finally, before averaging, the signals were baseline-
167 corrected regarding the time interval -0.5 to 0 seconds relative to the stimulus onset. The signals
168 recorded at the vertex electrode Cz referenced to the average of the two earlobes (A1A2) were
169 used to identify and characterize the latency and amplitude of the negative-positive complex
170 elicited by heat stimulation at the scalp vertex (N2-P2 complex). The signals recorded at the
171 contralateral electrode (T3/4) referenced to Fz were averaged and used to identify and
172 characterize the latency and amplitude of the earlier negative response maximal over central-
173 temporal electrodes contralateral to the stimulated limb (N1 wave). The signal-to-noise ratio of
174 the N2-P2 complex was computed as the ratio between peak-to-peak signal amplitude in the
175 post-stimulus time window (0 to 1 second relative to stimulation onset) and the peak-to-peak
176 signal amplitude in the pre-stimulus time window (-0.5 to 0 second relative to stimulation
177 onset).

178 **Statistics.** Paired comparisons were used to compare the perceptual and EEG responses to laser
179 and contact heat stimuli. Beforehand, a Shapiro-Wilk test for normality of distribution was
180 performed on each dataset. The normality of the distribution was set at $p > 0.05$. Then, paired-
181 sample *t*-tests were performed to compare intensities of perception, peak latencies of N1, N2
182 and P2, and peak amplitudes of N1, N2 and P2 elicited by laser stimulation as compared to
183 contact heat stimulation. Statistical significance was set at $p < 0.05$.

184 Experiment 2: Perception-matched laser and contact heat stimuli

185 In Experiment 2, the aim was to identify the target temperature of a contact heat stimulus
186 generated by the TCSII which would generate a sensation of similar intensity than the sensation
187 produced by a 55°C laser stimuli delivered by the LSD. To this aim, we used an adaptive
188 staircase method with the subjective report of the compared intensity of perception between
189 both devices at varying temperatures for the TCSII. All other parameters of stimulation were
190 identical to Experiment 1.

191 **Procedure.** Thermal stimuli were applied to the left and right volar forearms of the subjects in
192 two separate sessions. The side of the stimulated forearm for the first session and the order of
193 the stimuli (LSD or TCSII) were randomized between participants. The LSD target temperature
194 was set to 55°C and remained unchanged throughout the experiment. At the beginning of each
195 session, the target temperature of the TCSII was arbitrarily set to 60°C. Pairs of stimuli (contact
196 heat stimulus followed by a laser heat stimulus or laser heat stimulus followed by a contact heat
197 stimulus) were delivered on the same forearm, at separate locations and using a self-paced ISI
198 of at least 7 seconds. After each stimulation pair, the subject was asked which of the two stimuli
199 was the most or the less intense among the two. If the TCSII stimulus was perceived as more
200 intense than the LSD stimulus, the target temperature of the TCSII for the next trial was
201 decreased by 1°C; else, it was increased by 1°C. To avoid any burn injury, the maximum
202 allowable temperature delivered by the TCSII was set to 70°C. The target of the LSD and the
203 probe of the TCSII were slightly displaced after each trial. The pairs of stimuli were repeated
204 until achievement of four staircase reversals. A reversal is defined as the occurrence of a change
205 in the comparative perception after a modification of the TCSII stimulus temperature, i.e., when
206 a stimulus comes from being described as less (or more) intense to being described as more
207 (or less) intense (than the comparison stimulus).

208 **Statistics.** The threshold temperatures at each forearm (i.e., the temperature at which perception
209 of the stimuli delivered with the TCSII matched the perception of the 55°C LSD stimulus) were
210 obtained at single-subject level by averaging the temperatures at which the four staircase
211 reversals occurred. A paired sample *t*-test was done to check for a difference between the results
212 obtained at both volar forearms. An absence of significant difference between both volar
213 forearms would allow computation of the average threshold-temperature across both forearms
214 of all subjects.

215 Experiment 3: Comparing brain responses elicited by perception-matched stimuli

216 In Experiment 3, the aim was to compare the EEG responses elicited by laser and contact heat
217 stimulation using stimuli matched in terms of the intensity of perception. The target temperature
218 of the LSD was set to 55°C. The target temperature of the TCSII was set to 62°C based on the
219 results of Experiment 2 (see example of temperature time courses of these stimuli in Figure 1).
220 In addition, to better match passive cooling of the skin following laser stimulation, high-speed
221 post-stimulus cooling of the skin using the micro Peltier elements was deactivated. All other
222 stimulation characteristics were identical to those used in Experiment 1. EEG responses were
223 recorded, analyzed, and compared such as in Experiment 1.

224

225 <<Insert Figure 1 around here >>

226

227 Experiment 4: Computational model of the laser and contact heat stimulations to simulate heat
228 transfer in the skin

229 A computational model was implemented to investigate the differences between the LSD and
230 TCSII stimulation devices. The model was based on the finite element method (FEM) and
231 implemented in COMSOL Multiphysics 5.5 (COMSOL A/S, Stockholm, Sweden). Generally,
232 the model was based on the model developed and validated in (Frahm et al., 2020). However,

233 the model was converted to a 3D model with a length and width of 50 mm, this was done to
234 allow modelling the non-symmetric TCSII probe. The thickness of the tissue layers as well as
235 the optical and thermal parameters were based on (Frahm et al., 2020).

236 The LSD model mimicked the almost flat beam profile with a beam diameter of approximately
237 12 mm. Absorption of the laser photons was modelled using the Beer-Lamberts equation
238 (Frahm et al., 2010, 2020; Marchandise et al., 2014). The power of the laser stimulation was
239 based on the experimentally used values. The LSD model was simulated for the stimulation
240 temperature of 55°C (Figure 1).

241 The TCSII model was based on the 15 Peltier elements at the skin surface. The simulated
242 temperature of these elements was based on the temperature **time courses** measured during data
243 collection. The TCSII model was simulated for both stimulation temperatures of 55°C and
244 62°C.

245 The models were meshed using a swept mesh approach in COMSOL. The LSD model consisted
246 of 380,510 mesh elements and 1,560,079 degrees of freedom. The TCSII model consisted of
247 407,550 mesh elements and 1,669,071 degrees of freedom. The models were solved using time
248 steps of 10 ms. The solution time for each model was approximately 16 hours on a standard
249 personal computer (Intel i7 6600u, 20GB ram).

250 After solving the models, the temperature at the dermo-epidermal junction (DEJ), was extracted
251 to obtain an estimate of the temperature to which the nociceptors were exposed to during
252 stimulation (Frahm et al., 2010). The tissue volume above threshold (46 °C (Churyukanov et
253 al., 2012)) was integrated over time for both models (only within the vital layers, i.e., excluding
254 the stratum corneum where no nerve fibers are located).

255

256 **RESULTS**

257 Experiment 1: Brain responses to laser and contact heat stimuli matched in terms of stimulation
258 intensity

259 An N1 wave was identified by visual inspection in 9/12 patients following laser stimulation
260 (55°C) and in 8/12 patients following contact heat stimulation (55°C). A clear N2-P2 complex
261 was identified by visual inspection in all participants (12/12) following laser stimulation and in
262 9/12 participants following contact heat stimulation (Supplementary material S1).

263 A Shapiro-Wilk test for normality of distribution was performed and passed for each dataset
264 ($p > .05$) (See Supplementary material S3)

265 Amplitude and latency of the elicited responses, as well as the intensity of perception and the
266 results of the paired comparison are reported in Table 1 and Figure 2.

267

268 <<Insert Table 1 around here >>

269

270 **LEP and CHEP amplitudes.** Mean amplitudes of the N1 wave were $-2.73 \pm 2.24 \mu\text{V}$ and -1.8
271 $\pm 1.82 \mu\text{V}$ for the LSD and the TCSII, respectively. Differences in N1 amplitude between the
272 two devices were not significant ($p = .3975$). Mean amplitudes of the N2-P2 complex were 29.95
273 ± 11.05 and $14.92 \pm 4.83 \mu\text{V}$ for the LSD and the TCSII, respectively. The difference in
274 amplitude between the magnitude of the N2-P2 complex elicited by laser and contact heat
275 stimulation was significant ($p = .0026$).

276 **LEP and CHEP latencies.** Mean latencies of the N1 wave were 204 ± 43 ms and 251 ± 78 ms
277 for the LSD and the TCSII, respectively. Differences in N1 latencies between both devices were
278 statistically significant ($p = .0076$). Mean latencies of the N2 wave were 238 ± 57 ms and $303 \pm$
279 82 ms for the LSD and the TCSII, respectively. Mean latencies of the P2 wave were 382 ± 56
280 ms and 454 ± 83 ms for the LSD and the TCSII, respectively. Differences in latencies between
281 both devices were significant for both N2 wave ($p = .0068$) and the P2 wave ($p = .008$).

282 **Signal to Noise Ratio.** Averaged SNR was 6.04 and 2.50 for laser-evoked and contact heat-
283 evoked responses, respectively. Hence, the SNR was 2.42 times greater with the LSD than with
284 the TCSII.

285 **Intensity of perception.** All stimuli were clearly perceived, but the intensity of the elicited
286 sensations was greater for the LSD than for the TCSII. On the NRS, the average rating of the
287 intensity of perception was 61.46 ± 19.34 and 46.63 ± 16.84 for the LSD and the TCSII,
288 respectively (Table 1 and Figure 2). Differences in intensity of perception between both devices
289 were statistically significant ($p < .0001$). Regarding the quality of the elicited sensations, 11/12
290 participants described the laser stimulus as painful, and only 6/12 qualified the contact heat
291 stimulus as painful.

292 <<Insert Figure 2 around here >>

293

294 Experiment 2: Perception-matched laser and contact heat stimuli

295 For 2/10 participants, the temperature at which contact heat produced a similar sensation in
296 terms of intensity of perception as the 55°C laser stimuli could not be estimated at one of their
297 two forearms because the maximal allowable temperature (70°C) was reached before obtaining
298 four reversals.

299 The average threshold temperature obtained across participants was $61.5 \pm 1.8^\circ\text{C}$. No
300 statistically significant difference ($p = .663$) was found between the threshold temperatures at
301 right and left volar forearms (Figure 3A).

302

303 Experiment 3: Comparing brain responses elicited by perception-matched laser heat and 304 contact heat stimuli

305 An N1 wave was identified by visual inspection in 10/12 patients following laser stimulation
306 (55°C) and in 11/12 patients following contact heat stimulation (62°C). A clear N2-P2 complex

307 was identified by visual inspection in 11/12 participants following laser stimulation (LSD) and
308 in 12/12 participants following contact heat stimulation (TCSII) (Supplementary material S2).

309 A Shapiro-Wilk test for normality of distribution was performed and passed for each dataset
310 ($p > .05$) (See Supplementary material S3)

311 Amplitude and latency of the elicited responses, as well as the intensity of perception and the
312 results of the paired comparison are reported in Table 2 (see also Figure 3)

313

314 << Insert Table 2 around here >>

315

316 **LEP and CHEP amplitudes.** Mean amplitudes of the N1 wave were $-2.8 \pm 3.7 \mu\text{V}$ and -2.8
317 $\pm 2.9 \mu\text{V}$ for the LSD and the TCSII, respectively. Differences in N1 amplitudes between both
318 devices were not significant ($p = .991$). Mean amplitudes of the N2-P2 complex were 37.2 ± 15.2
319 μV and $36.5 \pm 2.8 \mu\text{V}$ for the LSD and the TCSII, respectively. Differences in amplitudes
320 between laser and contact heat stimulation were not significant ($p = .711$).

321 **LEP and CHEP latencies.** Mean latencies of the N1 wave were $218 \pm 29 \text{ ms}$ and $272 \pm 7 \text{ ms}$
322 for the LSD and the TCSII, respectively. Differences in latencies between both devices were
323 significant ($p < .001$). Mean latencies of the N2 wave were $273 \pm 19 \text{ ms}$ and $328 \pm 15 \text{ ms}$ for the
324 LSD and the TCSII, respectively. Mean latencies of the P2 wave were $413 \pm 32 \text{ ms}$ and 449 ± 40
325 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices were
326 statistically significant for both the N2 wave ($p < .001$) and the P2 wave ($p < .001$).

327 **Signal to Noise Ratio.** The average SNR was 4.79 and 4.76 for the LSD and the TCSII,
328 respectively. The SNR ratio between LSD and TCSII was 1.006.

329 **Intensity of perception.** On the NRS, the average rating of the intensity of perception was 61
330 ± 10 for the LSD and 59 ± 9 for the TCSII. No statistically significant difference was found
331 between the two devices ($p = .08$).

332

333 Experiment 4: Computational model of the stimulations to simulate heat transfer in the skin

334 The models for laser (LSD) and contact heat (TCSII) stimulation of the skin showed that the
335 55°C laser stimuli and the 62 °C contact heat stimuli resulted in very similar maximum
336 temperatures at the DEJ (Figure 4).

337

338 << Insert Figure 4 around here >>

339

340 Notably, at the DEJ, the A δ fiber threshold (46 °C) was reached approximately 80 ms after the
341 onset of the 62°C TCSII stimulus, and approximately 40 ms after the onset of the 55°C LSD,
342 indicating that nociceptor activation was delayed following TCSII as compared to LSD. To
343 evaluate whether this delay for contact heat stimulation at the DEJ could be reduced by
344 increasing the temperature slope, we simulated a contact heat stimulus with an infinitely fast
345 skin surface heating ramp (30°C increase in less than 0.01 s). As shown in Figure 4, this did not
346 markedly reduce the delay. The time required for DEJ temperature to reach the A δ fiber
347 threshold of 46°C remained approximately 80 ms.

348 The maximal volume of tissue that reached the theoretical activation threshold of A δ fibers
349 (defined arbitrarily as 46°C) was 5.5 mm³ for the LSD 55°C, 4.6 mm³ for the TCSII 55°C, 9.7
350 mm³ for the TCSII 62 °C and 11.8 mm³ for the TCSII 62°C with an infinitely steep ramp (Figure
351 4). In contrast, the total volume of activated tissue across time, corresponding the area under
352 the curve (AUC) were 1.2 mm³*s for the LSD 55 °C stimulation, 0.5 mm³*s for the TCSII 55°C
353 stimulation, 1.4 mm³*s for the TCSII 62°C stimulation, and 2.3 mm³*s for the TCSII 62°C
354 stimulation with an infinitely steep ramp.

355 The spatial temperature distribution for the LSD and TCSII stimulator are depicted in Figure 5.
356 Overall, the TCSII stimulated a larger area, but due to the design of the probe it is not a uniform
357 area, neither at the skin surface nor at the DEJ.

358

359 << Insert Figure 5 around here >>

360

361 **DISCUSSION**

362 The aim of this study was to compare the event-related brain potentials elicited by contact heat
363 stimulation delivered using a very steep heating ramp to the event-related brain potentials
364 elicited by infrared laser stimulation, and to explain potential reasons for the observed
365 differences in amplitude and latency.

366 When target temperature of the stimulated skin surface was matched (Experiment 1), contact
367 heat stimuli elicited EEG responses having markedly lower amplitudes, delayed latencies and
368 a lower SNR as compared to the responses elicited by laser stimulation. Furthermore, contact
369 heat stimuli were perceived less intense than laser heat stimuli. In contrast, when stimuli were
370 matched in terms of perceived intensity (Experiment 2), contact heat stimuli and laser heat
371 stimuli elicited EEG responses having similar amplitudes and SNR (Experiment 3). However,
372 there remained a clear increase in latency of the responses to contact heat as compared to radiant
373 heat.

374 Modelling heat transfer to the skin exposed to CO₂-laser radiant heat vs contact heat showed
375 that these differences can be explained entirely by the differences in heat transfer to the skin
376 (Experiment 4). In the case of CO₂ laser stimulation, the irradiated energy is absorbed within
377 the superficial layers of the skin, leading to direct and immediate heating below the surface of
378 the skin, in closer proximity to where the heat-sensitive free nerve endings are located, i.e., at
379 the level of the DEJ. During contact heat stimulation with a thermode placed against the skin,

380 heating at the depth of the free nerve endings relies entirely on thermal conduction from the
381 skin surface to the nociceptors and is therefore limited by the intrinsic thermal inertia of the
382 skin. This means that – as compared to radiant CO₂ laser heat stimulation – contact heat
383 stimulation yields a greater gradient between surface and depth temperature, as well as a delay
384 between peak temperature at skin surface and at the depth of the dermal-epidermal junction.
385 Those factors appear to explain why contact heat stimulation requires a greater skin surface
386 temperature to elicit a response of similar magnitude as laser heat stimulation, as well as the
387 delayed responses to contact heat as compared to laser heat stimulation.

388 The results of the modelization of heat transfer to the skin also showed that the maximal volume
389 above threshold was very similar for the 55°C laser heat stimulus and the 55°C contact heat
390 stimulus, whereas the AUC was very similar for the 55°C laser heat stimulus and the 62°C
391 contact heat stimulus. The fact that the two measures are not directly proportional is explained
392 by differences in the heating and cooling time courses (including the fact that the contact probe
393 was actively returned to baseline temperature at a rate of 300°C/s for the 55°C contact heat
394 stimulus and not for the 62°C contact heat stimulus).

395 The finding that stimuli with a similar AUC are perceived as equally intense even though they
396 differ markedly in terms of maximal volume of activated tissue suggests that the intensity of
397 perception is not determined solely by the number of activated afferents (which should be
398 proportional to the maximal volume of activated tissue) but also by the duration of this
399 activation, i.e., the volume of tissue above threshold across time.

400 Finally, the results of the present study were obtained for stimulation of the volar forearm and
401 should, therefore, be applicable for stimulation sites with a similar thickness of the stratum and
402 a similar depth of the DEJ. It would thus not be applicable for stimulation of the hand palm, but
403 could be applicable for stimulation of the hand dorsum. However, another point to take into
404 consideration is that contact between the probe and the skin – and, therefore, thermal

405 conductivity between the probe and the skin – is improved when the probe is applied against
406 skin overlying soft tissues such as muscle as compared to skin overlying harder and more
407 irregular structures such as the bones and tendons of the hand.

408

409 CONCLUSION

410 In summary, this validation study shows that it is possible to record robust nociceptive-heat
411 ERPs using a high-speed heating contact thermode, namely the TCSII. The amplitude and SNR
412 of the observed brain responses are comparable to those obtained with the gold-standard,
413 namely the CO₂-laser, provided that target skin surface temperature is adjusted to account for
414 the greater gradient between skin surface and temperature at the depth of the dermal-epidermal
415 junction for contact heat stimulation (for example 62°C contact heat stimulation vs 55°C radiant
416 heat stimulation). Latencies of the responses elicited by contact heat are slightly delayed relative
417 to the latencies elicited by radiant heat, due to the heating mechanism which relies on thermal
418 conduction and is therefore limited by the intrinsic thermal inertia of the skin.

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486

487 **AUTHORS CONTRIBUTIONS**

488 Study design : NL, EP, AM ; Data acquisition : NL, EP ; Data analysis : NL, EP, KSF, AM ;
489 Data interpretation : NL, EP, KSF, AM ; Manuscript drafting : NL, EP, KSF, AM ; All authors
490 have approved the final paper.

491

492 **ACKNOWLEDGMENTS**

493 NL is a post-doctoral researcher of the F.R.S.-Fonds National pour la Recherche Scientifique
494 (FNRS), Belgium. KSF is supported by the Danish National Research Foundation (DNRF121).
495 We thank André Dufour from University of Strasbourg for conception of the thermal cutaneous
496 stimulator and technical support.

497

498 **CONFLICT OF INTEREST STATEMENT**

499 None of the authors have potential conflicts of interest to be disclosed

500

501 **TABLES**

502 **Table 1.** Intensity of perception, amplitudes and latencies of the ERPs elicited by laser (LSD)
 503 and contact heat (TCSII) stimulation at 55°C (Experiment 1).

	LSD (55°C)	TCSII (55°C)	p-value
Visual inspection	N1 : 9/12 (75%)	N1 : 8/12 (67%)	-
(identification rate)	N2-P2 : 12/12 (100%)	N2-P2 : 9/12 (75%)	-
N1 Amplitude (µV)	-2.73 ± 2.24	-1.8 ± 1.82	p = 0.3975 (ns)
N2 Amplitude (µV)	-13.27 ± 6.56	-5.76 ± 1.43	p = 0.0175 *
P2 Amplitude (µV)	16.68 ± 7.03	9.162 ± 4.62	p = 0.0008 ***
N2-P2 Amplitude (µV)	29.95 ± 11.05	14.92 ± 4.83	p = 0.0026 **
N1 Latency (ms)	204 ± 43	251 ± 78	p = 0.0076 **
N2 Latency (ms)	238 ± 57	303 ± 82	p = 0.0068 **
P2 Latency (ms)	382 ± 56	454 ± 83	p = 0.008 **
Intensity of perception (NRS)	61.46 ± 19.34	46.63 ± 16.84	p < 0.0001 ***

504 *Note:* Average values and standard deviation of intensity of perception, amplitude and latencies obtained
 505 in the experiment. The last column indicates the p value of the paired-sample *t*-tests testing for the
 506 difference between the TCSII and the LSD. *** p < .001, ** p < .01, *p<.05, (ns) Non Significant for p
 507 value threshold set at .05 (paired-sample *t*-tests). µV = microvolts; ms = milliseconds; NRS = Numerical
 508 Rating Scale.

509

510

511 **Table 2.** Intensity of perception, amplitudes and latencies of the ERPs elicited by laser
 512 stimulation (LSD; 55°C) and contact heat (TCSII; 62°C) stimulation.

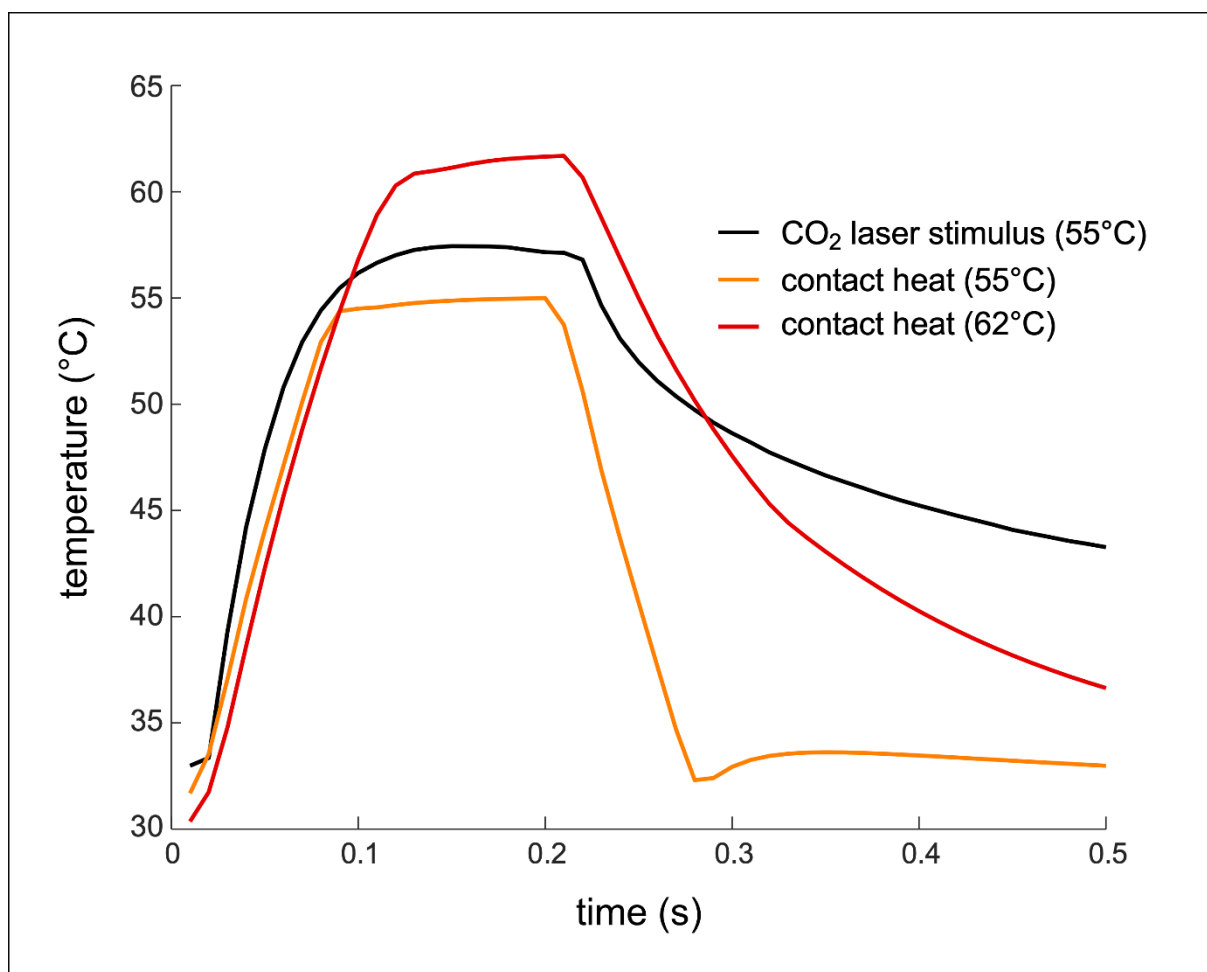
	LSD (55°C)	TCSII (62°C)	Value of p
<i>Visual</i> inspection	N1 : 10/12 (83%)	N1 : 11/12 (92%)	-
(identification rate)	N2-P2 : 11/12 (92%)	N2-P2 : 12/12 (100%)	-
N1 Amplitude (μV)	-2.83 ± 3.72	-2.84 ± 2.93	p = 0.991 (ns)
N2 Amplitude (μV)	-18.19 ± 8.67	-16.09 ± 6.23	p = 0.157 (ns)
P2 Amplitude (μV)	19.01 ± 8.426	20.43 ± 7.56	p = 0.310 (ns)
N2-P2 Amplitude (μV)	37.21 ± 15.16	36.53 ± 12.82	p = 0.711 (ns)
N1 Latency (ms)	217.5 ± 29.02	271.8 ± 27.43	p < 0.001 ***
N2 Latency (ms)	273.2 ± 19.43	328.1 ± 15.23	p < 0.001 ***

P2 Latency (ms)	412.5 ± 32.48	448.5 ± 39.95	p < 0.001 ***
Intensity of perception (NRS)	61.29 ± 10.23	58.67 ± 8.74	p = 0.080 (ns)

513 *Note:* Average values and standard deviation of intensity of perception, amplitude and latencies of laser-
514 and contact heat-evoked potentials obtained in Experiment 3. The last column indicates the p value of
515 the paired-sample *t*-test testing for the difference between laser (LSD) and contact heat (TCSII)
516 stimulation. *** p < .001, (ns) Non Significant for p value threshold set at .05. μV = microvolts; ms =
517 milliseconds; NRS = Numerical Rating Scale.

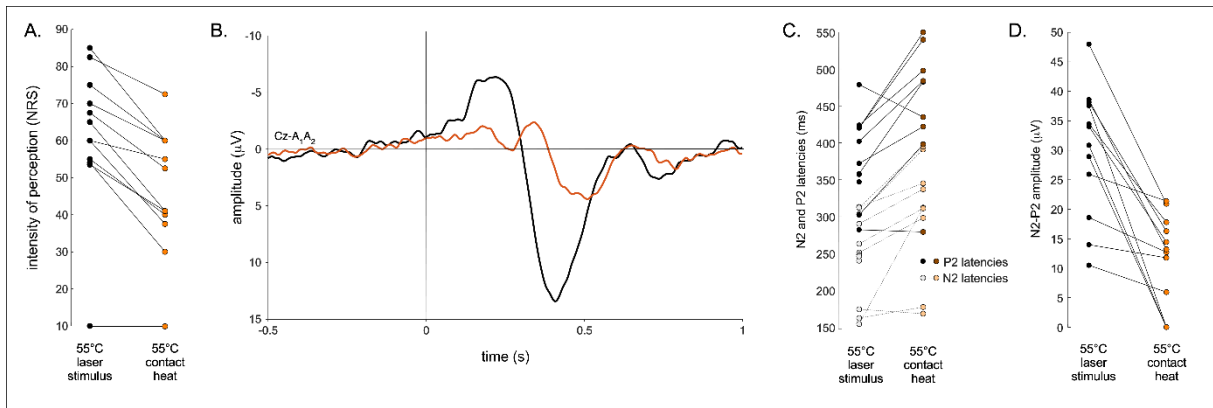
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521
 522 **Figure 1.** Mean time course of skin surface temperature for CO₂ laser heat stimuli and contact heat
 523 delivered in Experiments 1 and 3. In Experiment 1, both stimulators were set to reach a target skin
 524 surface temperature of 55°C, and to maintain that target temperature for a total duration of 200 ms. In
 525 Experiment 3, the target temperature for laser stimulation was 55°C, while the target temperature for
 526 contact heat stimulation was set to 62°C. X-axis: time relative to onset of the stimulus. Y-axis:
 527 temperature measured by the thermal sensor of the temperature-controlled laser stimulator, and
 528 measured by the thermocouples of the contact heat stimulator.

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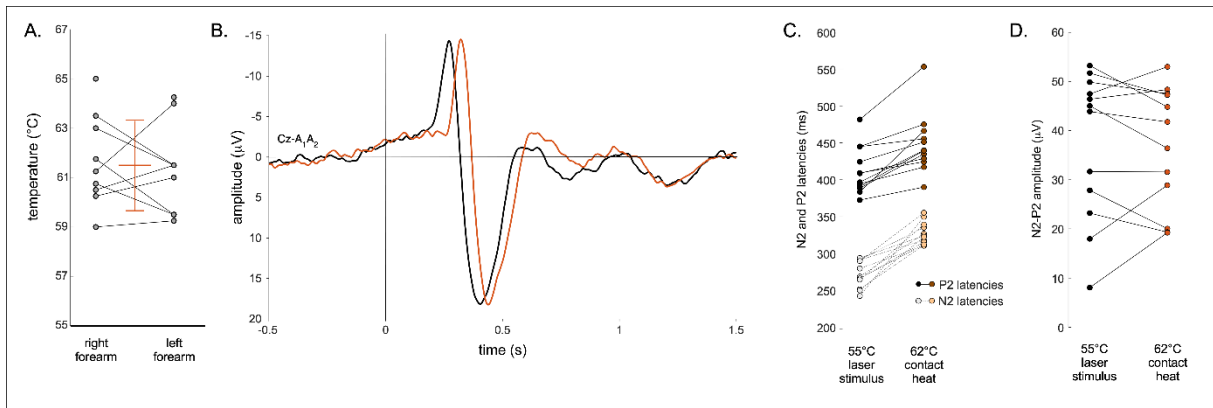


530

531 **Figure 2. Experiment 1.** A. Intensity of the perception elicited by laser and contact heat stimulation of
 532 the volar forearm at 55°C. Laser stimuli were perceived as significantly more intense than contact heat
 533 stimuli ($p < .001$; paired sample t -test). B. Group-level average laser-evoked and contact heat-evoked
 534 potentials elicited by the 55°C stimuli (electrode Cz vs. A1-A2). C. Single-subject N2 and P2 latencies,
 535 and N2-P2 amplitudes elicited by the 55°C laser and contact heat stimuli. Note the later latencies and
 536 lower amplitudes of the responses elicited by contact heat.

537

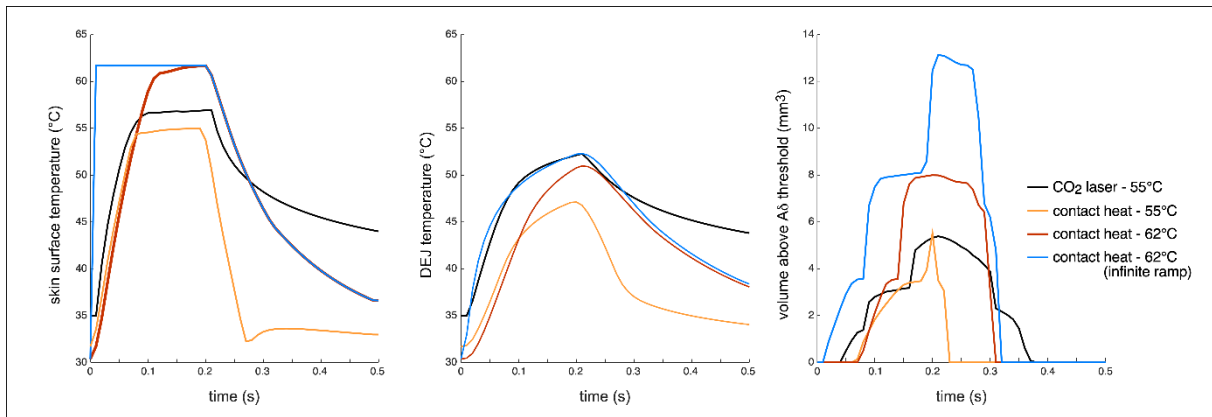
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539

540 **Figure 3.** A. Experiment 2. Single-subject stimulation temperatures required for contact heat stimuli to
 541 elicit a sensation perceived as equally intense as a 55°C CO₂ laser heat stimulus at the left volar forearm,
 542 the right volar forearm and averaged across the two forearms. B. Experiment 3. Group-level average of
 543 the event-related potentials elicited by 55°C CO₂ laser heat stimulation and 62°C contact heat
 544 stimulation (electrode Cz vs. A1-A2). C. Single-subject N2 and P2 latencies, and N2-P2 amplitudes
 545 elicited by the 55°C laser stimuli and the 62°C contact heat stimuli. Note the similar amplitudes of the
 546 responses elicited by contact heat and laser heat when the temperature of the contact heat stimulus is
 547 increased to match the intensity of the percept elicited by laser stimulation. Also note that the latency of
 548 the response elicited by contact heat remains delayed relative to the laser-evoked response.

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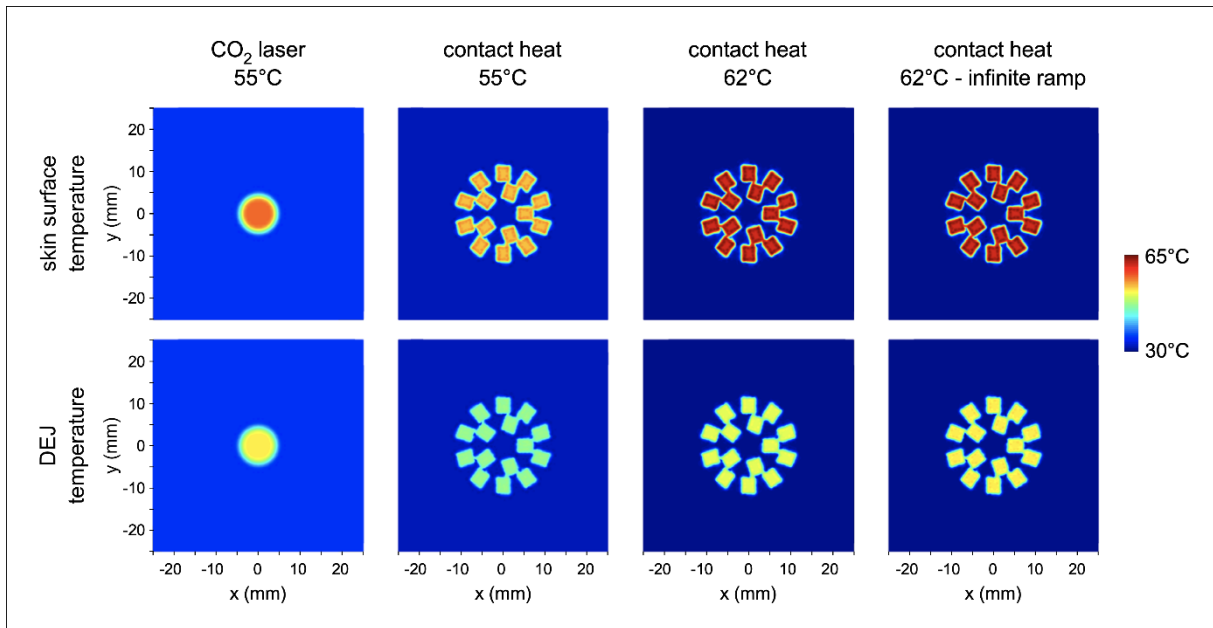


550

551 **Figure 4. Experiment 4.** Time course of temperature at skin surface (A) and at the dermo-epidermal
 552 junction (B) following CO₂ laser stimulation and contact heat stimulation. The data were obtained by
 553 simulation using a 55°C laser stimulus, a 55°C contact heat stimulus and a 62°C contact heat stimulus
 554 with a 300°C/s heating ramp, and a 62°C contact heat stimulus using an infinitely steep heating ramp.
 555 Note that, as compared to the temperature time course for a 55°C laser heat stimuli, temperature at the
 556 dermo-epidermal junction is lower for a 55°C contact heat stimulus, and similar for a 62°C contact heat
 557 stimulus. C. Estimated tissue volume above Aδ fiber threshold (46 °C) for a simulated 55°C laser
 558 stimulus, a 55°C contact heat stimulus and a 62°C contact heat stimulus with a 300°C/s heating ramp,
 559 and a 62°C contact heat stimulus using an infinitely steep heating ramp.

560

561



562

563 **Figure 5.** Simulated spatial temperature distribution at the skin surface at the *dermo-epidermal junction*
 564 *for 55°C laser stimulation, a 55°C contact heat stimulus and a 62°C contact heat stimulus with a 300°C/s*
 565 *heating ramp, and a 62°C contact heat stimulus using an infinitely steep heating ramp. The figure depicts*
 566 *the temperature distribution at the end of the stimulation (0.2 s). The temperature scale is in °C.*

567

568 **Supplementary Material - Figure S1.** Individual EEG responses elicited by LSD at 55°C (LEPs, left
 569 panel) and TCSII at 55°C (CHEPs, right panel) in Experiment 1. N2 responses are indicated by a first
 570 red circle, and the P2 response by the second one. N2-P2 responses were identified in all subjects for
 571 LSD at 55°C and in 9/12 subjects for TCSII at 55°C.

572 **Supplementary Material - Figure S2.** Individual EEG responses elicited by LSD at 55°C (LEPs, left
 573 panel) and TCSII at 62°C (CHEPs, right panel) in Experiment 3. N2 responses are indicated by a first
 574 red circle, and the P2 response by the second one. N2-P2 responses were identified in all subjects for
 575 LSD at 55°C and in 9/12 subjects for TCSII at 55°C

576 **Supplementary Material - Table S1.** Test of the normality of the distribution of the values obtained,
 577 using a Shapiro-Wilk test. p-values of each test are reported in this table. The normality test was
 578 considered as passed with an p-value ≥ 0.05
 579

	Experiment 1		Experiment 3	
	LSD (55°C)	TCSII (55°C)	LSD (55°C)	TCSII (62°C)
N1 Amplitude	p = 0.3135	p = 0.7908	p = 0.7313	p = 0.9838
N2 Amplitude	p = 0.6516	p = 0.2338	p = 0.5546	p = 0.397
P2 Amplitude	p = 0.995	p = 0.3508	p = 0.7203	p = 0.4052
N2-P2 Amplitude	p = 0.6666	p = 0.7643	p = 0,1121	p = 0,1297
N1 Latency	p = 0.0601	p = 0.1442	p = 0.7649	p = 0.6941
N2 Latency	p = 0.218	p = 0.1065	p = 0.2271	p = 0.1767
P2 Latency	p = 0.6903	p = 0.3575	p = 0.2062	p = 0,0621
Intensity of perception (NRS)	p = 0.291	p = 0.5646	p = 0.885	p = 0.1959

580