High-speed heating of the skin using a contact thermode elicits brain responses comparable to CO2 laser-evoked potentials

Lejeune, Nicolas; Petrossova, Eva; Frahm, Ken Steffen; Mouraux, André

Published in:
Clinical Neurophysiology

DOI (link to publication from Publisher):
10.1016/j.clinph.2022.11.008

Publication date:
2023

Document Version
Accepted author manuscript, peer reviewed version

High-speed heating of the skin using a contact thermode elicits comparable brain responses to those elicited by a CO_2-laser device

Nicolas Lejeune[1,2,3], Eva Petrossova[1], Ken Steffen Frahm[4], André Mouraux[1]

[1] Institute of NeuroScience, UCLouvain, 53, Avenue E. Mounier, 1200 Brussels, Belgium

[2] GIGA-Consciousness, GIGA research center (B34, +1), University of Liège, Avenue de l'Hôpital 1, 4000 Liège, Belgium.

[3] Centre du cerveau², University Hospital of Liège (B34, +1), Avenue de l'Hôpital 1, 4000 Liège, Belgium.

[4] Integrative Neuroscience, Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

Corresponding author:
Name: Nicolas Lejeune
Mailing address: nicolas.lejeune@uliege.be
Address: 53, Avenue E. Mounier, 1200 Brussels, Belgium.
Telephone number : +32 10 430 249.
https://orcid.org/0000-0001-6059-606X

Number of words: 4265 words
Number of tables and figures: 2 tables and 8 figures
Number of words in structured abstract : 196 words

ABSTRACT

OBJECTIVE

To compare nociceptive event-related brain potentials elicited by a high-speed contact-thermode vs. an infrared CO_2 laser stimulator.

METHODS

Contact heat-evoked potentials (CHEPs) and CO_2 laser-evoked potentials (LEPs) were recorded in healthy volunteers using a high-speed contact-thermode (>200°C/s) and a temperature-controlled CO_2 laser. In separate experiments, stimuli were matched in terms of target surface temperature (55°C) and intensity of perception. A finite-element model (FEM) of skin heat transfer was used to explain observed differences.

RESULTS

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

CONCLUSION

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

SIGNIFICANCE

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

KEYWORDS

Nociception; Pain; Thermal stimulation; Electroencephalography

HIGHLIGHTS

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

INTRODUCTION

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

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

The aim of the present study was to compare CHEPs elicited by very steep heating ramps of 300°C/s to LEPs elicited by infrared CO_2-laser stimulation, and to assess whether reliable responses can be obtained at single-subject level, as required for clinical diagnosis. Stimuli were delivered to the volar forearm. The study consisted in four parts. In Experiment 1, we compared the EEG responses elicited by radiant heat and contact heat stimuli matched in terms of target skin surface temperature, stimulated surface area and stimulus duration. In Experiment 2, the target temperatures used for radiant heat and contact heat stimulation were adjusted to match their intensity of perception. In Experiment 3, we compared LEPs and CHEPs elicited by such perception-matched stimuli. Finally, in Experiment 4, we utilized a computational model of radiant heat and contact heat stimulation of the skin to compare the temperature time course at the dermal-epidermal junction (DEJ), where the majority of heat-sensitive free nerve endings are located, to investigate if the observed differences between LEPs and CHEPs can be explained by differences in heat transfer within the skin.

METHODS

Participants

The three first experiments of the study were conducted in healthy subjects, while Experiment 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 CO_2 laser stimulation.** In all three experiments, radiant heat stimuli
93 were generated using a temperature-controlled CO_2 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 CO_2 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 mm^2 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 mm^2 each. The baseline temperature can be set to the neutral

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

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

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

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

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

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

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

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

Experiment 2: Perception-matched laser and contact heat stimuli

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

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

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

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

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

<<Insert Figure 1 around here >>

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

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

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

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

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

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

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

RESULTS

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

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

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

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

<<Insert Table 1 around here >>

LEP and CHEP amplitudes. Mean amplitudes of the N1 wave were -2.73 ± 2.24 µV and -1.8 ± 1.82 µV for the LSD and the TCSII, respectively. Differences in N1 amplitude between the two devices were not significant ($p=.3975$). Mean amplitudes of the N2-P2 complex were 29.95 ± 11.05 and 14.92 ± 4.83 µV for the LSD and the TCSII, respectively. The difference in amplitude between the magnitude of the N2-P2 complex elicited by laser and contact heat stimulation was significant ($p=.0026$).

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

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

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

<<Insert Figure 2 around here >>

Experiment 2: Perception-matched laser and contact heat stimuli

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

The average threshold temperature obtained across participants was 61.5 ±1.8°C. No statistically significant difference ($p = .663$) was found between the threshold temperatures at right and left volar forearms (Figure 3A).

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

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

was identified by visual inspection in 11/12 participants following laser stimulation (LSD) and in 12/12 participants following contact heat stimulation (TCSII) (Supplementary material S2). A Shapiro-Wilk test for normality of distribution was performed and passed for each dataset ($p>.05$) (See Supplementary material S3)

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

<< Insert Table 2 around here >>

LEP and CHEP amplitudes. Mean amplitudes of the N1 wave were -2.8 ±3.7 µV and -2.8 ±2.9 µV for the LSD and the TCSII, respectively. Differences in N1 amplitudes between both devices were not significant ($p=.991$). Mean amplitudes of the N2-P2 complex were 37.2 ±15.2 µV and 36.5 ±2.8 µV for the LSD and the TCSII, respectively. Differences in amplitudes between laser and contact heat stimulation were not significant ($p=.711$).

LEP and CHEP latencies. Mean latencies of the N1 wave were 218 ±29 ms and 272 ±7 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices were significant ($p<.001$). Mean latencies of the N2 wave were 273 ±19 ms and 328 ±15 ms for the LSD and the TCSII, respectively. Mean latencies of the P2 wave were 413 ±32 ms and 449 ±40 ms for the LSD and the TCSII, respectively. Differences in latencies between both devices were statistically significant for both the N2 wave ($p<.001$) and the P2 wave ($p<.001$).

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

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

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

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

<< Insert Figure 4 around here >>

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

The maximal volume of tissue that reached the theoretical activation threshold of Aδ fibers (defined arbitrarily as 46°C) was 5.5 mm^3 for the LSD 55°C, 4.6 mm^3 for the TCSII 55°C, 9.7 mm^3 for the TCSII 62 °C and 11.8 mm^3 for the TCSII 62°C with an infinitely steep ramp (Figure 4). In contrast, the total volume of activated tissue across time, corresponding the area under the curve (AUC) were 1.2 mm^3*s for the LSD 55 °C stimulation, 0.5 mm^3*s for the TCSII 55°C stimulation, 1.4 mm^3*s for the TCSII 62°C stimulation, and 2.3 mm^3*s for the TCSII 62°C stimulation with an infinitely steep ramp.

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

<< Insert Figure 5 around here >>

DISCUSSION

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

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

Modelling heat transfer to the skin exposed to CO_2-laser radiant heat vs contact heat showed that these differences can be explained entirely by the differences in heat transfer to the skin (Experiment 4). In the case of CO_2 laser stimulation, the irradiated energy is absorbed within the superficial layers of the skin, leading to direct and immediate heating below the surface of the skin, in closer proximity to where the heat-sensitive free nerve endings are located, i.e., at the level of the DEJ. During contact heat stimulation with a thermode placed against the skin,

heating at the depth of the free nerve endings relies entirely on thermal conduction from the skin surface to the nociceptors and is therefore limited by the intrinsic thermal inertia of the skin. This means that – as compared to radiant CO_2 laser heat stimulation – contact heat stimulation yields a greater gradient between surface and depth temperature, as well as a delay between peak temperature at skin surface and at the depth of the dermal-epidermal junction. Those factors appear to explain why contact heat stimulation requires a greater skin surface temperature to elicit a response of similar magnitude as laser heat stimulation, as well as the delayed responses to contact heat as compared to laser heat stimulation.

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

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

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

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

CONCLUSION

In summary, this validation study shows that it is possible to record robust nociceptive-heat ERPs using a high-speed heating contact thermode, namely the TCSII. The amplitude and SNR of the observed brain responses are comparable to those obtained with the gold-standard, namely the CO_2-laser, provided that target skin surface temperature is adjusted to account for the greater gradient between skin surface and temperature at the depth of the dermal-epidermal junction for contact heat stimulation (for example 62°C contact heat stimulation vs 55°C radiant heat stimulation). Latencies of the responses elicited by contact heat are slightly delayed relative to the latencies elicited by radiant heat, due to the heating mechanism which relies on thermal conduction and is therefore limited by the intrinsic thermal inertia of the skin.

REFERENCES

Atherton, D. D., Facer, P., Roberts, K. M., Misra, V. P., Chizh, B. A., Bountra, C., & Anand, P. (2007). Use of the novel Contact Heat Evoked Potential Stimulator (CHEPS) for the assessment of small fibre neuropathy: Correlations with skin flare responses and intra-epidermal nerve fibre counts. *BMC Neurology*, *7*, 21. https://doi.org/10.1186/1471-2377-7-21

Baumgärtner, U., Cruccu, G., Iannetti, G. D., & Treede, R.-D. (2005). Laser guns and hot plates. *Pain*, *116*(1–2), 1–3. https://doi.org/10.1016/j.pain.2005.04.021

Churyukanov, M., Plaghki, L., Legrain, V., & Mouraux, A. (2012). Thermal Detection Thresholds of Aδ- and C-Fibre Afferents Activated by Brief CO2 Laser Pulses Applied onto the Human Hairy Skin. *PLoS ONE*, *7*(4), 1–10. https://doi.org/10.1371/journal.pone.0035817

Cruccu, G., Aminoff, M. J., Curio, G., Guerit, J. M., Kakigi, R., Mauguiere, F., Rossini, P. M., Treede, R.-D., & Garcia-Larrea, L. (2008). Recommendations for the clinical use of somatosensory-evoked potentials. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *119*(8), 1705–1719. https://doi.org/10.1016/j.clinph.2008.03.016

De Keyser, R., van den Broeke, E. N., Courtin, A., Dufour, A., & Mouraux, A. (2018). Event-related brain potentials elicited by high-speed cooling of the skin: A robust and non-painful method to assess the spinothalamic system in humans. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *129*(5), 1011–1019. https://doi.org/10.1016/j.clinph.2018.02.123

Frahm, K. S., Andersen, O. K., Arendt-Nielsen, L., & Mørch, C. D. (2010). Spatial temperature distribution in human hairy and glabrous skin after infrared CO2 laser radiation. *Biomedical Engineering Online*, *9*(1), 69. https://doi.org/10.1186/1475-925X-9-69

Frahm, K. S., Gervasio, S., Arguissain, F., & Mouraux, A. (2020). New insights into cutaneous laser stimulation – dependency on skin and laser type. *Neuroscience*, *448*, 71–84. https://doi.org/10.1016/j.neuroscience.2020.09.021

Greffrath, W., Baumgärtner, U., & Treede, R.-D. (2007). Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *Pain*, *132*(3), 301–311. https://doi.org/10.1016/j.pain.2007.04.026

Inui, K., Tran, T. D., Hoshiyama, M., & Kakigi, R. (2002). Preferential stimulation of Aδ fibers by intra-epidermal needle electrode in humans. *Pain*, *96*(3), 247–252. https://doi.org/10.1016/S0304-3959(01)00453-5

Leone, C., Dufour, A., Di Stefano, G., Fasolino, A., Di Lionardo, A., La Cesa, S., Galosi, E., Valeriani, M., Nolano, M., Cruccu, G., & Truini, A. (2019). Cooling the skin for assessing small-fibre function. *Pain*, *160*(9), 1967–1975. https://doi.org/10.1097/j.pain.0000000000001584

Marchandise, E., Mouraux, A., Plaghki, L., & Henrotte, F. (2014). Finite element analysis of thermal laser skin stimulation for a finer characterization of the nociceptive system. *J.Neurosci.Methods*, *223*(1872-678X (Electronic)), 1–10. https://doi.org/10.1016/j.jneumeth.2013.11.010

Mouraux, A., & Iannetti, G. D. (2008). Across-trial averaging of event-related EEG responses and beyond. *Magnetic Resonance Imaging*, *26*(7), 1041–1054. https://doi.org/10.1016/j.mri.2008.01.011

Mouraux, A., & Iannetti, G. D. (2018). The search for pain biomarkers in the human brain. *Brain: A Journal of Neurology*, *141*(12), 3290–3307. https://doi.org/10.1093/brain/awy281

Mouraux, A., Iannetti, G. D., & Plaghki, L. (2010). Low intensity intra-epidermal electrical stimulation can activate Aδ-nociceptors selectively. *Pain*, *150*(1), 199–207. https://doi.org/10.1016/j.pain.2010.04.026

Mulders, D., de Bodt, C., Lejeune, N., Courtin, A., Liberati, G., Verleysen, M., & Mouraux, A. (2020). Dynamics of the perception and EEG signals triggered by tonic warm and cool stimulation. *PLoS ONE*, *15*(4). https://doi.org/10.1371/journal.pone.0231698

Nahra, H., & Plaghki, L. (2003). The effects of A-fiber pressure block on perception and neurophysiological correlates of brief non-painful and painful CO2 laser stimuli in humans. *European Journal of Pain (London, England)*, *7*(2), 189–199. https://doi.org/10.1016/S1090-3801(02)00099-X

Plaghki, L., & Mouraux, A. (2003). How do we selectively activate skin nociceptors with a high power infrared laser? Physiology and biophysics of laser stimulation. *Neurophysiologie Clinique = Clinical Neurophysiology*, *33*(6), 269–277.

Treede, R. D., Meyer, R. A., Raja, S. N., & Campbell, J. N. (1995). Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. *The Journal of Physiology*, *483*(Pt 3), 747–758.

Treede, R.-D., Lorenz, J., & Baumgärtner, U. (2003). Clinical usefulness of laser-evoked potentials. *Neurophysiologie Clinique/Clinical Neurophysiology*, *33*(6), 303–314. https://doi.org/10.1016/j.neucli.2003.10.009

AUTHORS CONTRIBUTIONS

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

ACKNOWLEDGMENTS

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

CONFLICT OF INTEREST STATEMENT

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

TABLES

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

	LSD (55°C)	TCSII (55°C)	p-value
Visual inspection (identification rate)	N1 : 9/12 (75%) N2-P2 : 12/12 (100%)	N1 : 8/12 (67%) 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 ***

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

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
Visual inspection (identification rate)	N1 : 10/12 (83%) N2-P2 : 11/12 (92%)	N1 : 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)

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

FIGURE LEGENDS

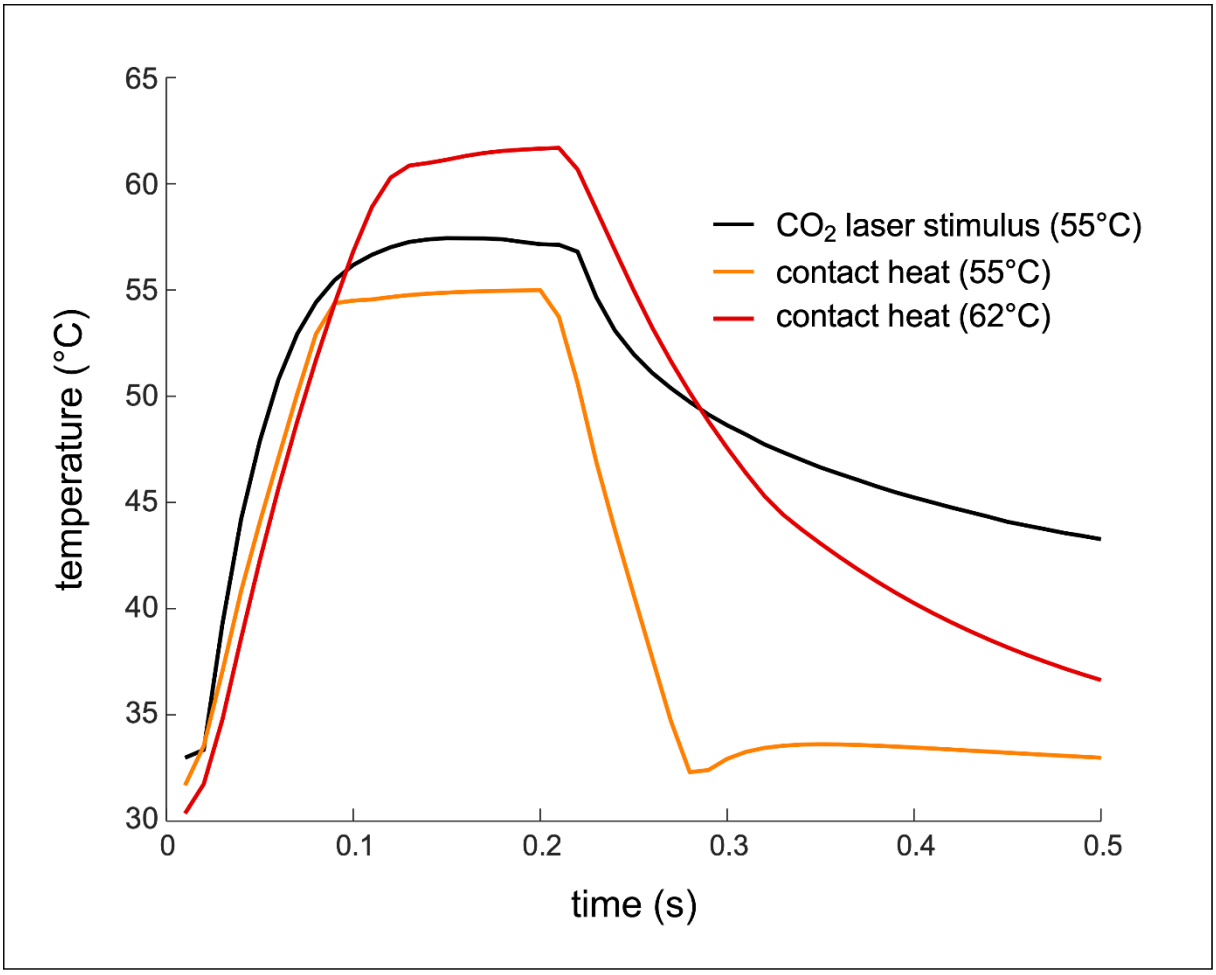

Figure 1. Mean time course of skin surface temperature for CO_2 laser heat stimuli and contact heat delivered in Experiments 1 and 3. In Experiment 1, both stimulators were set to reach a target skin surface temperature of 55°C, and to maintain that target temperature for a total duration of 200 ms. In Experiment 3, the target temperature for laser stimulation was 55°C, while the target temperature for contact heat stimulation was set to 62°C. X-axis: time relative to onset of the stimulus. Y-axis: temperature measured by the thermal sensor of the temperature-controlled laser stimulator, and measured by the thermocouples of the contact heat stimulator.

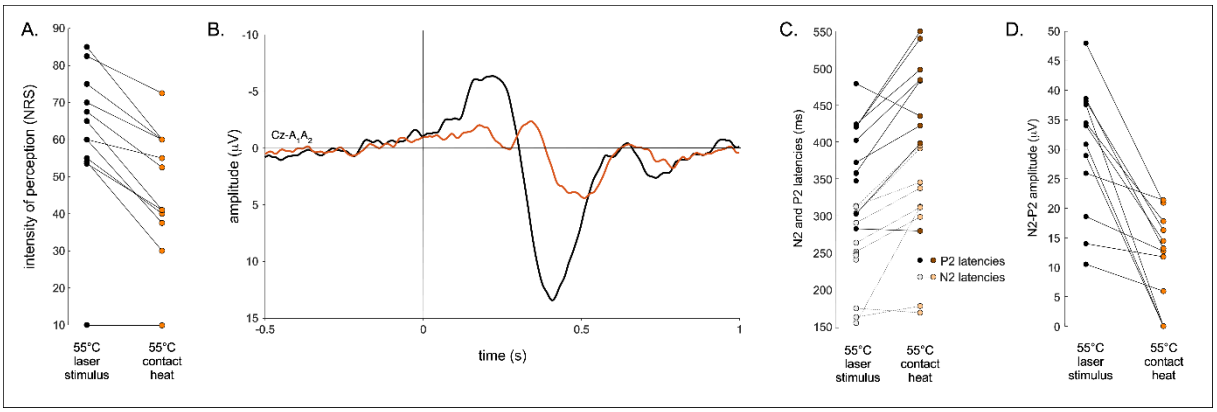

Figure 2. Experiment 1. A. Intensity of the perception elicited by laser and contact heat stimulation of the volar forearm at 55°C. Laser stimuli were perceived as significantly more intense than contact heat stimuli ($p < .001$; paired sample *t*-test). B. Group-level average laser-evoked and contact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

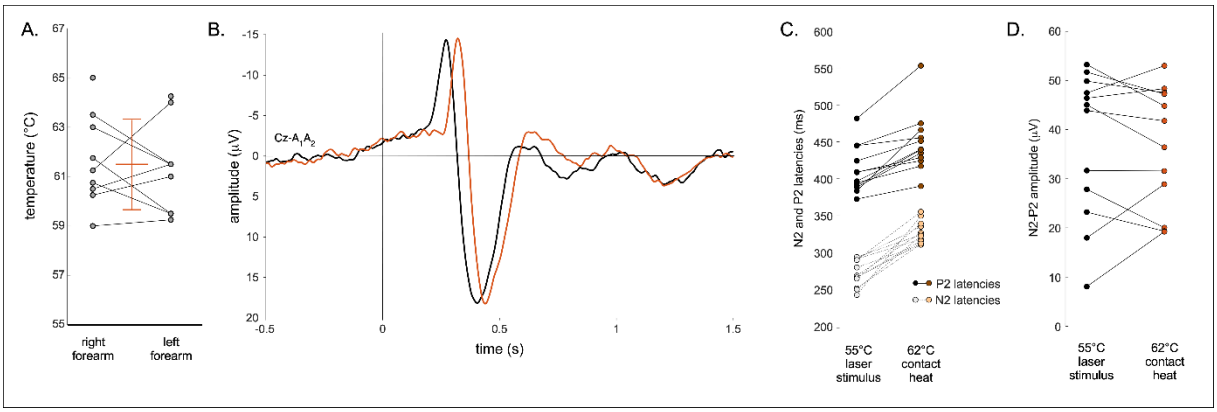

Figure 3. A. Experiment 2. Single-subject stimulation temperatures required for contact heat stimuli to elicit a sensation perceived as equally intense as a 55°C CO_2 laser heat stimulus at the left volar forearm, the right volar forearm and averaged across the two forearms. B. Experiment 3. Group-level average of the event-related potentials elicited by 55°C CO_2 laser heat stimulation and 62°C contact heat stimulation (electrode Cz vs. A1-A2). C. Single-subject N2 and P2 latencies, and N2-P2 amplitudes elicited by the 55°C laser stimuli and the 62°C contact heat stimuli. Note the similar amplitudes of the responses elicited by contact heat and laser heat when the temperature of the contact heat stimulus is increased to match the intensity of the percept elicited by laser stimulation. Also note that the latency of the response elicited by contact heat remains delayed relative to the laser-evoked response.

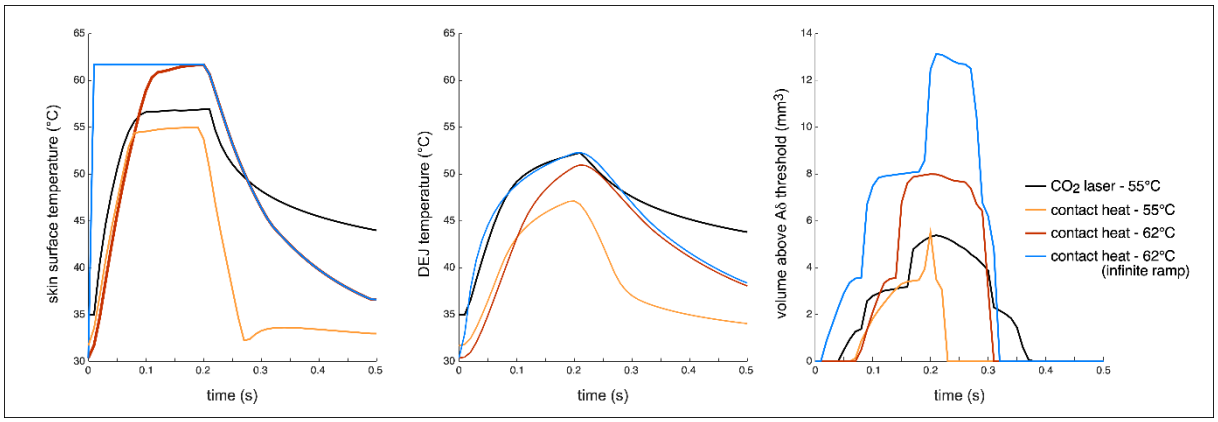

Figure 4. Experiment 4. Time course of temperature at skin surface (A) and at the dermo-epidermal junction (B) following CO_2 laser stimulation and contact heat stimulation. The data were obtained by simulation using a 55°C laser stimulus, a 55°C contact heat stimulus and a 62°C contact heat stimulus with a 300°C/s heating ramp, and a 62°C contact heat stimulus using an infinitely steep heating ramp. Note that, as compared to the temperature time course for a 55°C laser heat stimuli, temperature at the dermo-epidermal junction is lower for a 55°C contact heat stimulus, and similar for a 62°C contact heat stimulus. C. Estimated tissue volume above Aδ fiber threshold (46 °C) for a simulated 55°C laser stimulus, a 55°C contact heat stimulus and a 62°C contact heat stimulus with a 300°C/s heating ramp, and a 62°C contact heat stimulus using an infinitely steep heating ramp.

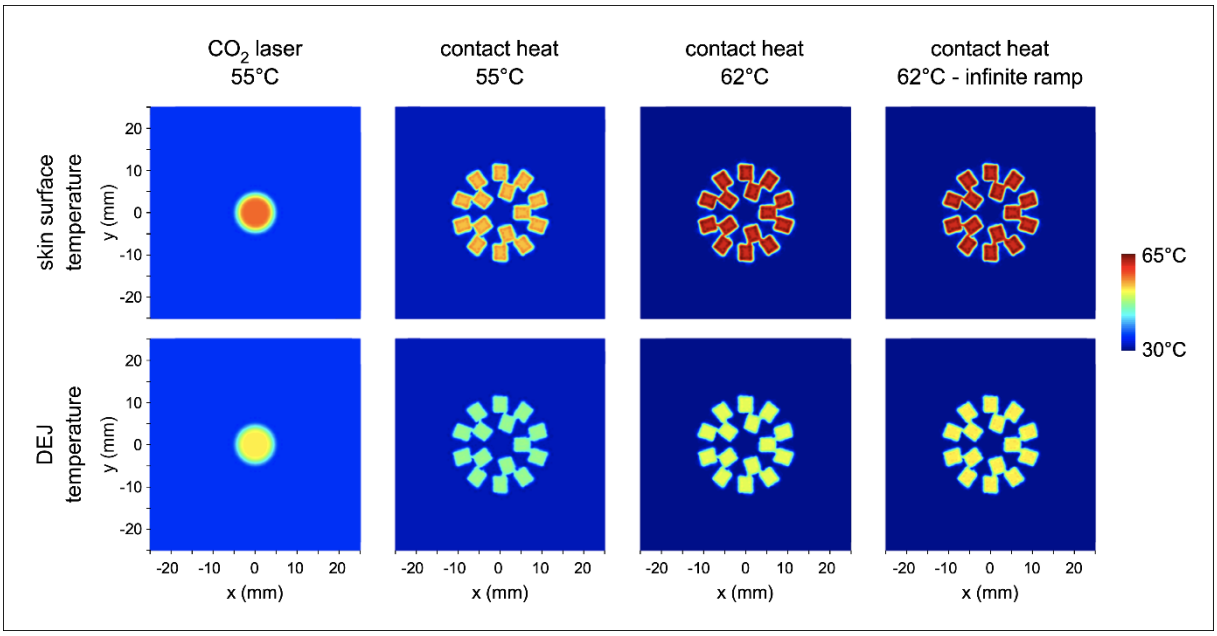

Figure 5. Simulated spatial temperature distribution at the skin surface at the *dermo-epidermal junction for 55°C laser stimulation,* a 55°C contact heat stimulus and a 62°C contact heat stimulus with a 300°C/s heating ramp, and a 62°C contact heat stimulus using an infinitely steep heating ramp. The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

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

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

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

	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