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# Cold-evoked potentials in clinical practice: A head-to-head contrast with laser-evoked responses

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-Running head: Head-to-head contrast between LEPs and CEPs

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**-Significance:** Recording of cold-evoked potentials is a well-tolerated, inexpensive and easy-to-use procedure that can be helpful in the diagnosis of abnormalities in the thin fiber-spinothalamic pathways. Supplementing LEPs with CEPs allows consolidating the diagnosis and, for some patients suffering from symptoms limited only to cold, CEPs but not LEPs may allow the diagnosis of thin fiber pathology. Optimal CEP recording conditions are of importance to overcome the low signal-to-noise ratio and habituation phenomena, which are less favorable than with LEPs.

## Abstract

**Background:** Innocuous cooling of the skin activates cold-specific A $\delta$  fibers, and hence the recording of cold-evoked potentials (CEPs) may improve the objective assessment of human thermo-nociceptive function. While feasibility of CEP recordings in healthy humans has been reported, their reliability and diagnostic use in clinical conditions have not been documented.

**Methods:** Here we report the results of CEP recordings in 60 consecutive patients with suspected neuropathic pain, compared with laser-evoked potentials (LEPs) which are the gold-standard for thermo-algesic instrumental assessment.

**Results:** CEP recording was a well-tolerated procedure, with only ~15 min of surplus in exam duration. Reproducibility and signal-to-noise ratio of CEPs were lower than those of LEPs, in particular for distal lower limbs. While laser responses were interpretable in all patients, CEPs interpretation was inconclusive in 5/60 because of artifacts or lack of response on the unaffected side. Both techniques yielded concordant results in 73% of the patients. In 12 patients CEPs yielded abnormal values while LEPs remained within normal limits; 3 of these patients had clinical symptoms limited to cold sensations, including cold-heat transformation.

**Conclusions:** CEPs appear as a useful technique for exploring pain/temperature systems. Advantages are low cost of equipment and innocuity. Disadvantages are low signal-to-noise ratio for lower limb stimulation, and sensitivity to fatigue/habituation. Joint recording of CEPs and LEPs can increase the sensitivity of neurophysiological techniques to thin fiber-spinothalamic lesions, in particular when abnormalities of cold perception predominate.

## Introduction:

Diagnosis of neuropathic pain relies on a neurologically plausible distribution of pain in regions with abnormal somatosensory function (Treede et al 2008, Finnerup et al 2016). This implies that for patients with suspected neuropathic pain (NP), it is essential to have clinical and instrumental tools to assess the functionality of somatosensory pathways, and particularly the thin-fiber/spinothalamic system which is most specifically related to NP development (Boivie et al 1989, Truini et al 2013).

Heat-evoked potentials triggered by contact thermodes or laser pulses are the gold-standard for the assessment of thin-fiber/spinothalamic function. Through the specific activation of A $\delta$  and C receptors, spinothalamic tract neurons and corresponding thalamo-cortical networks, heat stimulations produce recordable cortical responses specific to the transmission of pain/temperature signals (Cruccu et al., 2010, La Cesa et al 2018, Convers et al 2020, Garcia-Larrea & Hagiwara 2018, De Schoenmacker et al 2021).

Innocuous cooling of the skin also activates A $\delta$  fibers, including a cold-specific A $\delta$ -spinothalamic population (Kenshalo and Duclaux 1977, Dostrovsky and Craig, 1996, Campero et al., 2001), and the recording of cold-evoked potentials (CEPs) would represent a highly appealing tool for the assessment of thermal function in neuropathic pain. CEPs were first demonstrated by Duclaux et al. (1974) and Jamal et al. (1989). However, the very slow temperature change in initial experiments yielded desynchronized and fragile responses, which were abandoned until the development of thermodes with more efficient cooling ramps (Hüllemann et al. 2016). Since then, a number of authors have recorded reliable CEPs in healthy subjects, and shown their ability to measure loss of function in experimental settings. Using cooling thermodes with rapid ramps of up to 300°C/s, Leone et al. (2019) estimated peripheral conduction velocity of cold-afferent signals at ~13 m/s, consistent with stimulation of A $\delta$  fibers, and showed CEPs to be delayed by 30 ms relative to laser-evoked responses.

In an effort to improve the technique and make it reliable enough for clinical use, important questions have been addressed in recent years, including the influence of skin type, fixed or variable probe position, inter-stimulus interval and aging, (Hüllemann et al. 2016, 2019, Rosner et al. 2019, De Keyser et al. 2018, Leone et al 2019, 2020, Lithfous et al 2022, Scheuren et al 2022). While these studies substantiate the potentialities of CEPs as a diagnostic tool, clinical studies in NP patients have been so far limited to selective case reports. Jamal et al. (1989) showed CEP abnormalities in two patients with, respectively, small fiber neuropathy and syringomyelia. Leone et al (2019) and Hüllemann et al. (2019) also recorded abnormal CEPs in respectively two and three patients with abnormal cold processing, and concluded that CEPs could provide complementary information to laser-evoked potential recordings. The main objective of our study was therefore to test the clinical use of CEPs in a large series of patients, and compare their applicability and diagnostic yield *versus* laser-evoked potentials (LEPs) in a head-to-head fashion. Here we report the results of the systematic recording of CEPs and LEPs in a cohort of 60 consecutive patients suspected of having neuropathic pain.

## Methods and Patients:

### 1- Patients:

Sixty consecutive patients (28 men, mean age:  $49 \pm 12.3$  years) with clinical suspicion of NP were referred by their algologist, neurologist or family doctor for electrophysiological assessment at Pierre Wertheimer Neurological Hospital in Lyon (France), between September 2017 and September 2022. Twenty patients had upper limb (UL) and/or trunk pain, 32 had lower limb (LL) pain and 8 facial pain. Thirty-six patients (61% of the sample) had unilateral pain allowing comparison with a contralateral normal, non-painful side, while pain was bilateral or diffuse in 39%. Before electrophysiological assessment, patients were classed as having possible, probable or definite NP according to the IASP criteria (Finnerup et al., 2016). Demographical and clinical data of the 60 patients are summarized in Table 1. Each of the procedures applied in this investigation was part of the routine clinical management of patients. The laser stimulus was CE-marked and approved by the local and national Ethics Committee (CPP Sud Est IV n° 2006-A00572-49). In accordance with French legislation, acquisition and publication of routine diagnostic data collected anonymously that does not change the regular management of patients does not need to be declared or submitted to a research ethics board.

### 2-Stimuli:

Somatosensory evoked potentials to infrared laser pulses and transient cold stimuli were recorded in each patient. For each modality, 20-30 stimuli were applied, with interstimulus interval (ISI) of  $10 \pm 2$  s. For each patient, the same number of laser and cold stimuli was delivered for the control and the painful side, to ensure a reliable comparison between both sides, and modalities. The order of stimulations (laser or cold) was counter-balanced between subjects (pseudo-randomisation).

#### *a) Laser stimulation:*

Cutaneous radiant heat stimuli of 5 ms duration were delivered by a Nd:YAP-laser (Neodimium:Yttrium-Aluminium-Perovskite; 1.34  $\mu\text{m}$  wavelength; 4 mm beam diameter ElEn®, Florence, Italy). A 10 m length optical fiber transmitted the laser beam from the generator to the stimulating probe. The Nd:YAP laser allowed to pre-set the energy density delivered (in  $\text{mJ}/\text{mm}^2$ ) and the pulse duration. A red helium-neon (He-Ne) laser confocal with the infrared beam was used to visually indicate the irradiated area. The stimulus intensity was kept stable during the whole recording session, at or slightly above the nociceptive threshold in each subject, determined just before the recording. This threshold corresponded to a level of 4 on a verbal numerical scale ranging from 0 to 10 (where 0= no sensation; 1= barely perceived; 2= lightly pricking, not painful; 3= clearly pricking, not painful; 4= barely painful, like pulling a hair (i.e. pain threshold); 5= painful, prompting to rub the skin; 6= very painful and distressing; 7 and more= strongly unpleasant pain). The energy density needed to reach pain thresholds (80-100  $\text{mJ}/\text{mm}^2$ ) was in the range of standard values established in our and other laboratories (Truini et al., 2007a,b; Perchet et al., 2008, 2012). To avoid skin

damage, fatigue or habituation of nociceptors, the heat spot was slightly shifted a few millimeters after each stimulus.

*b) Transient cold stimulation:*

Transient cold stimuli were delivered using a contact cold stimulator based on micro-Peltier elements (TCS II; QST.Lab, Strasbourg, France). The stimulation probe was round-shaped, with a flat surface of 30 mm diameter in contact with the skin, and consisted of 15 embedded micro-Peltier elements, divided into 5 equal stimulation areas. The TCS system allows adjusting the size of the stimulated surface by determining which Peltier elements are active during stimulation. Activation of all micro-Peltier elements resulted in a 90 mm<sup>2</sup> stimulation surface, which was systematically used in this work. Stimulus duration was 100 ms, with a target probe temperature of 0°C attained using a steep ramp of 300°C/s. The sensation perceived was innocuous, described as similar to a light rapid contact with an ice cube. Care was taken throughout the session to maintain good contact between the probe and the subject's skin. During the experiment, the probe, weighting 440 g, was slightly displaced after each stimulus. Thermal stimulation paradigms are included in routine electrophysiological tests in the Neurological hospital, and give rise to a report that is included in the medical files.

### **3- Clinical examination and recording procedures**

All patients underwent clinical neurological examination before initiating the recordings. Sensory examination integrated testing of lemniscal (Von Frey filaments, light touch, joint position and vibration senses, graphaesthesia) and spinothalamic functions (superficial pain and heat sensations). After the determination of the sensory and nociceptive thresholds, patients underwent electrodiagnostic procedures including the recording of scalp evoked potentials, reaction times and sympathetic skin responses in response to different types of stimulations in order to test different types of fibers. For patients with diffuse or quadri-distal pain, stimulations were addressed to the territory where pain was maximal.

The present study concerns exclusively the comparison of cold and laser-evoked scalp potentials.

Scalp recordings were obtained using 32 electrodes mounted on an electrode cap (WaveGuard, ANT® - Advanced Neuro Technologies), and referenced to the nose. A ground was placed on the mid-forehead. Skin impedance was kept below 5 kΩ. Blinks and saccades were recorded on frontopolar electrodes. The EEG signal was continuously recorded, amplified and band-pass filtered (non-phase shifting -3 dB cutoff at 0.3–30 Hz; 12dB roll off slope) and written to hard disk (Advanced Neuro Technologies, ANT®). Epoching of the EEG, selective averaging and recordings analysis were performed off-line using Brain Vision® (Brain Products®, Munich, Germany). Patients were asked to move slightly the index finger upon the perception of each stimulus, reaction times being recorded with bipolar surface electrodes attached to the hand.

#### **4- Evoked potentials (EPs):**

Prior to averaging, the EEG was segmented into 1000 ms epochs, and each single EEG segment was visually analyzed to reject those contaminated with ocular artifacts (blink or saccades) or EMG activity with amplitude exceeding  $\pm 50 \mu\text{V}$ . This was performed by two different examiners and checked by a third one in case of discrepancy. Epochs containing blinks impacting the main analysis window (the N2-P2 latency, 100-500 ms) were discarded even if their amplitude did not reach the  $50 \mu\text{V}$  threshold. For both laser and cold stimuli, the evoked responses were analyzed over a 1000 ms epoch, including a pre-stimulus baseline of 100 ms. For the two modalities of stimulation, we assessed the two main scalp components appearing in the 100 – 450 ms window, commonly labeled “N2” and “P2”, or “vertex potentials” (Crucchi et al., 2008; Garcia-Larrea and Hagiwara, 2018; Leone et al., 2019; Hüllemann et al., 2019). Components were defined in terms of scalp topography and sequential development. N2 corresponded to the negative vertex wave maximal over the midline and culminating between 120 and 230 ms in healthy controls. “P2”, or the positive component of the vertex complex, was the positive wave following immediately the N2 wave, culminating between 250 and 450 ms in healthy controls.

LEPs were considered abnormal if N2/P2 response amplitude to stimulation of the affected, painful side was depressed by at least 30% relative to the normal side (or by 2.5 SDs relative to the average value in normal controls) and/or peak latency of vertex responses was delayed by at least 30 ms (Beydoun et al., 1993; Garcia-Larrea et al., 2010; Vartiainen et al., 2016). CEP data in patients were interpreted with reference to our local normative data bank (n=30 subjects, age-range 20 – 62 years, mean age:  $38 \pm 14$  years) and abnormality also considered when values diverged by at least 2.5 SDs from mean normal data. Normal limits of latency and amplitude for LEPs and CEPs used in this study are summarized in Table 2.

#### **5- Statistical analysis:**

The N2 and P2 peak-latencies and N2-P2 peak-to-peak amplitude were analyzed on the vertex (Cz) electrode. This scalp site was used to analyze responses to both types of stimuli in a given patient.

##### *a-Individual level analysis:*

After classification of individual responses according to the above criteria, the association between diagnostic groups (normal / abnormal) using LEPs and CEPs was assessed by Chi-square analysis. Individual values of latency and amplitude obtained using the two modes of stimulation were subject to Pearson product-moment correlation. Correlations were first performed regardless of the stimulated territory and then by considering separately the responses from stimulation of the upper body (face, upper limb and trunk) and the lower body (abdomen, perineum and lower limbs). The correlation coefficients obtained for the upper body and for the lower body were compared statistically.

##### *b-Group level analysis:*

In patients with unilateral pain (n=36), responses were submitted to 3-way repeated-measures ANOVA, with type of stimulus (cold vs. laser) and side (control vs. affected) as within-subject factors, and affected territory (upper vs. lower body) as between-subject factor. When appropriate, the Geisser-Greenhouse (GG) correction was applied to degrees of freedom. A level of  $p < 0.05$  after GG correction was accepted as statistically significant. Post-hoc comparisons (t-tests) were performed when ANOVA yielded significant main results. For values that did not follow a normal distribution, the Wilcoxon test was added to the post-hoc tests. If a discrepancy between the two tests was observed, it was indicated in the results.

Paired sample t-tests were used to compare the responses obtained on the normal side for both stimulation modalities and to compare the responses obtained in normal subjects of our local database and on the control side of the patients.

## **Results**

### ***1- Description and morphology of responses:***

#### **- Laser-evoked potentials (LEPs):**

LEPs were formed by a main biphasic response over the midline with maximum amplitude at the vertex (Cz), often preceded by a lateralized response over the temporal areas, which was not used for diagnosis because of its small amplitude and inconsistent identification. Mean latencies and amplitudes of the midline response to stimulation of face, upper limbs/trunk and lower limbs are summarized in Table 2. There was no statistically significant difference between LEPs to stimulation of the unaffected side in the patients in this series in comparison with normative values of our lab (Perchet et al., 2008, 2012) and values provided by other studies using Nd:YAP lasers with the same parameters of stimulation (Cruccu et al., 2003; Frasson et al., 2021).

#### **- Cold-evoked potentials (CEPs):**

The morphology of CEPs was very similar to that of LEPs, also dominated by a biphasic midline response “N2-P2” complex with maximum amplitude at the vertex (Cz). Mean latencies and amplitudes to the three sites of stimulation are summarized in Table 2. As shown in Figure 1, while morphology to stimulation of the normal side were similar for LEPs and CEPs, latencies to cold stimulation were significantly delayed relative to those obtained after laser stimulation LEPs (N2:  $278 \pm 57$  ms vs.  $226 \pm 41$  ms,  $t(33) = -7.8$ ,  $p < 0.001$ ; P2:  $425 \pm 94$  ms vs.  $367 \pm 77$  ms,  $t(33) = -3.7$ ,  $p < 0.001$ ) respectively. Amplitudes were also significantly smaller to cold stimulation as compared to laser stimulation ( $13 \pm 8$   $\mu$ V vs.  $18 \pm 9$   $\mu$ V,  $t(33) = 3.8$ ,  $p < 0.001$ ) respectively. There was no statistically significant difference between CEPs to stimulation of the unaffected side in the patients in this series in comparison with normative values of our lab and values provided by other studies using cold stimulators with the same parameters of stimulation (De Keyser et al. 2018; Leone et al., 2019).

### ***2- Clinical feasibility and reproducibility of the responses:***

#### **- LEPs:**

The range of stimulus intensities was 80 to 100 mJ/mm<sup>2</sup> and the same intensity was always used for both sides. All patients reported clear pinprick sensations to stimulation of the non-affected territory and more variable sensations to stimulation of the affected region, ranging from barely noticed to hyperalgesic. LEPs were interpretable in 100% of cases after stimulation of either the normal or the affected territories, although they were often of lower amplitude after stimulation of lower limbs.

- CEPs:

Cold stimuli evoked a sensation comparable to “being touched by an ice cube”, that was not unpleasant. CEPs were not interpretable in 5/60 patients either because of (i) stimulation artifacts (in 2 patients stimulated on the face and T7 dermatome), or (ii) lack of response on the non-affected control area (3 patients stimulated on the lower limbs). Six patients showed low-amplitude CEPs (<5 $\mu$ V). To identify and analyze the CEP of these patients, several complementary procedures were used: search for a negative-positive complex within the latency window where the CEP was expected; comparison with responses recorded by the electrodes surrounding the vertex (C3, C4, CPz, P3, P4, Fz); checking the temporal correlation of scalp responses with the motor reaction time (EMG), and comparison with the LEPs obtained in the same patients to verify morphological similarity.

**3- Group comparison of LEPs vs. CEPs:**

Repeated measures three factor ANOVA showed significant effect of “type of stimulus” (cold or laser) ( $F(1,29)=24.1$ ,  $p<0.001$ ) and “stimulated territory” (upper or lower body) ( $F(1,29)=23.9$ ,  $p<0.001$ ) on N2 latency with a significant interaction between “stimulus type” and “stimulated territory” ( $F(1,29)= 4.8$ ,  $p=0.04$ ) explained by significantly shorter latencies for LEPs as compared to CEPs in the lower part of the body. The factor ‘side’ almost reached significance ( $F(1,29)= 3.1$ ,  $p=0.06$ ) with mean N2 latencies to stimulations of the affected side slightly later than those obtained on the control side (265ms vs. 255ms). On post-hoc analysis, N2 latencies were significantly longer with CEPs than LEPs (289ms vs. 232ms;  $t(29)= -4.9$ ,  $p<0.001$ ) respectively and for lower limbs than for upper limbs (289ms vs. 235ms;  $t(29)= -4.9$ ,  $p<0.001$ ) respectively (figure 6).

Repeated measures three factor ANOVA showed a significant effect on P2 latencies of the factors “type of stimulus” ( $F(1,29)=10.9$ ,  $p=0.003$ ), “stimulated territory” ( $F(1,29)=16.7$ ,  $p<0.001$ ) and “side” ( $F(1,29)= 5.3$ ,  $p=0.03$ ). On post-hoc analysis, P2 latencies were significantly later with CEPs than LEPs (435ms vs. 377ms;  $t(29)= -3.3$ ,  $p=0.003$ ) respectively and for lower than upper limbs (442ms vs. 384ms;  $t(29)= 4.1$ ,  $p<0.001$ ) respectively (figure 6). P2 latencies were also greater on the affected side compared to the control side (414ms vs. 393ms;  $t(29)= -2.3$ ,  $p=0.03$ ) respectively. The interaction between “type of stimulus” and “stimulated territory” was significant ( $F(1,29)= 4.3$ ,  $p=0.04$ ) which was explained by significantly longer P2 latencies of CEPs compared to LEPs in the lower parts of the body (492ms vs. 392ms;  $t(29)= -3.6$ ,  $p=0.006$ ) but lack of significant difference for the upper parts (380ms vs. 364ms;  $t(29)=-0.9$ ,  $p=0.9$ ).

Repeated measures 3-factor ANOVA showed a significant effect on the N2P2 amplitude of the cortical responses recorded at the vertex, of the factors “type of stimulus” ( $F(1, 29) = 16.5, p < 0.001$ ) and “side” ( $F(1, 29) = 11.6, p = 0.002$ ). On post-hoc analysis, N2P2 amplitude was smaller on CEPs compared to LEPs ( $12\mu\text{V}$  vs.  $16\mu\text{V}$ ;  $t(29) = 4.1, p < 0.001$ ) and in the affected side compared to the control side ( $12\mu\text{V}$  vs.  $16\mu\text{V}$ ;  $t(29) = 3.4, p = 0.002$ ) (Figures 2 and 6). There was no significant difference in N2P2 amplitude according to the factor “territory”. The interaction between the different factors was not significant either.

#### **4- Individual analysis of LEPs and CEPs**

##### *-Association between diagnostic classifications*

Chi-square analysis showed a significant association between the diagnostic classification of the responses to both techniques ( $\chi^2 = 12.1; p < 0.01$ ). Thus, in a majority of cases (40/55 patients; 73%) there was concordance between the diagnostic classification of the LEPs and CEPs (Figure 3 for examples). A discrepancy in the diagnostic classification was found in 27% of the sample (15/55 patients): in 3 of these cases CEPs were classified as normal while LEPs were abnormal, while in 12/15 cases of discrepancy it was the opposite (Figure 3). One of these 12 patients (n° 50) could be considered as a ‘false positive’ CEP, since on further examination pain proved to result from a mechanical ankle lesion without neuropathic component, and LEPs remained strictly normal. One patient (n°5) with a cervical plexus lesion after excision of a parotid tumor had CEPs and LEPs attenuated to stimulation of the affected side, but only CEPs reached the statistical threshold of 2.5 SDs below normal limits. One patient (n° 57) with normal LEPs but delayed CEPs was under evaluation for multiple sclerosis due to abnormal CSF findings and T2 hyper-intensities on spinal and brain MRIs. The isolated CEP abnormality of this patient was not considered as a ‘false positive’ since cold hypoesthesia has been suggested as a sign of MS-related central pain (Rivel et al 2022). Finally, 5 patients had different levels of altered sensory thresholds to cold, and 3 had cold-specific symptoms, including cold-heat transformation, which could be related to the CEP abnormalities.

When the upper and lower body territories were analyzed separately, Chi-square analysis showed very significant association between the diagnostic classification of the responses to both techniques when applied to *upper body parts* ( $\chi^2 = 12.2; p < 0.01$ ). In a vast majority of cases (22/26 patients; 85%) there was concordance between the diagnostic classification from LEPs and CEPs. A discrepancy in classification was found in 15% of the sample (4/26 patients): in one of them the LEPs were classified as normal and the CEPs as abnormal and in the 3 others it was the opposite. When data from the lower body was analysed separately, although Chi-square analysis showed again a significant association between the two diagnostic classifications ( $\chi^2 = 5.3; p < 0.05$ ), the percentage of divergent results significantly increased to 38% of the sample (11/29 patients): in all of them LEPs were classified as normal and the CEPs as abnormal.

##### *- Correlational analyses:*

There was a significant positive correlation between the values of N2, P2 latencies and N2P2 peak-to-peak amplitude obtained for LEPs and CEPs (N2 latency:  $r = 0.620; p < 0.001; 95\% \text{CI} = [0.429; 0.758]$ ; P2

latency:  $r=0.479$ ;  $p<0.001$ ; 95%CI= [0.242; 0.662] and N2P2 amplitude:  $r= 0.405$ ;  $p= 0.003$ ; 95%CI= [0.152; 0.609]. When the different territories were considered separately, correlations between latencies and amplitudes persisted for the upper body segments (face, trunk and upper limbs). Conversely, for the lower limbs, there was no significant correlation between N2 and P2 latencies but only a significant correlation for N2P2 amplitude ( $r=0.558$ ;  $p<0.001$ ; 95%CI= [0.254; 0.762]) (Figure 4). The respective correlation coefficients for N2 latencies were significantly different ( $r= 0.837$  vs.  $r= 0.016$ ,  $U= 4.36$ ). Similar results were obtained in correlation data of CEP and LEP P2 latencies ( $r= 0.666$  vs.  $r= 0.169$ ;  $U=2.31$ ). Conversely, no statistical difference was observed between the N2P2 amplitude correlations obtained for the upper and lower parts of the body ( $r= 0.398$  vs.  $r= 0.558$ ;  $U= 0.76$ ).

## **Discussion:**

The recording of cold-evoked potentials (CEPs) proved feasible under routine clinical conditions in patients with possible, probable or definite neuropathic pain (NP); acquisition of CEPs represented only about 15 min of surplus in exam duration. CEP recordings could be performed technically in each patient and cold stimuli were always well tolerated; however, the reproducibility of responses and the signal-to-noise ratio were in general lower than for responses to conventional laser stimuli (Figure 5). Thus, while responses to laser were interpretable in every patient, those to cold stimuli could not be reliably assessed for technical or procedural reasons in 5/60 patients (8% of the sample), due to high-voltage artifacts or lack of sizeable response to stimulation of the unaffected side. In six other cases, CEP responses were of very low amplitude and could only be safely interpreted by their morphological similarity to the LEPs, and their latency concordance with the simultaneously recorded motor reactions. This implies that in 11/60 (18%) of this sample CEPs could not have been clinically assessed with confidence had they been recorded alone. This may contrast with previous studies in healthy volunteers reporting that cold-evoked responses could be systematically recorded in all subjects under very different conditions (e.g. De Keyser et al., 2018; Leone et al., 2019; Rosner et al., 2019). These reports, however, were based on stimuli delivered to young, medication-free healthy volunteers stimulated in the face, cervical dermatomes or upper limbs, and the results are hardly comparable to the recordings presented here, obtained from clinical patients of older age and, for most of them, medicated with psychotropic drugs. Even under such difficult settings, CEPs proved readily interpretable in 82% of cases, hence supporting their technical feasibility in routine clinical environments.

Cortical responses were in general of lower amplitude and longer latency for CEPs compared to LEPs, most probably due to technical differences between the two modes of stimulation. Despite using a thermode with ultra-rapid cooling ramps of 300 °C/sec, such cooling rates cannot be compared with the temperature ramps induced by laser pulses, which can reach 10,000 °C/sec (Plaghki and Mouraux, 2003). Laser-induced temperature ramps allow using very short duration pulses of 5 ms or less, which activate simultaneously the set of thermal responding fibers. In contrast, the duration of cold stimuli was 100 ms, which entails the progressive activation of receptors with different response thresholds, hence inducing a desynchronized

afferent volley. Such differences increase with the distance between the stimulated region and the cortex, and accordingly were maximal for stimulation of the lower limbs. As illustrated in Figures 2 and 5, the stability and quality of responses to cold stimuli applied to the face and upper limbs were quite similar to those of LEPs, whereas their signal-to-noise ratio and inter-trial stability highly decreased relative to laser stimuli when lower limbs were stimulated.

These differences in cortical responses translated to correlation analyses: correlations between LEP and CEP parameters were robust and highly significant for stimulation of the upper body (face, trunk and upper limbs), while significance was less robust when the lower limbs were considered in isolation, mainly due to a high dispersion of CEP values.

Despite the differences outlined above, there were clear similarities between both techniques not only in terms of morphology and scalp distribution (Figure 1) but also in diagnostic yield. There was a very significant association in the dichotomic (normal / abnormal) patient classification via CEPs and LEPs, (Figure 3), with 73% classification concordance. Mismatches were observed in 27% of cases and were unevenly distributed, with 3/15 patients having abnormal LEPs but normal CEPs and 12/15 the reverse, i.e. abnormal CEPs –mostly to stimulation of lower limbs– in the presence of LEPs within physiological limits. Thus, the majority of patients presenting a classification discordance had normal LEPs but abnormal CEPs, which may suggest a superior sensitivity of the latter to detect subtle or highly specific abnormalities of transmission. It should be noted that CEPs appear to be technically less reliable than LEPs, because of their increased susceptibility to drowsiness, fatigue or habituation. In addition, we detected 1 clear false positive, suggesting that abnormal CEPs may not always be due to the underlying pathology. However, as suggested by results from patients n° 56 and 58, CEPs can also provide additional and potentially critical information in certain patients, such as those with predominant or specific impairment of the cold transmission system.

While in rodents and primates a proportion of A $\delta$  fibers may be involved in both cold and warm information transmission (Dubner et al. 1975, Cain et al. 2001, Campero et al., 2001), innocuous cooling appears to be conveyed by a separate cold-specific A $\delta$  population in both humans and non-human primates (Kenshalo and Duclaux 1977), which does not contribute to the detection of noxious heat (Dubner et al. 1975) and may be transmitted separately within the spinothalamic system to supraspinal structures (Dostrovsky and Craig 1996, Bowsher 2005). Differences between membrane receptors sensitive to cooling (TRPA1, TRPM8) and heating of the skin (TRPV1-4, TRPM2/4/5) are well recognized, and a number of specific conditions such as oxaliplatin, paclitaxel or ciguatera-related neuropathies are characterized by cold allodynia (Patel et al., 2015; Chaumette et al., 2018; Zanata et al., 2021) while others such as erythromelalgia produce exclusively heat-related symptoms (Genebriera et al. 2012). Three patients with selectively abnormal CEPs in our series had a specific alteration of cold sensation and two of them had a very specific alteration of cold sensation, termed “cold-heat” transformation, whereby low-temperature cold stimuli evoke an unpleasant burning sensation. Such rare type of altered perception is seen in cases of peripheral neuropathy and is thought to reflect a selective alteration of cold A $\delta$  channels, since it can be reproduced in healthy subjects by selectively

blocking A $\delta$  afferents (Yarnitsky and Ochoa 1990). Thus, although the number of patients examined in this study does not allow strong generalization on this respect, the elective abnormality of responses to cold may signal in some cases their superior sensitivity to detect subtle or highly specific abnormalities of transmission involving exclusively the subset of afferent channels activated by cold stimuli. Larger patients' samples with long follow-up remain needed to assess these possibilities.

## **Limitations**

The results presented herein emanate from routine clinical procedures, and hence the interpretation of results was not blinded, but established with explicit knowledge of the type of exam. Convergence of interpretation between at least two of the authors was however ensured, and results submitted to a third party in case of unclear or doubtful results. Interpretation of all exams was performed before any statistical analysis of the group data, hence reasonably ensuring the absence of systematic bias.

Also of notice, although all patients were sent for diagnostic tests under a suspicion of neuropathic pain, both LEPs and CEPs remained within the normal range in around half of the patients. This was due to the fact that a number of patients reported painful symptoms of doubtful interpretation (e.g. from questionnaires) but which suggested the possibility of NP, and were addressed to the lab to verify objectively the absence of somatosensory transmission abnormalities, and hence formally dismiss the suspicion of NP. In most of these patients, results were normal for both techniques, and substantiated the absence of somatosensory abnormalities in small fibers / spinothalamic paths.

Finally, since patients were essentially referred to us for suspicion of neuropathic pain, they were for most of them under pharmacological treatment including psychotropic drugs such as antidepressants, anxiolytic or anticonvulsant drugs. While some of these drugs are known to affect LEP parameters, their effects on CEPs is still unknown, and might be enhanced due to the potentially lower robustness of CEPs relative to LEPs.

## **Conclusions**

CEPs proved feasible in clinical routine. Their characteristics, in particular their latency, are clearly compatible with the activation of A $\delta$  fibers, and therefore they can be used to objectify a peripheral or central abnormality in the thin fibers / spinothalamic pathways. The advantages of CEPs over LEPs are the lower cost of the equipment and the absence of pain induced by the stimulus (except in rare cases of neuropathy with cold-heat transformation). The disadvantages are related to the same characteristics, since the finer sensation results in a lower signal-to-noise ratio compared to LEPs, especially for lower limb stimulation, and a greater sensitivity to fatigue and habituation. These drawbacks prevent opting for CEPs over LEPs or CHEPs as a

gold standard for the instrumental assessment of thin fibre / spinothalamic abnormalities. Nonetheless, CEPs appear to be a useful addition to the arsenal of techniques for exploring pain/temperature systems, and a proportion of patients showed abnormalities to cold stimuli that were not detected by LEPs. Since cold and heat pathways may be partially dissociated, the joint recording of CEPs and LEPs is likely to increase the instrumental sensitivity to detect objectively lesions involving A $\delta$  / spinothalamic pathways.

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## Legends for illustrations and tables:

Figure 1: Sequential maps of grand-averaged LEPs (black lines) and CEPs (grey lines) between 80 and 500 ms post-stimulus in one representative patient (n°17). Each map reflects the average activity within a 30 ms window. In the upper part are depicted the traces recorded at Cz scalp site. Responses to laser (black) and cold (grey) stimuli are superimposed. Note the similar scalp distribution of the LEPs and CEPs, but the smaller amplitudes and delayed latencies of the CEPs.

Figure 2: Grand averaged vertex potentials in the 36 patients with unilateral symptoms, in response to laser (LEPs; left panel) and cold stimuli (CEPs; right panel). Responses to stimulation of the unaffected side (black line) and the painful side (grey line) are superimposed, surrounded by their 95% confidence intervals. The upper panel depicts grand averaged responses to stimulation at all body locations. The lower panel shows differential grand averages of responses to stimulation of the upper body (UB) and the lower body (LB), surrounded by 95% confidence intervals.

Figure 3: Single-patient examples of vertex LEPs (left side) and CEPs (right side), showing different patterns of abnormality. Responses to stimulation of the normal side (black lines) and the painful side (grey lines) are shown superimposed. The uppermost panel shows abnormal responses to both laser and cold stimulation of the affected side. The middle and lower panels show examples of LEP / CEP discordances, with either pathological LEPs but normal CEPs (middle panel) or the reverse (bottom panel).

Figure 4: Correlations between CEPs and LEPs latencies and amplitudes to stimulation of the upper and lower parts of the body.

Figure 5: Examples of vertex LEPs and CEPs from three different patients to stimulation of the hand (upper) (patient n°21), knee (middle) (patient n°40) and foot (lower) (patient n°41). Consecutive averages of 3 stimulations (grey lines) are superimposed to the total average of 20 stimuli (black line), to illustrate the differential inter-trial jitter of CEPs and LEPs. Note the greater jitter for cold stimulations (traces on the right side) and the existence of a gradient of this jitter, greater for the stimulations to knee and foot.

Figure 6: A: N2 and P2 latencies boxplots of LEPs and CEPs for the upper and lower parts of the body. B: N2P2 amplitude boxplot of LEPs and CEPs and of control and affected sides. \*\*= p<0.05 et \*\*\*= p<0.001.

Table 1: Clinical and demographic characteristics of the patient's sample. Abbreviations: F: female, M: male, V1,2,3: trigeminal branches, C: cervical, T: thoracic, L: lumbar, S: sacral, L: left, R: right, ENT: ear, nose and throat, CRPS: complex regional pain syndrome, MS: multiple sclerosis, NP: neuropathic pain, N: normal, Abn: abnormal, NE: not evaluable for technical reasons or absence on both sides, NSAID: non-steroidal anti-

inflammatory drugs, TENS: transcutaneous Electrical Nerve Stimulation, PCT: paracetamol, MDMA: 3,4-méthylènedioxy-N-méthylamphétamine (ecstasy), FBSS: Failed Back Surgery Syndrome, DMA: dynamic mechanic allodynia.

Table 2: Values  $\pm$  SEM of the peak latencies, and peak-to-peak amplitudes of the laser and cold evoked-responses in the different sites of stimulation and for the affected and unaffected sides.

**Table 1:**

Patient		Age	Localisation of pain	Characteristics of pain	Underlying or suspected condition	LEPs	CEPs	Medication
1	F	55	V1-V2 (R)	electrical discharges	neurovascular conflict at trigeminal exit zone	N	N	zolmatriptan, anafranil, PCT
2	M	53	V2-V3 (R>L)	continuous pain background + paroxysmal pain	Polyneuropathy, Charcot-Marie-Tooth syndrome	Ab	Ab	carbamazepine, anafranil
3	M	52	V2 (R)	continuous pain background + paroxysmal pain	Right maxillary lymphoma	N	N	amitriptyline, lidocaine, clomipramine, duloxetine
4	F	57	V1 (R)	tactile and warm allodynia	Meningioma of cavernous sinus	Ab	NE	PCT,codeine
5	M	44	C3 (L)	continuous pain background + electrical discharges	Neuroma exeresis of the left parotid	N	Ab	anafranil, pregabalin, tramadol, ketamine
6	M	64	V3 (L)	permanent burning	post oral surgery labial nerve lesion	Ab	N	gabapentine
7	M	41	C2-C3 (R)	burning + electrical discharges	iatrogenic spinal nerve injury during ENT surgery	N	N	pregabalin, PCT
8	F	64	hemiface (R)	dynamic mechanical allodynia	right cerebellar peduncle and perimesencephalon hematoma	Ab	Ab	ketoprofen, PCT
9	M	36	hemibody (L)	dynamic allodynia	Stroke	Ab	Ab	gabapentin, duloxetine, baclofen
10	M	66	C6 (L)	painful cold, electrical discharges, allodynia	Right paramedian ischemic stroke in the brainstem	Ab	Ab	PCT, gabapentin, clonazepam
11	F	47	C6 (Bil; D>G)	squeezing	Insulinorequiring diabetes-related neuropathy ; carpal tunnel syndrome	N	N	PCT
12	F	38	C6 (L)	electrical dysesthesia, burning, squeezing	post-surgery carpal tunnel nerve lesion	N	N	no
13	M	47	upper limbs, prox. & distal	squeezing, movement-related	lupus - related neuropathy	Ab	Ab	PCT, pregabalin, tramadol, venlafaxine
14	M	68	upper limbs distal and lower limbs prox.	burning, dysesthesia	search for fine fiber injury in a context of great fatigue. Monoclonal IGG-lambda peak	Ab	Ab	clonazepam, pregabalin
15	M	52	upper limbs	dysesthesia		Ab	Ab	no
16	F	58	upper limb (R)	burning, stabbing, twisting	Left Wallenberg syndrome	Ab	N	gabapentin, duloxetine, carbamazepine, tramadol
17	F	31	upper limb (R)	allodynia	Cavernous angioma C2-C4	Ab	Ab	pregabalin amitriptyline

18	F	46	T1 (L)	allodynia	uninformed	Ab	N	pregabalin, tramadol
19	M	37	T7 para-vertebral (R)	Uninformed	T8-T9 herniated disc	Ab	NE	pregabalin, lidocaine, capsaicin
20	M	71	subcostal (L)	inspiration-related	Cyriax syndrome	N	N	amitriptyline
21	F	28	upper and lower limbs	polymorphic, invasive pain	search for fine fiber injury in a context of endometriosis	N	N	cannabis
22	F	25	upper limbs(L>R), distal	Uninformed	Ehlers-Danlos III and cervical syringomyelia + Chiari malformation	N	N	lidocaine, lacosamide, pregabalin, clonazepam
23	M	33	T3-T8 (L)	burning, pricking, squeezing; focal hyperesthesia	post-traumatic intercostal nerve lesion	N	N	capsaicin
24	F	39	Upper limb (L)	burning	C3-T1 syringomyelia + Chiari (operated)	N	N	clomipramine alprazolam
25	M	48	T9-T10 (R)	squeezing, movement-related	Stroke (left thalamo capsular lacuna)	N	N	niflumic acid, niflumate
26	F	52	upper and lower limbs (upper>lower)	burning	search for fine fiber injury in a context of fibromyalgia	N	N	amitriptyline, gabapentin
27	F	50	neck and upper limbs	Uninformed	search for fine fiber injury in a context of C6-C7 syringomyelia	N	N	TENS, capsaicin, clonazepam
28	M	60	T6-T7 (L)	Uninformed	Stroke	N	N	lidocaine, pregabalin, clonazepam
29	F	47	inguinal and thigh regions (R)	hyperalgesia	search for fine fiber injury in a context of endometriosis	N	NE	ketamine, amitriptyline, opium, gabapentin, nefopam
30	F	39	sole of foot (L)	hyperalgesia	post-traumatic medial plantar nerve lesion	Ab	NE	pregabalin, NSAID tramadol, opium, PCT
31	M	76	sole of foot	Uninformed	search for fine fiber injury of lower limbs	Ab	Ab	PCT, amitriptyline, pregabalin, ketamine
32	M	51	L2 (L)	Uninformed	Arthrodesis	N	Ab	gabapentin, amitriptyline, NSAID, lidocaine
33	F	37	L2-L3 (R)	burning	search for fine fiber injury in a context of spinal anaesthesia for hemorrhoids	N	N	clomipramine, ketamine, capsaicin, TENS
34	M	49	cruralgia (R)	hyperalgesia, position-related	Arthrodesis extension to L2	Ab	Ab	pregabalin, clomipramine
	M	28	knee (R)	dynamic allodynia, burning, electrical discharges	peroneal nerve entrapment	N	N	opium, gabapentin, capsaicin, amitriptyline
36	F	66	L1 (ilio-inguinal R)	electrical discharges, movement-related	search for fine fiber injury in a context of cervical lesion	Ab	NE	PCT, opium, pregabalin, clomipramine, amitriptyline

37	M	57	calves	cramps, throbbing	Multiple spinal interventions, herniated discs L4-L5	Ab	Ab	morphine, PCT, ketoprofen
38	F	39	upper and lower limbs (lower>upper), distal	burning, dysesthesia	CRPS left knee, post trauma	N	Ab	opium, paracetamol, prazepam
39	F	52	lower limbs, distal	burning	search for fine fiber injury in a context of possible fibromyalgia	N	N	amitriptyline, opium, pregabaline, gabapentine, PCT, clonazepam
40	F	42	upper and lower limbs	Uninformed	search for fine fiber injury in a context of possible fibromyalgia	N	N	amitriptyline, ketamine, NSAID, propranolol
41	F	46	L5 (R)	Uninformed	Recurrent lumbosciatica with multiple operations	N	N	no
42	M	52	scrotum	electrical discharges	search for fine fiber injury in a context of bipolar disorder	N	Ab	gabapentine, opium, pregabaline, capsaicin, TENS
43	F	45	T10 (R)	burning, dysesthesia	Left thoracic spinal cord injury (T10)	Ab	Ab	pregabaline, amitriptyline
44	F	48	pelvic-perineal	stabbing	Multiple operations of the pelvis	N	N	morphine
45	M	44	lower limbs, distal	dysesthesia	search for fine fiber injury in a context of inguinal herniorraphy	N	N	nefopam
46	M	58	lower limbs, distal	throbbing	Multiple operations for left S1 radiculopathy	N	Ab	opium, pregabaline, gabapentine, duloxetine, ketamine, capsaicin, TENS
47	M	31	upper and lower limbs	Uninformed	search for fine fiber injury in a context of toxic exposure (MDMA)	N	Ab	pregabaline, opium, duloxetine
48	F	68	foot (L)	Uninformed	CRPS after Hallux Valgus surgery	N	Ab	no
49	F	71	Lower limbs, distal	burning	search for fine fiber injury in a context of knee prosthesis	Ab	Ab	gabapentine, opium, capsaicin, oxcarbazepine
50	F	38	foot and ankle(L)	squeezing, movement-related	Ankle sprain	N	Ab	capsaicin, amitriptyline, PCT, opium
51	M	27	knee (L)	terebrating	Road accident	N	N	nefopam, clomipramine
52	F	50	upper and lower limbs, proximal and distal	muscular	search for fine fiber injury in a context of suspected fibromyalgia	N	N	PCT, pregabaline, ropirinol
53	F	49	upper and lower limbs	dynamic allodynia, hyperalgesia	search for fine fiber injury in a context of suspected fibromyalgia	N	N	ropirinol, nefopam

54	F	69	ileo inguinal (R )	Uninformed	search for fine fiber injury in a context of lumbosciatica	Ab	Ab	PCT, opium
55	F	48	upper and lower limbs, distal	burning, throbbing	search for fine fiber injury in a context of rheumatoid arthritis	N	N	PCT
56	M	41	lower limbs, distal	Uninformed	search for fine fiber injury in a context of chemotherapy for stomach cancer	N	Ab	amitriptyline, pregabalin
57	F	43	upper and lower limbs	burning, tingling, throwing	Multiple spinal interventions, probable MS	N	Ab	clonazepam, pregabalin, nefopam
58	M	62	lower limbs, proximal and distal	burning	Spinal cord injury and sequelae of lower limb pyramidal syndrome	N	Ab	no
59	M	64	perineum	burning	left pudendal nerve anomaly	N	Ab	capsaicin, gabapentine, oxcarbazepine
60	F	49	lower limbs	burning, electrical discharges	search for fine fiber injury in a context of diabetes	N	N	lidocaine, duloxetine, gabapentine, pregabalin

Table 2:

Side Stimulated site	LEPs					
	Normal			Painful		
	Face	UL and trunk	LL	Face	UL and trunk	LL
N2 latency (ms)	188±23	226±35	241±43	193±23	252±61	247±43
P2 latency (ms)	290±39	385±76	387±72	287±45	430±121	396±83
AN2P2 (µV)	20±7	16±9	18±9	13±7	13±8	14±7
Side Stimulated site	CEPs					
	Normal			Painful		
	Face	UL and trunk	LL	Face	UL and trunk	LL
N2 latency (ms)	205±16	260±40	316±44	243±46	264±35	330±74
P2 latency (ms)	328±40	377±53	489±81	412±112	392±50	491±102
AN2P2 (µV)	18±8	15±8	11±8	12±4	12±6	10±8

Figure 1:

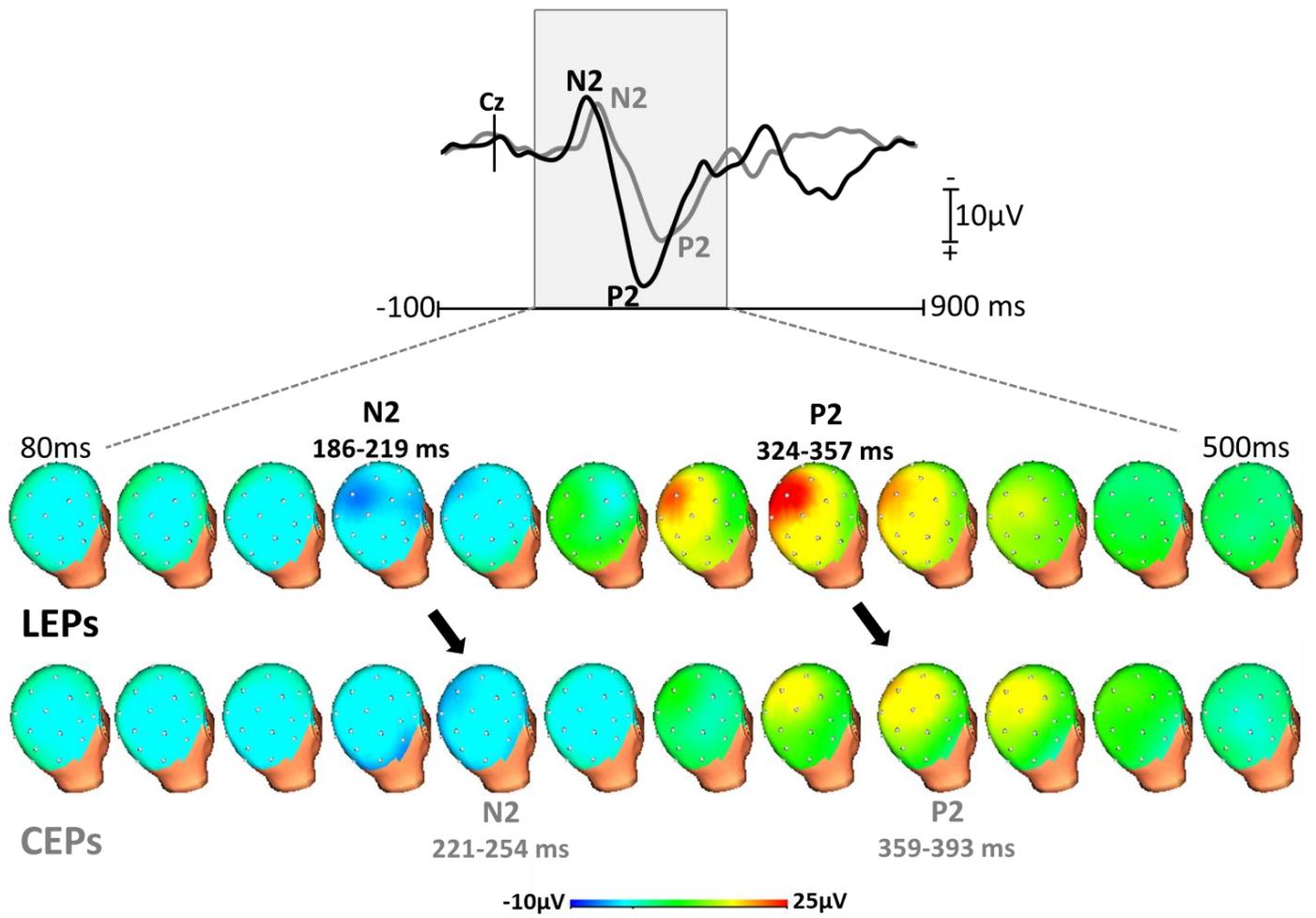


Figure 2:

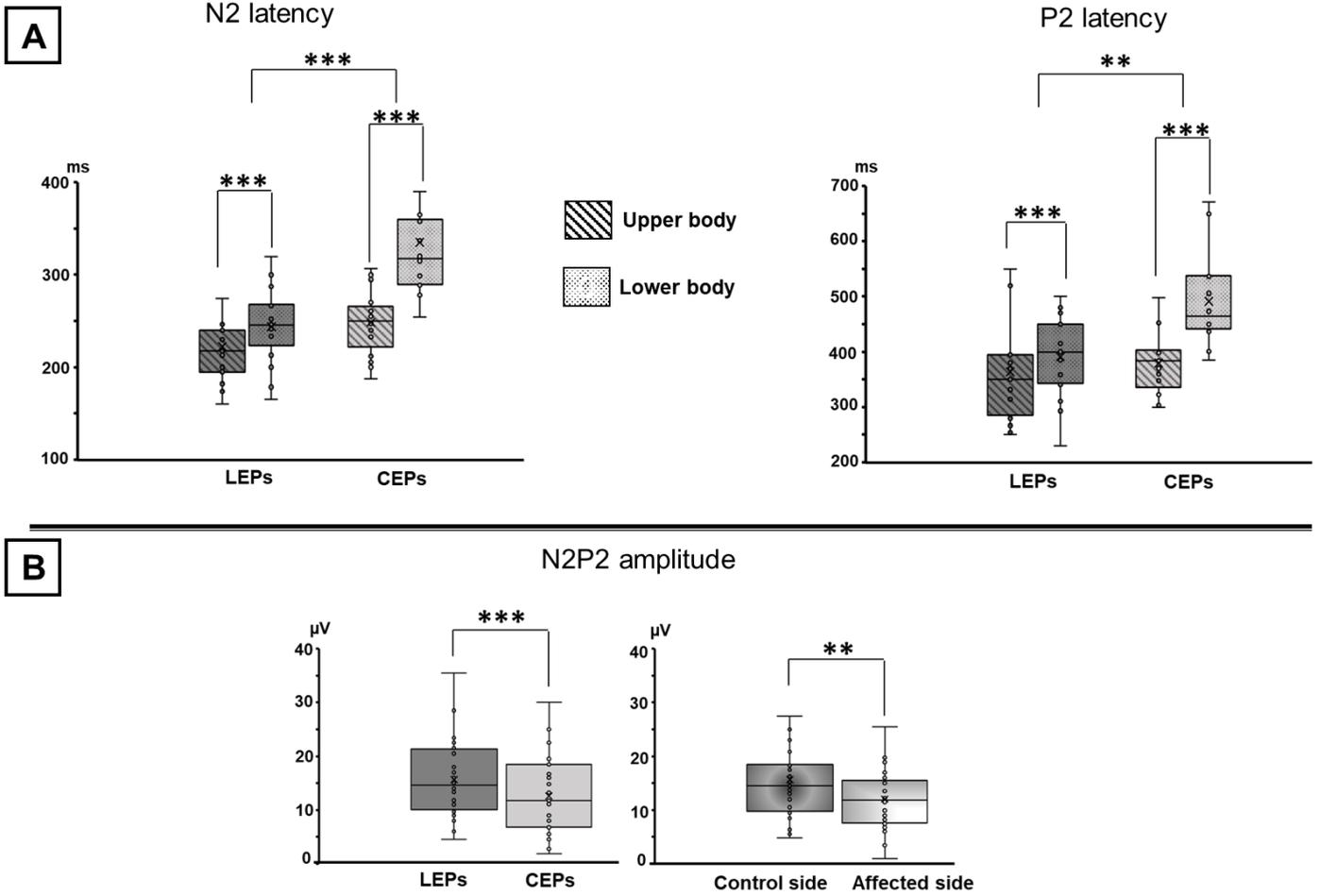


Figure 3:

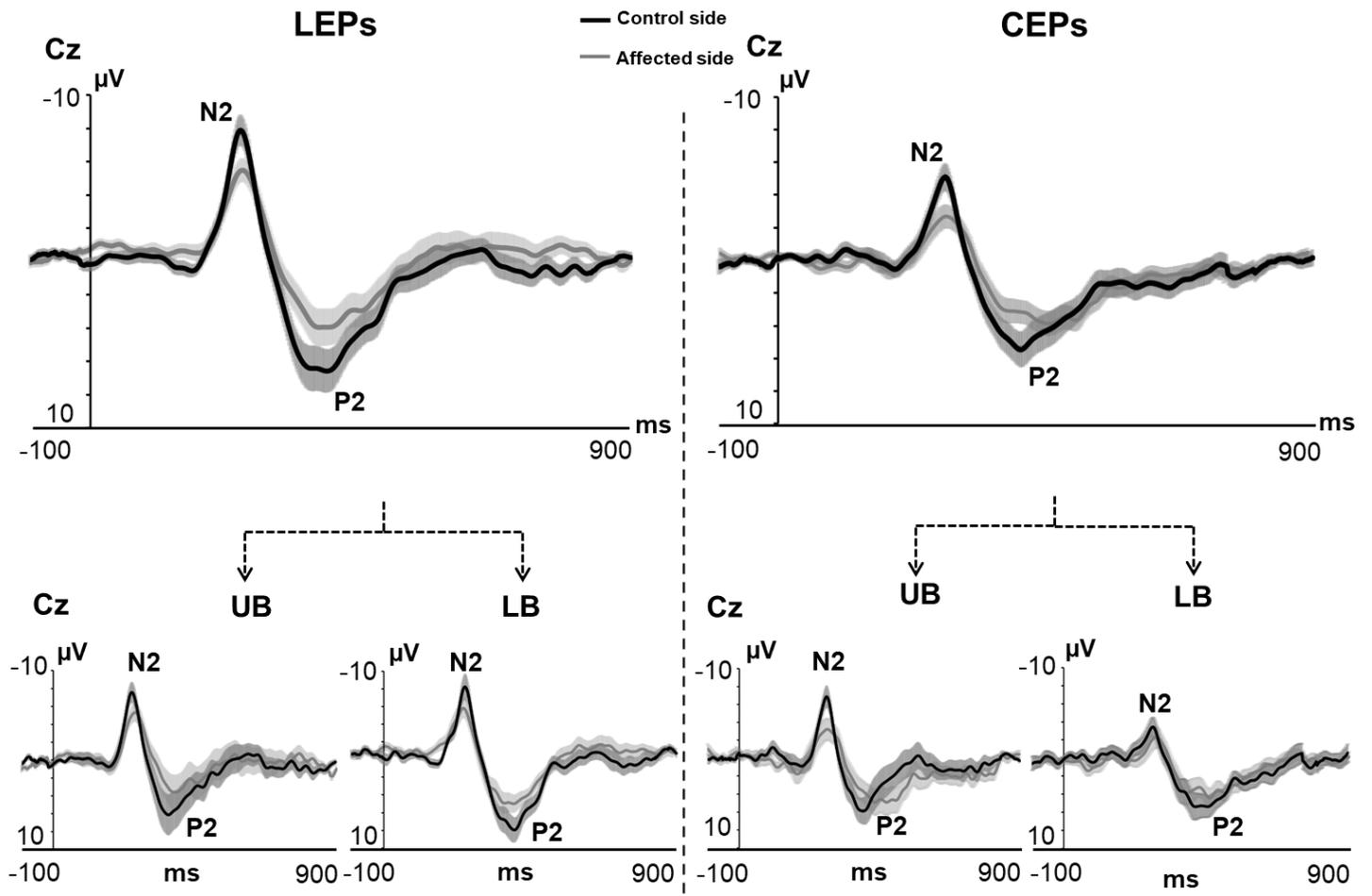


Figure 4 :

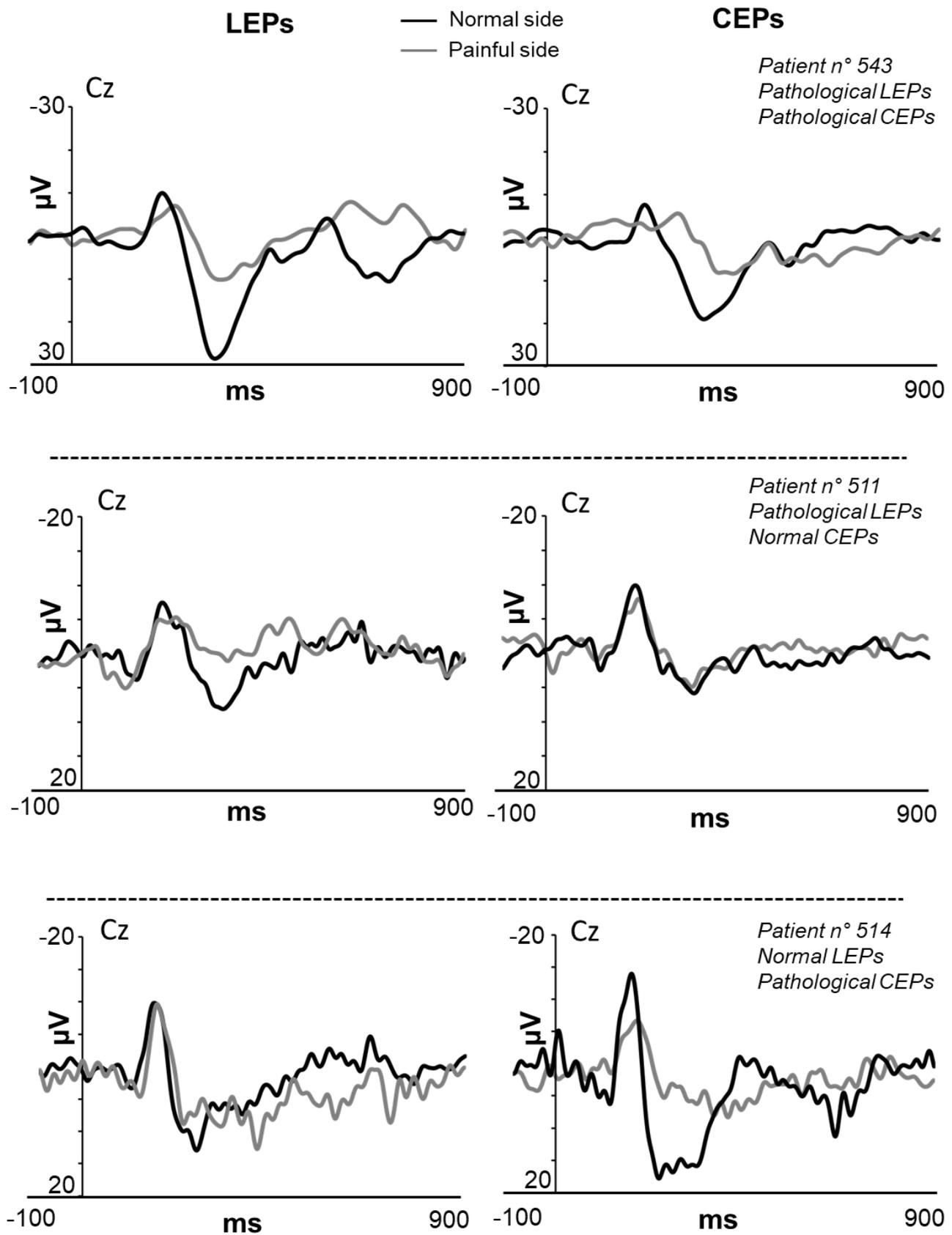


Figure 4:

Figure 5:

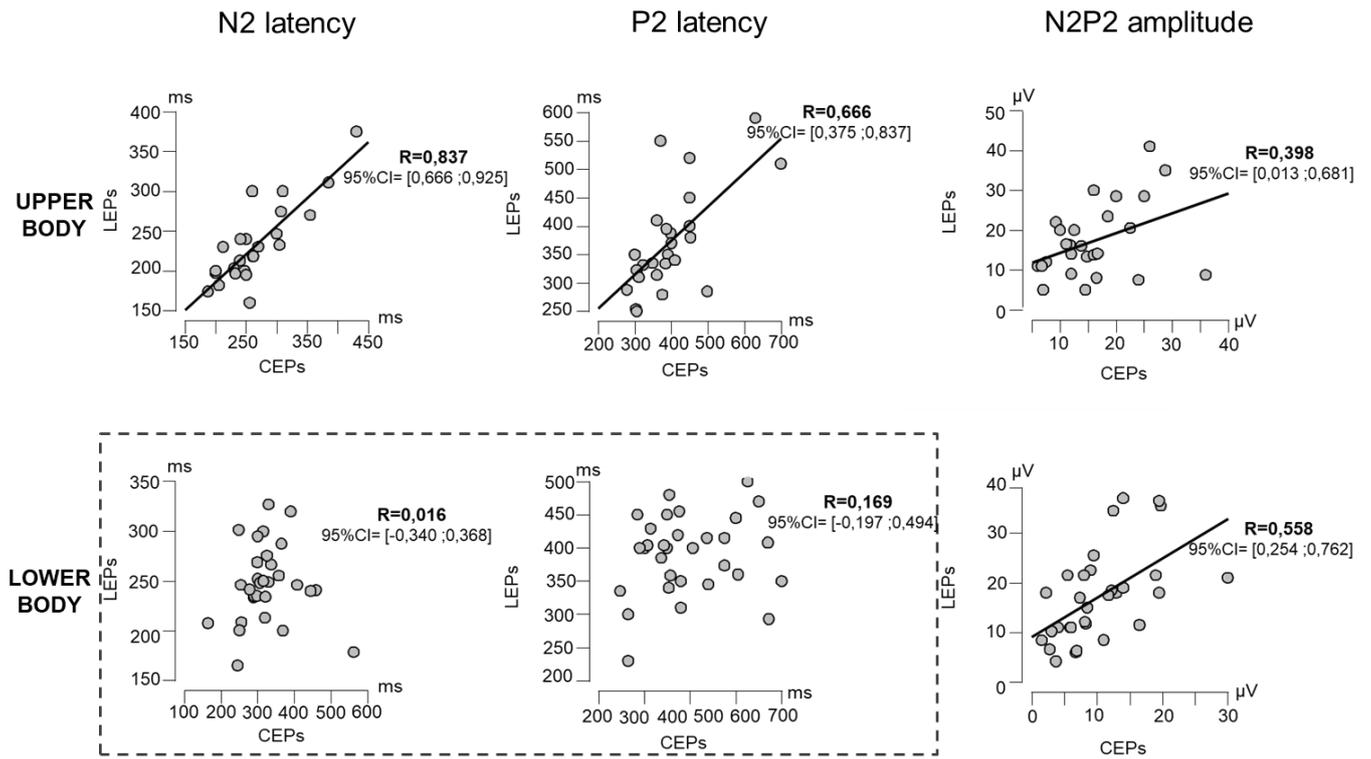


Figure 5:

Figure 6:

