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The new micropatterned interdigitated electrode for selective assessment of the nociceptive system

--Manuscript Draft--

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Abstract:	Background In this neurophysiological study, we aimed at verifying the nociceptive selectivity of the new, micropatterned electrode (150IDE), recently designed to generate an electric field limited to the intraepidermal free nerve endings. Methods Using the new 150IDE we recorded evoked potentials after stimulation of the face and hand dorsum in 22 healthy participants and in patients with exemplary conditions selectively affecting the nociceptive system. We also measured the peripheral conduction velocity at the upper arm and verified the nociceptive selectivity of 150IDE assessing the effect of a selective block of nociceptive nerve fibres of radial nerve with local anaesthetic infiltration. In healthy participants and in patients we have also compared the 150IDE-evoked potentials with laser-evoked potentials. Results In healthy participants, the 150IDE evoked pinprick sensation and reproducible scalp potentials, with latency similar to laser-evoked potentials. The mean peripheral conduction velocity, estimated at the upper arm, was 12 m/s. The selective nociceptive fibre block of the radial nerve abolished the scalp potentials elicited by the 150IDE stimulation. In patients, the 150IDE-evoked potentials reliably detected the selective damage of the nociceptive system. Conclusions Our neurophysiological study shows that this new 150IDE provides selective information on nociceptive system.
Additional Information:	

Question	Response
Significance Below please give a paragraph entitled **"Significance"**, indicating the main aspects where this work adds significantly to existing knowledge in the field, and if appropriate to clinical practice. The signifiance statement should be short, attention-grabbing,non-redundant with the conclusions and rigorously in line with the contents of the full article. It should not exceed 80 words and will be added to the end of the abstract at the time of typesetting. This paragraph will NOT count to the abstract's total word limit of 250 words. The statement "Significance" also applies to Review papers.	Our neurophysiological study in healthy humans and patients with exemplary conditions selectively affecting nociceptive system shows that the new 150IDE might be an useful tool for investigating nociceptive fibres in patients with neuropathic pain.

SECTION EDITOR/ Interesting study promoting a new electrical stimulation device to test small fibre function, that may be potentially useful in clinical and Basic Research. There are a number of methodology concerns (both from the technical and clinical points of view) the authors should clarify/comment on.

Reviewer #1: In this manuscript, the authors report on the results of examining the reliability of using the electrode described as the 150IDE for the assessment of conduction in the nociceptive pathway in healthy and diseased persons. As the authors state, it is important that a relatively simple method can be accepted for testing nociceptive system functions and, according to the results reported here, this electrode might as well comply with the necessary requirements. However, there are many unclear points in the manuscript.

The first concern that this reviewer has is the number of subjects studied. Although 22 healthy subjects were enrolled, only a few of them underwent some of the most important tests for the assessment of nociceptive function: conduction velocity in the nociceptive system was examined in only 5 subjects and the effects of conduction block in nociceptive fibers in 4. The consequences of a disease were only tested in 3 patients…. (and, in the Results section, the reader knows that evoked potentials were obtained in only 13 subjects out of the 22 initially recruited). These numbers are too low for meaningful results, even if the data obtained were consistent among all subjects.

Whereas the two main experiments (experiments 1 and 2) were conducted in 10 and 13 subjects, experiments 3 and 4 involved a smaller sample of subjects. Such experiments, however, were conceived with a validation purpose and provided homogeneous results at inter-individual level, in agreement with findings from the main experiments.

As per the patients, we decided to enrol only three subjects with representative damages of the nociceptive system. Given that we focused on healthy subjects, the enrolment of these patients was meant to analyse a selected number of exemplary cases, paving the way to future studies targeting patients.

We realize however that the numbers of subjects included in this study may be a potential limitation. We therefore acknowledge this limitation in the Discussion (Page 15).

There are some methodological issues that, added to the small number of subjects tested, complicate the potential relevance of the reported findings:
Has the device any connector to the electrical source? What system is used to control the stimulus duration, intensity and number of pulses? The stimulus duration should be 10 ms (10 stimuli at 1000 Hz). How is this stimulation setting triggered and controlled?

A dedicated pulse generator (Bionen, Italy) allowed the delivery of a 10-pulse train at 1kHz, lasting 10 ms. The intensity was modulated through a Constant Current Stimulator, model DS7A, connected to the pulse generator. The signal was recorded through an EEG device. According to this Rewiever's suggestion, we added these details in the Method (Page 6).

The stimulus duration for single stimuli for testing the radial nerve SNAP was 0.1 ms in both electrodes. However, both, the stimulated surface and the distance anode/cathode were obviously very different in both electrodes. What would be the effect of these differences on SNAP recordings?

The short distance between anode and cathode of the 150IDE generates an electric field confined within 100 µm of depth in the skin, thus selectively activating intraepidermal free nerve endings. At this given distance and with low stimulus intensity, the recording of sensory action potentials related to Aβ-fibres is not expected. However, high stimulation intensity elicits low-amplitude sensory action potential. We have now included a comment on the peculiar characteristics of 150IDE and the relationship between its design and the sensory action potential recording in the Discussion (Page 13).

Methodological differences were also present when recording the scalp potentials: Apart from the expected change in site of stimulation of the laser beam (while the 150IDE electrode was kept in

the same place), the number of traces recorded for future average was different. For the 150IDE, the authors recorded 30 responses while for the laser stimuli they recorded 20 traces. The averaging of a different number of evoked potentials can indeed cause differences in the resulting response. Why the authors did chose to have these differences? What consequences might them have had?

We collected a different number of trials for the two techniques due to the different types of stimulation and possible low signal-to-noise ratio during the recording. We preferred to collect no more than 20 laser stimuli for each site of stimulation to avoid an excessive skin heating and possible burning lesions. Besides, the higher number of stimuli with the 150IDE was chosen to mitigate a possible lower signal-to-noise ratio associated with this new technique, in comparison with the well-known laser-evoked potential recording. Admittedly, the different number of trials may have influenced our findings, possibly hampering the amplitude differences between the two nociceptive evoked potentials. This reasoning was added in the Discussion (Page 15).

There are also unclear points in the Results section. The authors state that, in Experiment 1, the 150IDE electrode stimulation '…did not evoke any Aβ-mediated sensory action potentials.' and they cite figure 2 to corroborate the statement. However, Figure 2 shows that a SNAP of a sizeable amplitude was indeed recorded with stimuli of 9 mA intensity or higher in the majority of subjects, as the whiskers (which meaning is not described in the figure legend), do not show a floor effect. This means, indeed, that some Abeta fibers were stimulated at this intensity, which they recognize in the Discussion section. The authors should rephrase their statement in the second paragraph of the Results section.

Admittedly, 150IDE stimulation at 9 mA intensity evoked a sensory action potential in several participants (6 out of the 10 subjects). Hence we amended the Results as follows: "…low intensity stimulation (below 9 mA) with 150IDE of the radial nerve territory did not evoke any Aβ fibre-mediated sensory action potential" (Page 10), and the Discussion as reported hereafter: "…stimulation intensities lower than 9 mA were unable to evoke Aβ-fibre mediated sensory action potentials in all participants" (Page 13).

Whiskers represent standard deviation. We have now amended the legend of figure 2.

Some statements in the Results section do not add up: In experiment 1, perception threshold (examined in hand dorsum, as per protocol) was 3.2 +/-0.9. However, low intensity stimulation of the same area was 6.6. This corresponds to more than 2 times perception threshold intensity, not to the expected (and stated) 1 to 1.5 times perception threshold intensity.

We apologize for this trivial mistake. We thank this Reviewer for giving us the opportunity to amend the text. The 150IDE low stimulation intensity ranged between 2-2.5 times the perceptive threshold. We have now amended the whole text.

If 6.6 is an intensity between 1 and 1.5 times perception threshold intensity, I guess that some subjects could have not been really stimulated at 5-6.5 times perception threshold, which would possibly be beyond 25 mA, said to be the maximum stimulus intensity that the device was capable of administrating. Surprisingly enough, the authors report in the same paragraph that the intensities used for 'high intensity stimulation' (5 to 6.5 times perception threshold intensity), stating that this was 18.6 +/-4.4 mA for hand dorsum stimulation. Again, the numbers do not add up, as it is impossible to find a factor that gives the perception threshold intensity which 1 to 1.5 times is 6.6 mA and which 5 to 6.5 times is 18.6 mA.

We thank this Reviewer for pointing out a misleading information. At low intensity, we used a stimulation intensity equal to 2-2.5 times the perceptive threshold for both the perioral and the hand dorsum regions.

For the experiments at high stimulation intensity we basically followed the subjects' perception. In the perioral region, we used an intensity of 10-12.5 times the perceptive threshold, evoking a distinct painless electrical shock-like sensation (in this region, lower stimulation intensity induced ambiguous sensation).

Conversely, at the hand dorsum, we used 5-6.5 times the perceptive threshold, given that in all subjects this stimulation intensity evoked a distinct painless electrical shock-like sensation.

In the high-intensity experiments, we have used different multiple of perceptive threshold to stimulate the perioral region and the hand, because in the perioral region it was particularly difficult to elicit distinct electrical shock-like sensations; the subjects usually perceived ambiguous, mixed pinprick and electrical shock-like, sensations for intensity lower than 10 times the perceptive threshold. This phenomenon might be due to the peculiar characteristics of skin innervation of the perioral region. In this site, the intraepidermal nerve fibre density is particularly high, thus probably contributing to persistent pinprick sensation. Admittedly, the different skin characteristics at the perioral region and hand dorsum may further influence the perception at high stimulation intensity.

The text was amended accordingly (Page 7).

There were 3 subjects whose perception threshold was too high, but this does not provide an explanation for the numbers not adding up. I still cannot find a perception threshold value that can be calculated using the data supplied in the Results section.

Intensity values given for perioral region are clear, as they may correspond to a perception threshold value of 2.25 or 2.26 (even if this figure is not given in the manuscript). In my view, the authors should work carefully on how to transmit to the reader their observations: Do the values given for hand dorsum perception threshold in experiment 1 contain those of the 3 subjects with too high a perception threshold? If these three subjects were excluded from the recording of evoked potentials, they should probably be excluded also from all experiments, but on what rationale? The 3 subjects with high hand-dorsum perception threshold, did they have also a high perioral perception threshold? and, if this was the case, how come that the numbers given for the perioral region are indeed clear and those for the hand-dorsum are not?

These three subjects did not participate in the experiment 1.

We excluded these participants from experiment 2 given that they had a high perceptive threshold (9-10 mA) and perceived an ambiguous (pinprick/electrical) sensations at 2-2.5 times the perceptive threshold. We have now reported in the text these values. Their perceptive threshold from perioral region was not collected, given that they were excluded. These clarifications have been added in the text (Page 11).

In experiment 3, the authors calculated conduction velocity in the fibers stimulated by the 150IDE. This resulted from stimulating in 3 different points along the upper limb (hand dorsum, forearm and upper arm). In this regard, there are two important questions with no answers in the manuscript. Are the readers forced to understand that all 13 subjects that had evoked potentials to hand dorsum stimulation had them also to forearm and upper arm stimulation? What stimulus intensities were used in the three stimulation points? If authors used the same stimulus intensity, the values would be probably at different multiples of perception threshold for each stimulation point. If the authors used the intensity corresponding to low-intensity stimuli for the hand dorsum, this reader may doubt that all stimuli in the forearm and upper arm could be equally effective as those in the hand dorsum. If the intensity used was the one corresponding to the high-intensity stimuli in the hand dorsum, I have difficulties understanding how the results could compare with those obtained with laser stimulation.

We realized that some methodological details in this section were missing.

The experiments assessing the conduction velocity was performed in five subjects. In the other participants we did not record 150IDE evoked potentials after forearm and proximal arm stimulation. However, we may assume that in the other 13 subjects, the reproducibility of the 150IDE evoked potentials after forearm and shoulder stimulation is probably similar.

In these five subjects, we detected the hand perceptive threshold and then we stimulated the three sites with the same intensity (calculated as 2-2.5 times the hand perceptive threshold), corresponding to 6.9 ± 0.9 mA (for the three sites). Hence, in this experiment we

did not calculate the perspective threshold for the forearm and proximal arm (we cannot exclude that the stimulation intensity used in these two sites corresponds to a variable multiple of perceptive threshold, different from the hand). However, all subjects reported a clear pinprick sensation in the three body sites and the stimulus evoked reproducible scalp potentials. This information has been added in the text (Methods section: Page 8; Results section: page 11).

Minor: in the first line of Results for Experiment 4, the word 'evoked' is equivocal. I assume that the authors mean 'induced'.
The text was changed accordingly (Page 12).

Reviewer #2: This study aimed to testify the nociceptive selectivity of the micropatterned electrode (150IDE). Authors performed a series of experiments on both healthy subjects and patients and compared the performance of the 150IDE with laser stimulation. The paper is well written, and well organized. In addition, the results are reliable and conclusive. I have some comments, which may help further improve the manuscript.

1. "Laser stimulation was set at relatively high intensity (102-153 mJ/mm2)". It's not clear how was the stimulus intensity determined, especially considering that the solid relationship between stimulus intensity and LEP amplitude and latency.

Stimulation intensity was 2-2.5 times the perceptive threshold. We have now added this information in the text (Page 7, line 13).

2. "Trials contaminated by eye blinks and movements were rejected. Epochs with amplitude ... were rejected". Authors should report the rate of the rejection, especially considering that the number of trials in each condition was quite limited.

The range of rejection rate was 0-20%. We have now reported this information in the text (Page 11, line 19).

3. Peak latency and amplitude of N1 was measured from T3 referenced to Fz, of N2 and P2 from Cz referenced to nasion (Nz). The choice of the active and reference electrodes is not compatible with several previous studies. Nose was normally used as the reference to measure peak latency and amplitude of N2 and P2. In addition, C3 would be more optimal in detecting the N1 wave (e.g., Figure 4, top left plot), as pointed out in Valentini et al., NeuroImage 2012 (The primary somatosensory cortex contributes to the latest part of the cortical response elicited by nociceptive somatosensory stimuli in humans).

We agree with this Reviewer that the parietal electrode is equally valid for the recording of the lateralized component. However, following our consolidated clinical practice, we prefer to record N1 using Tc referenced to the frontal area (Fz). This recording method is in line with many previous studies (it is worth mentioning that most of the reference values for LEPs used this recording).

Similarly, we believe that the nose as reference for the vertex component is equivalent to Nz. Both references are routinely used in clinical practice. We believe that Cz referenced to Nz does not change findings.

4. The stimulus intensity was not specified in Experiments 3 and 4 in the Method section.

Stimulus intensity with 150IDE, set at 2-2.5 time the perceptive threshold, was 6.9 ± 0.9 mA in experiment 3 and 6.8 ± 0.3 mA in experiment 4. Laser stimulation was set at relatively high intensity (102-153 mJ/mm^2). These information are now reported in the text (Pages 11 and 12).

5. Statistical analysis, "paired t-test" should be changed to "paired-sample t-test".

We amended the text accordingly (Page 10).

6. Results section. The latencies of all LEP peaks (especially for hand stimulation) are too shorter than previous publications. Very strange. Authors should double check their data.

We have now double-checked our data. We admit that the latencies of hand-LEPs are earlier than those reported in most studies. However, these latencies are close to the lowest values of our normative ranges (Di Stefano et al., 2017). We believe that the early latency of hand-LEPs could be explained by the young age of the included population. We have now reported in the text (page 14) that in our subjects hand-LEPs have an earlier latency in comparison with previous studies, and these early latencies might be explained with the young age of the subjects included in our study.

7. Some important and relevant references should be cited, e.g., Mouraux et al., Pain 2010 (Low intensity intra-epidermal electrical stimulation can activate Aδ-nociceptors selectively).

We have now added in the text this reference (Page 4).

8. In both Tables, the amplitudes of N1 and N2 should be negative. In addition, there are two "Table 1", and no "Table 2" in the submitted file.

Negative values are now reported in Tables 1 and 2.

Professor Luis Garcia-Larrea

Editor-in-Chief European Journal of Pain

Dear Professor Garcia-Larrea,

Please pass on our sincere thanks to the Reviewers for their helpful suggestions. We amended the text according to their comments. Each point is addressed in bold. Additions in the manuscript are highlighted in red.

We hope that the revised manuscript is now suitable for publication in your esteemed Journal.

On behalf of all authors,

Andrea Truini

Abstract

Background: In this neurophysiological study, we aimed at verifying the nociceptive selectivity of the new, micropatterned electrode (150IDE), recently designed to generate an electric field limited to the intraepidermal free nerve endings.

Methods: Using the new 150IDE we recorded evoked potentials after stimulation of the face and hand dorsum in 22 healthy participants and in patients with exemplary conditions selectively affecting the nociceptive system. We also measured the peripheral conduction velocity at the upper arm and verified the nociceptive selectivity of 150IDE assessing the effect of a selective block of nociceptive nerve fibres of radial nerve with local anaesthetic infiltration. In healthy participants and in patients we have also compared the 150IDE-evoked potentials with laser-evoked potentials.

Results: In healthy participants, the 150IDE evoked pinprick sensation and reproducible scalp potentials, with latency similar to laser-evoked potentials. The mean peripheral conduction velocity, estimated at the upper arm, was 12 m/s. The selective nociceptive fibre block of the radial nerve abolished the scalp potentials elicited by the 150IDE stimulation. In patients, the 150IDE-evoked potentials reliably detected the selective damage of the nociceptive system.

Conclusions: Our neurophysiological study shows that this new 150IDE provides selective information on nociceptive system.

The new micropatterned interdigitated electrode for selective assessment of the nociceptive system

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The other Authors have no competing interest to declare

Significance: 150IDE is a promising new tool for investigating nociceptive system in patients with neuropathic pain.

Keywords: pain, small-fibre neuropathy, neuropathic pain

Abstract

Background: In this neurophysiological study, we aimed at verifying the nociceptive selectivity of the new, micropatterned electrode (150IDE), recently designed to generate an electric field limited to the intraepidermal free nerve endings.

Methods: Using the new 150IDE we recorded evoked potentials after stimulation of the face and hand dorsum in 22 healthy participants and in patients with exemplary conditions selectively affecting the nociceptive system. We also measured the peripheral conduction velocity at the upper arm and verified the nociceptive selectivity of 150IDE assessing the effect of a selective block of nociceptive nerve fibres of radial nerve with local anaesthetic infiltration. In healthy participants and in patients we have also compared the 150IDE-evoked potentials with laser-evoked potentials.

Results: In healthy participants, the 150IDE evoked pinprick sensation and reproducible scalp potentials, with latency similar to laser-evoked potentials. The mean peripheral conduction velocity, estimated at the upper arm, was 12 m/s. The selective nociceptive fibre block of the radial nerve abolished the scalp potentials elicited by the 150IDE stimulation. In patients, the 150IDE-evoked potentials reliably detected the selective damage of the nociceptive system.

Conclusions: Our neurophysiological study shows that this new 150IDE provides selective information on nociceptive system.

Introduction

The current neurophysiological assessment of the nociceptive system function relies on recording heat mediated evoked potentials, i.e. laser-evoked potentials and contact heat evoked potentials, both mediated by Aδ-fibres (Cruccu et al., 2008; Haanpaa et al., 2011; Garcia-Larrea, 2012; Treede et al., 2003, Truini et al., 2007; Hüllemann et al., 2019). Although the nociceptive specificity and the diagnostic accuracy of these two techniques are widely agreed, their clinical usefulness is currently limited due to their high cost and the possible safety concerns for laser stimulation.

Over the last years, clinical research has devised special surface concentric electrodes (consisting of a central cathode and an external anode ring) and intra-epidermal electrodes supposed to deliver electrical stimuli only in the superficial epidermal layers, where the Aδ-fibre related nociceptors lay (Katsarava et al., 2006; Mouraux et al., 2010). Although some clinical studies used surface concentric electrode-related evoked potentials as an objective measure of nociceptive system function (Yoon et al., 2011; Üçeyler et al., 2013), recent studies demonstrated that this electrode invariably co-actives Aβ-fibres (La Cesa et al., 2018; Perchet et al., 2012; de Tommaso et al., 2011).

However, electrical stimulation is a safe, easy-to-use and cheap technique, and thus a reliable nociceptive-specific electrode would improve the assessment of the nociceptive system function in clinical practice. Accordingly, a new surface micropatterned interdigitated electrode for selective stimulation of the nociceptive fibres has been recently designed (Leandri et al., 2018). Its nociceptive specificity depends on the peculiar interdigitated conformation (IDE) of this electrode, made of conductive rails arranged in a comb-like micropattern, situated only 150 µm apart (150IDE) and alternately connected to the opposite poles of the stimulator. The short distance between anode and cathode generates an electric field confined within 100 µm of depth in the skin, thus selectively activating intraepidermal free nerve endings (Leandri et al., 2018).

In this neurophysiological study in healthy humans and patients with exemplary conditions selectively affecting nociceptive system, we aimed at testing the reliability of the 150IDE for the assessment of nociceptive system. To do so, we compared 150IDE-evoked potentials and laser-

evoked potentials in healthy participants and verified the accuracy of the 150IDE-evoked potentials in disclosing selective damage of the nociceptive system in patients with peripheral and central nervous system diseases.

Methods

Study cohort and design

Between January and December 2018, we consecutively enrolled 22 healthy participants among hospital personnel (aged 23–36 years, 11 females, 11 males) and three patients with exemplary peripheral and central nervous system diseases, selectively affecting the nociceptive system.

Healthy participants had no clinical history, symptoms or signs of peripheral or central nervous system disorders or other medical conditions. They participated in four distinct experiments. In experiment 1, using different intensities of stimulation, we tested quality and intensity of perception elicited by the 150IDE. We also verified whether the 150IDE could elicit a peripheral sensory action potential, suggesting a co-activation of non-nociceptive large myelinated fibres. In experiment 2 we compared scalp potentials evoked by 150IDE and laser-evoked potentials after stimulation of the face and the hand dorsum. In experiment 3, in order to estimate the conduction velocity of the activated fibres, we recorded the 150IDE-evoked potentials after stimulating the hand, the forearm and the proximal arm (with a fixed distance of 20 cm). In experiment 4 we verified the nociceptive selectivity of 150IDE by assessing the effect of a selective block of the nociceptive nerve fibres with local anaesthetic infiltration.

In the three patients with selective nociceptive system damage due to peripheral and central nervous system diseases, we investigated the 150IDE selectivity, by comparing the 150IDE- with laser-evoked potentials.

All participants gave their written informed consent. The research was approved by the local Institutional Review Board.

150IDE stimulation

The 150IDE used in these experiments, based on a prototype recently designed (Leandri et al., 2018 and Italian Patent n.1425199), has been developed under licence by Bionen (Florence, Italy). It consists of a glass epoxy substrate, with a micropattern of gold conductor rails interdigitated in a double comb-like fashion. This electrode covers an overall area of 10 x 10 mm. Each 150IDE stimulus consisted of a burst of 10 electrical pulses of 0.2 ms duration at 1 kHz (Leandri et al., 2018).

A dedicated pulse generator (Bionen, Italy) allowed the delivery of the high-frequency stimulation. The intensity was modulated through a Constant Current Stimulator (DS7A, Digitimer Ltd, UK), triggered by the pulse generator. Before stimulation, the skin was gently cleansed with cotton wool moistened with ethanol then left to dry. In order to avoid electric shorting of the electrode rails no conductive gel was used.

Experiment 1- Psychometric measures and sensory action potentials in healthy participants

This experiment was carried out in 10 participants (aged 23–30 years, 4 females, 6 males). It consisted of two parts. In the first part, we applied the 150IDE stimulation to the hairy skin of the right-hand dorsum, between the first and second metacarpal bone, to investigate the perceptive threshold and assess subjective perception as a function of the stimulus intensity, ranging from 0 to 25 mA. After each stimulus, participants were asked to provide a description of the perception quality (pre-codified as pinprick, electrical shock like or ambiguous sensation) and to quantify the stimulus intensity using a numerical rating scale (NRS) ranging from 0 to 10 (0 = no sensation, 10 = most intense imaginable sensation).

In the second part of experiment 1, using surface electrodes, we recorded orthodromically superficial radial nerve sensory action potentials at increasing stimulation intensities with both 150IDE (single pulse stimulation, 0.1 ms duration) and standard bipolar electrode (single pulse stimulation, 0.1 ms duration). The amplitude of sensory action potentials was measured at each stimulus intensity, ranging from 0 to 25 mA, with steps of 1 mA. For each step of stimulation intensity, 10 trials were recorded.

Experiment 2 - Scalp potentials in healthy participants

In 16 participants (aged 23–30 years, 10 females, 6 males) we recorded 150IDE-evoked potentials after stimulation of the perioral region and hand dorsum. In ten of these 16 participants, we also recorded laser-evoked potentials after stimulation of the same sites.

For the 150IDE-evoked potentials, we recorded two blocks of 30 stimuli for each site of stimulation. In the first block, we used a low-intensity stimulation, slightly above the pinprick detection threshold, corresponding to 2-2.5 times the perceptive threshold and evoking a distinct pinprick sensation. In the second block, we used a relatively high intensity stimulation, producing a distinct electrical shock-like sensation in all subjects, corresponding to 10-12.5 times the perceptive threshold in the perioral region and 5-6.5 times the perceptive threshold in the hand dorsum. The interstimulus time interval varied randomly, in the range of 10–15 seconds.

For the laser-evoked potentials, we used a Neodymium-YAP stimulator and recorded 20 trials for each site of stimulation. Laser stimulation was set at relatively high intensity (102-153 mJ/mm^2), corresponding to 2-2.5 times the perceptive threshold, short duration (5 ms), and small diameter (5 mm), eliciting a clear pinprick sensation. Laser pulses were delivered with an interstimulus time interval varying randomly, in the range of 10–15 s. To avoid skin burns, nociceptor fatigue and central habituation the laser beam was slightly shifted after each stimulus (Truini et al., 2010).

During scalp potential recording, participants laid on a couch and during the laser stimulation wore protective goggles. They were asked to keep their eyes open to avoid alpha contamination and gaze slightly downwards. We asked the subjects to focus their attention towards the stimulus by counting the number of delivered stimuli. The EEG was recorded using 32 Ag–AgCl scalp electrodes mounted in an elastic electrode cap and placed according to the International 10–20 system, referenced to the nose. Impedance was < 5kΩ. Electroculographic (EOG) signals were simultaneously recorded using surface electrodes.

EEG data were pre-processed using Letswave 6, a free signal-processing toolbox (http://www.nocions.org/letswave) (Mouraux and Iannetti, 2008). Continuous EEG data were filtered, with a fast-Fourier transform filter, in the range 1 to 30 Hz. EEG epochs were selected using a window of -200 to 1000 ms relative to stimulus onset and the baseline was corrected using

the prestimulus interval. Trials contaminated by eye blinks and movements were rejected. Epochs with amplitude values exceeding ±100 μV were rejected.

After artefact rejection, EEG epochs of each category (150IDE-evoked potentials after low- and high-intensity stimulation and laser-evoked potentials) were averaged, thus obtaining three averaged waveforms for each participant. We measured peak latency and amplitude of the lateralized component, recorded from T3 referenced to Fz, and of the vertex complex from Cz referenced to nasion (Nz). Then, a grand-average among subjects for each type of stimulus was performed.

Experiment 3 – Conduction velocity of the 150IDE-evoked potentials

In five subjects (aged 23–30 years, 3 females, 2 males) in order to calculate the peripheral conduction velocity, we recorded the 150IDE-evoked potentials after low-intensity stimulation of three sites of the upper limb (hand, forearm and proximal arm) at a distance of 20 cm from each other. The intensity of stimulation corresponded to 2-2.5 times the perceptive threshold detected from the hand dorsum, evoking a distinct pinprick sensation at the three sites. The recording order from stimulated areas was randomly alternated across the different participants. To estimate the conduction velocity, we used two methods. First, we divided the distance between the stimulated sites by the latency difference of the N2 components. Second, we calculated the reciprocal of the slope of the regression line for all the N2 latency values obtained at each site of stimulation (Cruccu et al., 2000).

Experiment 4 – Selective block of nociceptive fibres

In four participants (aged 23–35 years, 2 females, 2 males) we verified whether a selective block of nociceptive nerve fibres of the superficial radial nerve with near-nerve infiltration of lidocaine abolished the 150IDE-evoked potentials. Before the lidocaine block, we quantitatively assessed the mechanical detection threshold using a standardized set of von Frey hairs (Optihair2-Set, Marstock Nervtest), pinprick detection threshold with weighted pinprick stimuli (MRC Systems GmbH) and laser perceptive threshold. We also recorded orthodromically the radial nerve sensory action potential and evoked potentials related to laser and 150IDE stimulation of the hand dorsum in the

superficial radial nerve territory. The intensity of laser and 150IDE stimulation corresponded to 2-2.5 times the perceptive threshold.

After these baseline procedures, we injected 0.5 ml of lidocaine between the tendon of the brachioradialis and the radius just proximal to the styloid process of the radius to selectively block the nociceptive fibres of the superficial radial nerve. A few minutes after the lidocaine injection, we tested the mechanical and pinprick perception and recorded the superficial radial nerve sensory action potentials. Once obtained the evidence of a selective nociceptive block we recorded evoked potentials after 150IDE and laser stimulation.

Patients

In three patients with peripheral and central nervous system diseases associated with a selective damage of the nociceptive system, we investigated the 150IDE selectivity, by comparing 150IDE-evoked potentials with laser-evoked potentials.

Patient 1 (male, 50 years) had a small-fibre neuropathy associated with AL amyloidosis. He had thermal-pain sensory disturbances, including distally distributed neuropathic pain. The nerve conduction study showed spared sensory nerve action potentials and the skin biopsy showed a severe reduction of the intraepidermal nerve fibre density. Patient 2 (female, 45 years) had a syringomyelia involving the cervical spinal cord (between C6-C7), unrelated to Arnold-Chiari malformation, as assessed with magnetic resonance imaging. This patient complained of thermal-pain sensory deficits involving the cervical dermatomes. Patient 3 (male, aged 57 years) suffered from facial neuropathic pain due to a previous ischemic lesion involving the lateral part of the medulla (Wallenberg syndrome). This patient had thermal pain sensory deficits and suffered from neuropathic pain affecting the right side of the face.

In the three patients with a selective nociceptive system damage, we recorded evoked potentials after laser and low intensity 150IDE stimulation (2-2.5 times the perceptive threshold). In each patient, we stimulated the area of maximal thermal pain sensory deficit, and a homologous unaffected area (in the patient with small-fibre neuropathy we stimulated the clinically-unaffected hand dorsum, in the patient with syringomyelia we stimulated the upper thoracic dermatomes, in

the patient with Wallenberg syndrome we stimulated the contralateral perioral region). Twenty trials, separated by a 5–10 s inter-stimulus interval, were recorded from each stimulation site.

Statistical analysis

All data had normal distribution, as assessed with the D'Agostino & Pearson normality test. To test the differences between evoked potentials variables after low and high intensity 150IDE stimulation and between low intensity 150IDE and laser stimulation we used the paired-sample t-test.

To assess the conduction velocity of the involved fibres we calculated the goodness of the linear regression with r2 and its deviation from zero with F-test.

The <0.05 level was considered statistically significant. All results are reported as mean ± SD. For statistics and graphs we used Prism 8.0 (GraphPad, CA, USA).

Results

Experiment 1 - Psychometric measures and sensory action potentials in healthy participants

The 150IDE perceptive threshold at the hand was 3.2±0.9 mA. The stimulus-response curve showed that most participants perceived a clear pinprick sensation with stimulus intensity approximately lower than 10 mA (Figure 1).

The second part of this experiment showed that in all subjects, low intensity stimulation (below 9 mA) with 150IDE of the radial nerve territory did not evoke any Aβ-mediated sensory action potentials (Figure 2).

Experiment 2 - Scalp potentials in healthy participants

Low-intensity 150IDE stimulation of the perioral region and the hand dorsum (2.7±0.7 mA for the perioral region and 6.6±2.4 mA for the hand dorsum) elicited a clear pinprick sensation in 13 of 16 subjects (NRS: 2.9±1.1 for the perioral region and 3.1±0.6 for the hand dorsum). Conversely, with the high-intensity stimulation (13.6±5.3 mA for the perioral region and 18.6±4.4 mA for the hand dorsum) all participants reported a distinct electrical shock-like sensation (NRS: 4.8±2.3 for the perioral region and 5.4±2.4 for the hand dorsum).

In three subjects (aged 23–27 years, 1 female, 2 males) the low intensity 150IDE stimulation failed to evoke a distinct pinprick sensation after hand dorsum stimulation. These subjects had a high perceptive threshold (9-11 mA) and perceived mixed (pinprick/electrical) sensations at 2-2.5 times the perceptive threshold. These subjects therefore were excluded from all the evoked potential recordings, including the perioral stimulation.

In the remaining thirteen participants, both high and low intensity stimulation evoked a reproducible N2-P2 vertex complex (Figure 3, Table 1). The N2 latency after perioral region and hand dorsum stimulation was 142±24.6 and 169±23.1 ms with low intensity 150IDE stimulation, and 115.8±6 and 143.1±20.3 ms with high intensity stimulation. In 12 participants, 150IDE stimulation evoked a lateralized N1 component. The N1 latency after perioral region and hand dorsum stimulation was 100.5±13 and 131.6±22.4 ms with low intensity stimulation, and 88.5±11.5 and 115.0±21.7 ms with high intensity stimulation.

The latencies of the different scalp potentials were significantly longer after low intensity 150IDE stimulation than after high intensity stimulation ($p<0.05$) (Table 1).

Latency and amplitude of low-intensity 150IDE-evoked potentials were not significantly different from those of laser-evoked potentials ($p> 0.05$) (Figure 4, Table 2). The NRS related to laser stimulation (4.1±0.4), however, was significantly higher than that reported with IDE stimulation (3.0±1.1).

In the scalp potential recording, the artefact rejection rate ranged between 0% and 20% for both 150IDE and laser-evoked potentials.

Experiment 3 – Conduction velocity of the 150IDE evoked potentials

At low stimulation intensity, corresponding to 6.9±0.9 mA, all subjects reported a clear pinprick sensation in the three sites and the stimulus evoked reproducible scalp potentials. The mean value of the individual conduction velocity computed from each subject was 12.3±5.2 m/sec. The regression line calculated from N2 latencies of low-intensity 150IDE-evoked potentials from each stimulated site indicated a significant linear relationship between distance and time ($r2 = 0.4096$; F

= 9.017; p=0.01; Figure 5). The conduction velocity, indicated by the reciprocal of the slope of the regression line, was 11.70 m/sec (Figure 5).

Experiment 4 – Selective block of nociceptive fibres

In the four healthy participants, orthodromic radial nerve stimulation induced sensory action potentials (13.6±4.1 µV); stimulation of the superficial radial nerve territory with laser (102-153 mJ/mm^2) and low-intensity 150IDE (6.8 ± 0.3 mA) evoked the N2P2 complex (25.8±15.8 µV and 27±17.3 µV).

In all participants the superficial radial nerve block, using near-nerve lidocaine infiltration, abolished thermal-pain sensations as assessed with laser pulses and weighted pinprick stimuli. Conversely, the mechanical detection threshold, tested with a standardized set of von Frey hairs, was preserved. Accordingly, the evoked potentials related to laser and 150IDE stimulation were completely abolished while the superficial radial nerve sensory action potential amplitude did not differ from the baseline recording (13.8±4.5 µV) (Figure 6).

Patients

In all the three patients with selective damage of the nociceptive system, when the area of maximal thermal-pain sensory deficit was stimulated with laser and low intensity 150 IDE, no scalp evoked potentials could be recorded. Conversely, both laser-evoked potentials and low intensity 150 IDE-evoked potentials were preserved after stimulation of the unaffected area (Figure 7).

Discussion

In this neurophysiological study we showed that the new interdigitated electrode 150IDE, at low intensity stimulation, elicits distinct pinprick sensations, evokes scalp responses which are comparable to laser-evoked potentials, suggesting a conduction velocity consistent with Aδ nociceptive fibres. Another common feature is that both types of responses are abolished after near nerve lidocaine infiltration. In the three patients with exemplary peripheral and central nervous system diseases the 150 IDE-evoked potentials provided reliable information on the selective damage of the nociceptive system.

Findings in healthy participants

The stimulus-response curve showed that the 150IDE, at stimulation intensity lower than 10 mA, elicited a clear pinprick sensation in most participants. Similarly, stimulation intensities lower than 9 mA were unable to evoke Aβ-fibre mediated sensory action potentials in all participants. These findings suggest that, at low intensity, 150IDE selectively activates nociceptive nerve fibres, without co-activation of Aβ-fibres. Conductive rails arranged in a comb-like micropattern, situated 150 µm apart from each other and alternately connected to the opposite poles of the stimulator, generate an electric field limited to the most superficial skin layers. Hence, the peculiar 150IDE design makes this electrode relatively unsuitable for activating Aβ-fibres and evoking related sensory action potentials. Admittedly, at increasing intensities (higher than 9-10 mA) the 150IDE stimulation elicits ambiguous or electrical shock-like sensations and evokes sensory nerve action potentials, thus indicating that high intensity stimulation co-activates the Aβ-fibres, probably due to the spread of electric filed in the deeper skin areas, where the mechanoreceptors lay.

In a small proportion of subjects, at the lowest stimulus intensity able to evoke reproducible scalp potentials, the first perceived sensation was a mixed sensation (pinprick and electrical shock-like sensations) thus hampering the possibility of selective stimulation.

In the scalp potential recording we found that the 150IDE stimulation evoked large vertex complex (N2-P2), preceded by a lateralized negative component (N1). The N1 and N2 latencies were significantly longer after low stimulation intensity than after high stimulation intensity, thus implying that the high intensity stimulation may co-activate the large myelinated Aβ-fibres. The latency and amplitude of the 150IDE-evoked potentials after low intensity stimulation were comparable to those of laser-evoked potentials. The minor latency difference (this difference however did not reach the statistical significance) is probably explained by the different stimulation modality between 150IDE and laser stimulation. Whilst the 150IDE directly activates intraepidermal nerve fibres, the laser stimulation activates AMH receptors, with an estimated receptor activation time of about 20 ms (Perchet et al., 2008).

It is worth mentioning that in our study the mean latency of laser-evoked potentials after hand stimulation was earlier than that reported in several studies (Perchet et al., 2008). This finding is probably due to the young age of the included population, being the laser-evoked potential latency strongly related to age (Di Stefano et al., 2017).

To estimate the peripheral conduction velocity we stimulated the upper arm in three distinct sites and calculated the latency difference of the N2 component of 150IDE evoked potentials. Although this method of estimating the conduction velocity is intrinsically affected by the variability of the latency of vertex potentials, this approach is widely agreed and it has been applied in previous studies (Kakigi et al., 1991; Rossi et al., 2000). The conduction velocity related to the 150IDE-evoked potentials that we found (about 12 m/s) is compatible with small myelinated Aδ-fibre activation. Human and animal studies showed that the Aδ-fibres have a conduction velocity of 5-35 m/s (Cruccu et al., 2003, Fields, 1987). Admittedly, most studies investigating Aδ-fibre conduction velocity using laser stimulation reported slower conduction velocities (9 - 11 m/sec) (Rossi et al., 2000; Iannetti et al., 2003; Kakigi et al., 1991). However, laser stimulation of the hairy skin activates only Aδ-fibres related to mechano-heat receptors type II (AMH II). Conversely electrical stimulation *via* 150IDE activates indistinctly all intraepidermal Aδ-fibres, including AMH I-related nerve fibres, cold fibres and Aδ hair receptors, known to have a higher conduction velocity than AMH II-related nerve fibres (Treede et al., 1995; Koltzenburg et al., 1997; Leone et al., 2019).

The lidocaine infiltration near the radial nerve at the wrist left the mechanical detection threshold and the sensory action potential unaffected but abolished pinprick sensation, laser perception and the evoked potentials related to laser and 150IDE stimulation. These findings indicate that the evoked potentials elicited by low intensity 150IDE stimulation are selectively mediated by small myelinated Aδ-fibres.

Findings in patients

Patient 1 suffered from a small-fibre neuropathy manifesting with distally distributed neuropathic pain and thermal-pain sensory deficits. Nerve conduction study, skin biopsy and laser-evoked potentials showed the sparing of the Aβ-fibres, and the selective damage of small-fibres.

Accordingly, the 150IDE-evoked potentials were suppressed. Conversely, the 150IDE-evoked potentials after stimulation of the clinically unaffected hand dorsum were spared. Patient 2 had a cervical syringomyelia and suffered from a segmental and dissociated sensory loss. In this patient laser-evoked potentials as well as the 150IDE-evoked potentials were abnormal. However, both laser and 150 IDE evoked potentials were preserved after stimulation of the areas below the dissociated sensory disturbances, thus showing the selective damage of the decussating axons of nociceptive second order neurones, and the sparing of the spinothalamic tract. Patient 3 suffered from facial neuropathic pain due to a previous lateral medullary ischemic lesion. In this patient the 150IDE-evoked potentials, as well as laser-evoked potentials, were suppressed. These findings suggest that the 150IDE-evoked potentials are mediated by the trigeminal thermal-pain system in the medulla.

Limitations and future prospective

Although in this study we included 22 healthy participants, only a proportion of this sample participated in the experiments 3 and 4. However, we conceived these two experiments with a validation purpose; they provided homogeneous results at inter-individual level, in agreement with findings from the main experiments (experiments 1 and 2). As per the patients, we decided to enrol only three subjects with three representative damages of the nociceptive system. Since we focused on healthy subjects, the enrolment of patients was meant to analyse a selected number of explicative cases, paving the way to future studies targeting patients.

We collected a different number of trials for the laser- and the 150IDE-evoked potentials. We collected 20 trials for the laser and 30 trials the 150IDE stimulation, due to the two different types of stimulation and possible low signal-to-noise ratio for the new 150IDE-evoked potentials. We preferred to collect no more than 20 laser stimuli to avoid an excessive skin heating and possible burning lesions. We chose 30 stimuli for the 150IDE-evoked potential recording to increase a possibly low signal-to-noise ratio with this new technique. Admittedly, the different number of trials may have influenced our findings, possibly hampering the amplitude differences between the two nociceptive evoked potentials.

This new 150IDE electrode loses nociceptive selectivity with increasing intensity, as it would be expected with any device delivering any form of energy. At intensity approximately higher than 9 mA the 150IDE may also activate the Aβ-fibres. Furthermore, due to the specific design of the 150 IDE, the stimulation is strongly influenced by the skin condition. Matter of fact, the skin may be dry or moist, or the stratum corneum thin or thick. It follows that some subjects may have high perceptive thresholds; in these subjects the intensity needed to evoke scalp potentials exceeds the empirical limit of 10 mA, thus excluding the possibility of selective stimulation. Such technical issues may hamper the clinical usefulness of this new electrode. However, future studies may investigate whether 150IDE of larger area than the one we used may maximize the afferent input while keeping a low stimulation intensity.

Conclusion

Our neurophysiological study in healthy humans and patients with exemplary conditions selectively affecting nociceptive system shows that the new 150IDE might be a useful tool for the selective activation of nociceptive fibres. Some technical issues still limit its use in the everyday clinical practice; however, we believe that future technical refinement (e.g. large area electrodes) may improve its reliability also for clinical purposes.

Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

Study conception and design: A.T., M.L., G.D.S., and GC

Acquisition of data: G.D.S., A.D.L., S.L.C., G.D.P, A.F., E.G., C.L.,

Analysis and interpretation of data: A.T., G.D.S.

References

1. Cruccu, G., Iannetti, G.D., Agostino, R., Romaniello, A., Truini, A., Manfredi, M. (2000). Conduction velocity of the human spinothalamic tract as assessed by laser evoked potentials. *Neuroreport*, 11, 3029-3032.
2. Cruccu, G., Pennisi, E., Truini, A., Iannetti, G.D., Romaniello, A., Le Pera, D., De Armas, L., Leandri, M., Manfredi, M., Valeriani, M. (2003). Unmyelinated trigeminal pathways as assessed by laser stimuli in humans. *Brain*, 126, 2246-2256.
3. 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. *Clin Neurophysiol*, 119, 1705–1719**.**
4. de Tommaso, M., Santostasi, R., Devitofrancesco, V., Franco, G., Vecchio, E., Delussi, M., Livrea, P., Katzarava, Z. (2011). A comparative study of cortical responses evoked by transcutaneous electrical vs CO(2) laser stimulation. *Clin Neurophysiol*, 122, 2482-2487.
5. Di Stefano G, La Cesa S, Leone C, Pepe A, Galosi E, Fiorelli M, Valeriani M, Lacerenza M, Pergolini M, Biasiotta A, Cruccu G, Truini A. (2017) Diagnostic accuracy of laser-evoked potentials in diabetic neuropathy. *Pain*, 158:1100-1107.
6. Fields, H.L. (1987). Pain (pp 354). New York: McGraw-Hill.
7. Garcia-Larrea, L. (2012). Objective pain diagnostics: Clinical neurophysiology. *Neurophysiol Clin*, 42, 187–197.
8. Haanpää, M., Attal, N., Backonja, M., Baron, R., Bennett, M., Bouhassira, D., Cruccu, G., Hansson, P., Haythornthwaite, J.A., Iannetti, G.D., Jensen, T.S., Kauppila, T., Nurmikko, T.J., Rice, A.S., Rowbotham, M., Serra, J., Sommer, C., Smith, B.H., Treede, R.D. (2011). NeuPSIG guidelines on neuropathic pain assessment. *Pain,* 152, 14–27.
9. Hüllemann, P., Nerdal, A., Sendel, M., Dodurgali, D., Forstenpointner, J., Binder, A., Baron, R. (2019). Cold-evoked potentials versus contact heat-evoked potentials-Methodological considerations and clinical application. *Eur J Pain*, 23, 1209-1220.

10. Iannetti, G.D., Truini, A., Romaniello, A., Galeotti, F., Rizzo, C., Manfredi, M., Cruccu, G. (2003). Evidence of a specific spinal pathway for the sense of warmth in humans. *J Neurophysiol*, 89, 562-70.
11. Kakigi, R., Endo, C., Neshige, R., Kuroda, Y., Shibasaki, H. (1991). Estimation of conduction velocity of A delta fibers in humans. *Muscle Nerve*, 14, 1193-1196.
12. Katsarava, Z., Yaldizli, O., Voulkoudis, C., Diener, H.C., Kaube, H., Maschke, M. (2006). Pain related potentials by electrical stimulation of skin for detection of small-fiber neuropathy in HIV. *J Neurol*, 253, 1581–1584.
13. Koltzenburg, M., Stucky, C.L., Lewin, G.R. (1997). Receptive properties of mouse sensory neurons innervating hairy skin. *J Neurophysiol*, 78, 1841-1850.
14. La Cesa, S., Di Stefano, G., Leone, C., Pepe, A., Galosi, E., Alu, F., Fasolino, A., Cruccu, G., Valeriani, M., Truini, A. (2018) Skin denervation does not alter cortical potentials to surface concentric electrode stimulation: A comparison with laser evoked potentials and contact heat evoked potentials. *Eur J Pain,* 22,161-169.
15. Leandri, M., Marinelli, L., Siri, A., Pellegrino, L. (2018). Micropatterned surface electrode for massive selective stimulation of intraepidermal nociceptive fibres. *J Neurosci Methods*, 293, 17-26.
16. 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, 1967-1975.
17. Mouraux, A., Iannetti, G.D. (2008). Across-trial averaging of event related EEG responses and beyond. *Magn Reson Imaging*, 26, 1041–1054.
18. Mouraux A, Iannetti GD, Plaghki L. (2010). Low intensity intra-epidermal electrical stimulation can activate Aδ-nociceptors selectively. *Pain*, 150, 199-207.
19. Perchet, C., Godinho, F., Mazza, S., Frot, M., Legrain, V., Magnin, M., Garcia-Larrea, L. (2008). Evoked potentials to nociceptive stimuli delivered by CO2 or Nd:YAP lasers. *Clin Neurophysiol,* 119, 2615-2622.

20. Perchet, C., Frot, M., Charmarty, A., Flores, C., Mazza, S., Magnin, M., Garcia-Larrea, L. (2012). Do we activate specifically somatosensory thin fibres with the concentric planar electrode? A scalp and intracranial EEG study. *Pain*, 153, 1244–1252.
21. Rossi, P., Serrao, M., Amabile, G., Parisi, L., Pierelli, F., Pozzessere, G. (2000). A simple method for estimating conduction velocity of the spinothalamic tract in healthy humans. *Clin Neurophysiol*, 111, 1907-1915.
22. 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. *J Physiol*, 483, 747-758.
23. Treede, R.D., Lorenz, J., Baumgärtner, U. (2003). Clinical usefulness of laser-evoked potentials. *Neurophysiol Clin,* 33, 303-314.
24. Truini, A., Galeotti, F., Pennisi, E., Casa, F., Biasiotta, A., Cruccu, G. (2007). Trigeminal small-fibre function assessed with contact heat evoked potentials in humans. *Pain*, 132,102-107.
25. Truini, A., Panuccio, G., Galeotti, F., Maluccio, M.R., Sartucci, F., Avoli, M., Cruccu, G. (2010). Laser-evoked potentials as a tool for assessing the efficacy of antinociceptive drugs. *Eur J Pain*, 14, 222–225.
26. Üçeyler, N., Zeller, D., Kahn, A.K., Kewenig, S., Kittel-Schneider, S., Schmid, A., Casanova-Molla, J., Reiners, K., Sommer, C. (2013). Small fibre pathology in patients with fibromyalgia syndrome. *Brain,* 136, 1857–1867.
27. Yoon, M.S., Obermann, M., Dockweiler, C., Assert, R., Canbay, A., Haag, S., Gerken, G., Diener, H.C., Katsarava, Z. (2011). Sensory neuropathy in patients with cryoglobulin negative hepatitis-C infection. *J Neurol,* 258, 80–88.

Legends to Figures

Figure 1. Quality of perception. Quality of perception elicited by the 150IDE in healthy participants, using different intensities of stimulation. Y-axis: percentage of subjects; X-axis: stimulation intensity (mA).

Figure 2. Peripheral sensory action potential recordings. Amplitude of the superficial radial nerve sensory action potential, in healthy participants, using different intensity of stimulation, with 150IDE and standard electrode (single pulse, 0.1 ms duration). Y-axis: sensory action potential amplitude (μV) with standard deviation; X-axis: stimulation intensity (mA).

Figure 3. Comparison between low and high intensity 150IDE-evoked potentials. Vertex complex (N2-P2 recorded at Cz-Nz) of 150IDE-evoked potentials, after perioral and hand dorsum stimulation, by using a low-intensity stimulation, slightly above the pinprick detection threshold (light blue and blue lines), and a relatively high intensity stimulation, evoking an electrical shock sensation (grey and black lines). The superimposed thin lines represent the average of 30 trials in each healthy subject. The thick dotted line corresponds to the grand-average among subjects. The dotted lines indicate the N2 peak latency.

Figure 4. Comparison between low intensity 150IDE-evoked potentials and laser-evoked potentials. Lateralized component (N1 recorded at Tc-Fz) and vertex complex (N2-P2 recorded at Cz-Nz), of 150IDE-evoked potentials, after perioral stimulation, by using a low-intensity stimulation, slightly above the pinprick detection threshold (light blue and blue lines), and laser-evoked potentials (grey and black lines). The superimposed thin lines represent the average of 30 trials in each healthy subject. The thick dotted line corresponds to the grand-average among subjects. The dotted lines indicate the N1 and N2 peak latency.

Figure 5. Peripheral conduction velocity. The thin dashed lines represent the regressions of individual latencies in five subjects; the thick dotted line corresponds to the mean regression. Dots represent the mean values of the N2 latencies after stimulation of the three sites. The reciprocal of the slope of the mean regression (11.70 m/sec) indicates the mean conduction velocity.

Figure 6. Superficial radial nerve block. Recording of superficial radial nerve sensory action potential (SNAP), 150IDE-evoked potentials and laser-evoked potentials before and after selective superficial radial nerve block, using near-nerve lidocaine infiltration. The lidocaine block abolished laser-evoked potentials and 150IDE-evoked potentials, but left the radial SNAP

Figure 7. Comparison between low intensity 150 IDE- evoked potentials and laser-evoked potentials in patients with nociceptive system damage.

Skin innervation of the distal leg in a patient with small-fibre neuropathy related to amyloidosis, showing the intraepidermal nerve fibre loss. MRI image in patient with syringomyelia involving the cervical spinal cord. MRI image in patient with ischemic lesion involving the lateral part of the medulla (Wallenberg syndrome).

Vertex complex (N2-P2 recorded at Cz-Nz) of low intensity 150IDE-evoked potentials and laser-evoked potentials after stimulation of the healthy (red) and the affected (blue) areas. Each trace represents the average of 30 trials. Dotted lines indicate the stimulus onset. Both laser and low intensity 150IDE stimulation of the area of maximal thermal-pain sensory deficit failed to evoke scalp potentials.

Table

Table 1. Comparison between 150IDE-evoked potentials variables after low and high intensity stimulation

	Perioral region			Hand dorsum		
	High intensity stimulation	**Low intensity stimulation**	**p**	**High intensity stimulation**	**Low intensity stimulation**	**p**
N1 latency (ms)	88.5±11.5	100.5±13.4	0.001	115.0±21.7	131.6±22.4	0.0001
N1 amplitude (μV)	-9.1±6.5	-7.7±5.8	0.07	-7.9±5.0	-5.6±3.1	0.1
N2 latency (ms)	115.8±6	142.4±24.7	0.003	143.1±20.3	169.0±23.1	0.0004
P2 latency (ms)	234.0±25.5	251.0±38.7	0.01	259.9±34.9	278.0±51.8	0.1
N2 amplitude (μV)	-28.4±11.0	-15.6±8.1	0.0001	-17.9±9.0	-13.4±6.6	0.01
P2 amplitude (μV)	23.5±9.5	15.9±8.3	0.0002	18.1±9.3	12.1±7.7.1	0.01

Table

Table 2. Comparison between laser-evoked potentials and 150IDE-evoked potentials after low-intensity stimulation

	Perioral region			Hand dorsum		
	Laser stimulation	**Low intensity stimulation**	**p**	**Laser stimulation**	**Low intensity stimulation**	**p**
N1 latency (ms)	118.8 ±12.9	101.8 ± 15.6	0.4	136.1 ± 24.9	128.8 ± 14.4	0.1
N1 amplitude (μV)	-7.7 ± 3.6	-8.1 ± 6.8	0.5	-5.7 ± 0.6	-6.1 ± 3.2	0.09
N2 latency (ms)	150.3 ± 9.4	140.3 ± 28.3	0.1	187.9 ± 11.6	170.4 ± 22.7	0.2
P2 latency (ms)	256.4 ± 27.1	246.2 ± 41.0	0.4	298.9 ± 35.4	273.9 ± 52	0.3
N2 amplitude (μV)	-20.0 ± 6.3	-17.2 ± 8.6	0.2	-19 ± 8.2	-14.6 ± 7.4	0.6
P2 amplitude (μV)	21.3 ± 6.8	18.1 ± 8.4	0.2	19 ± 8.8	12.3 ± 8.4	0.2

Figure

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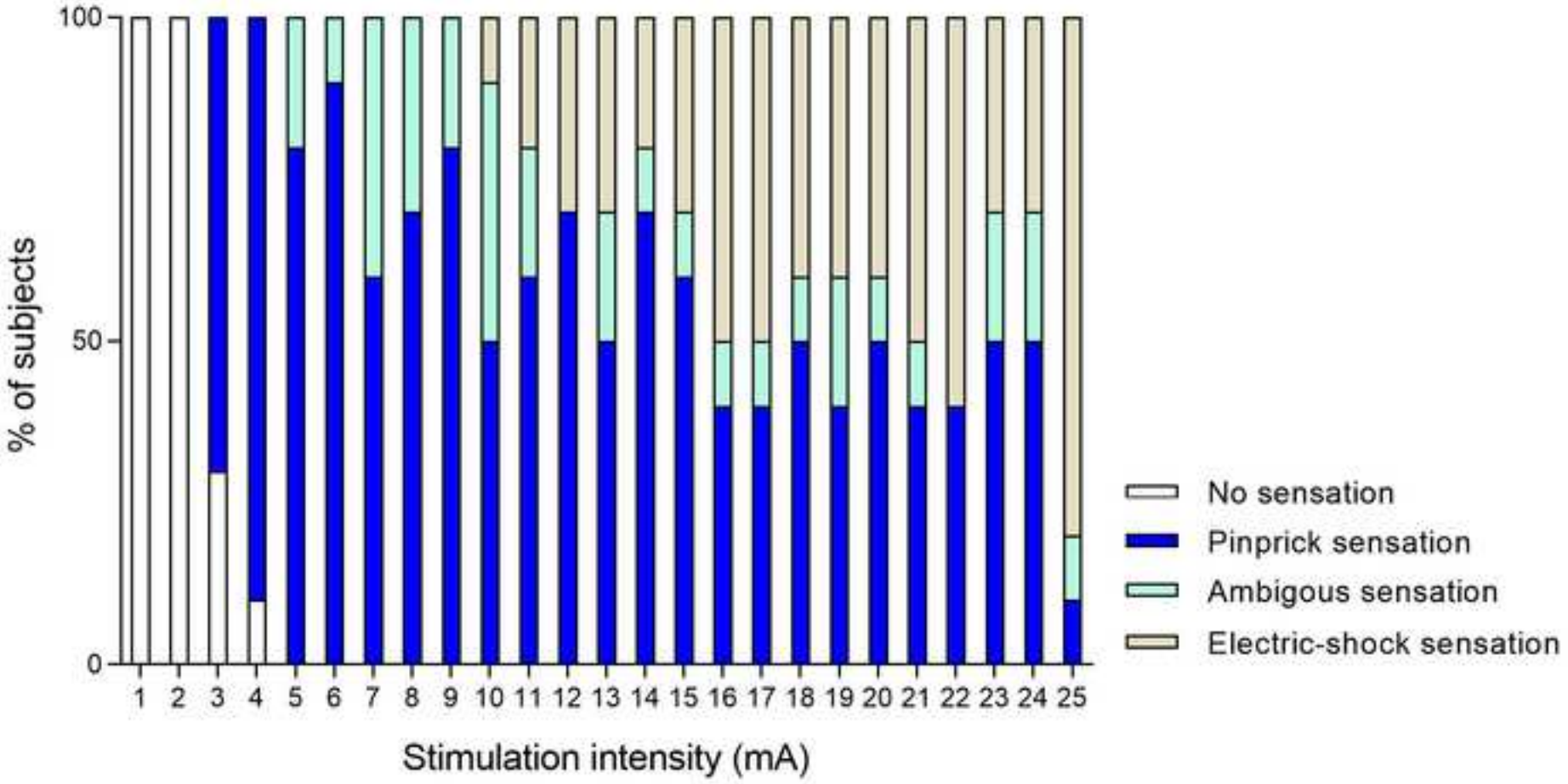

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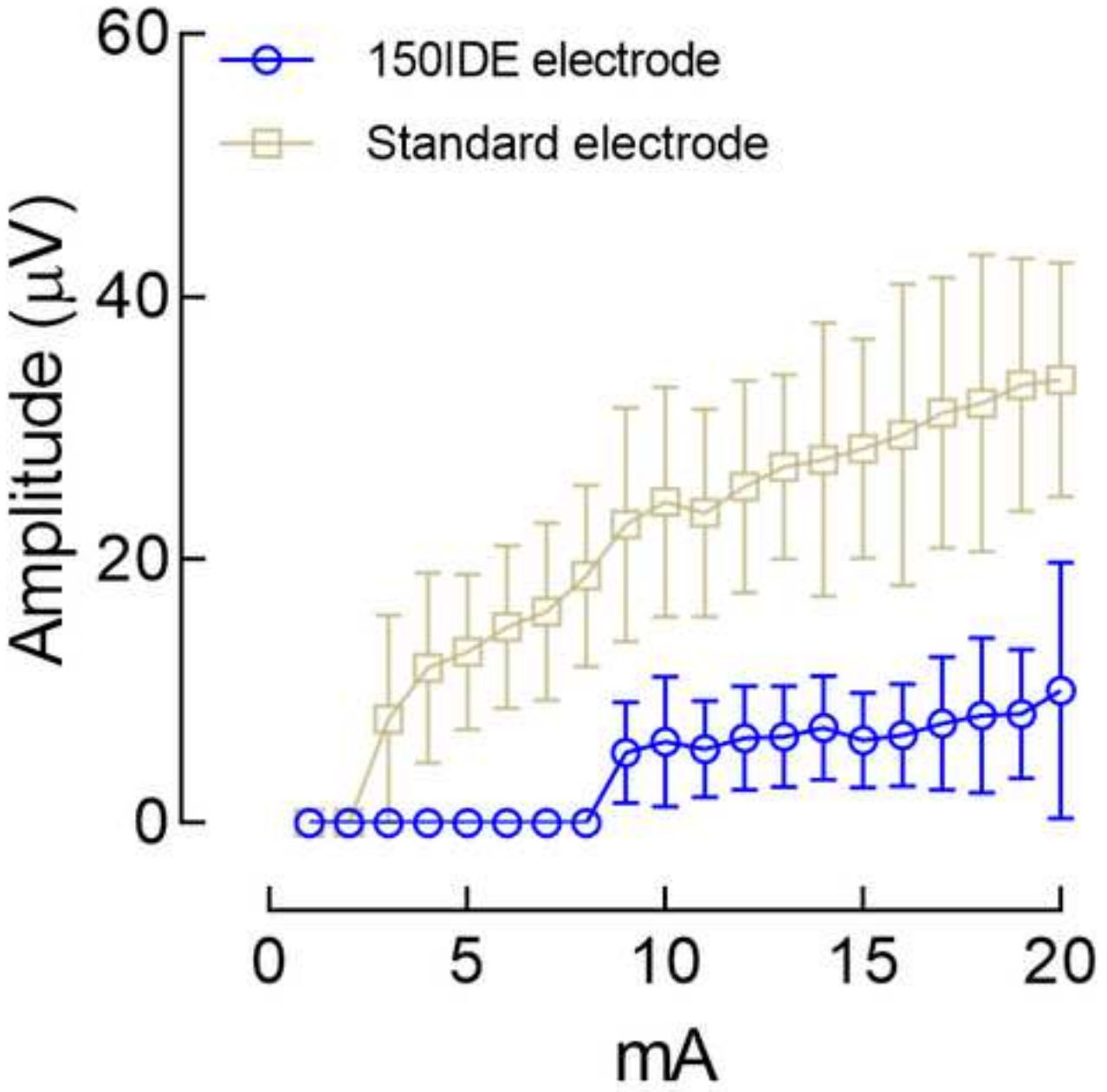

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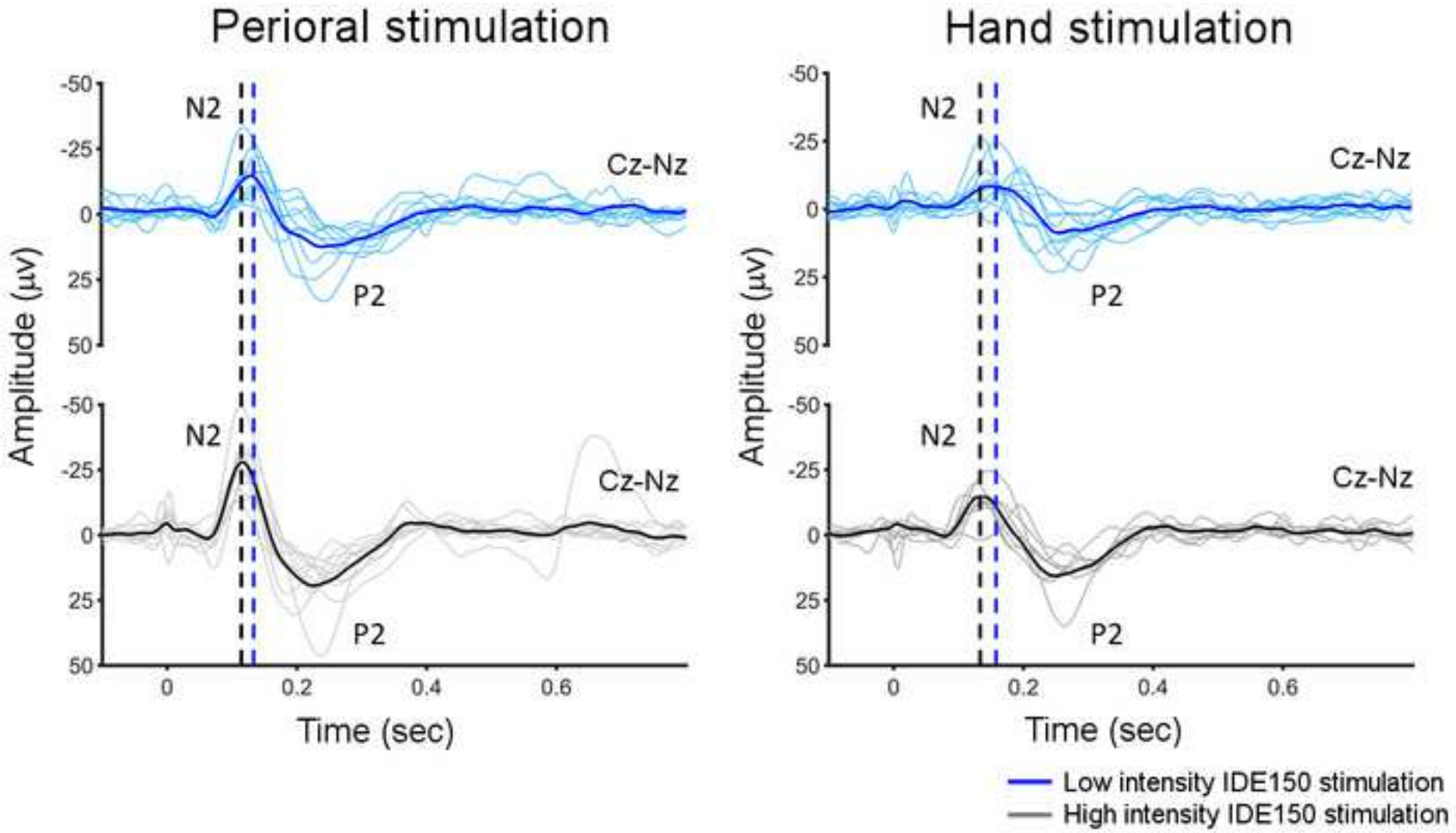

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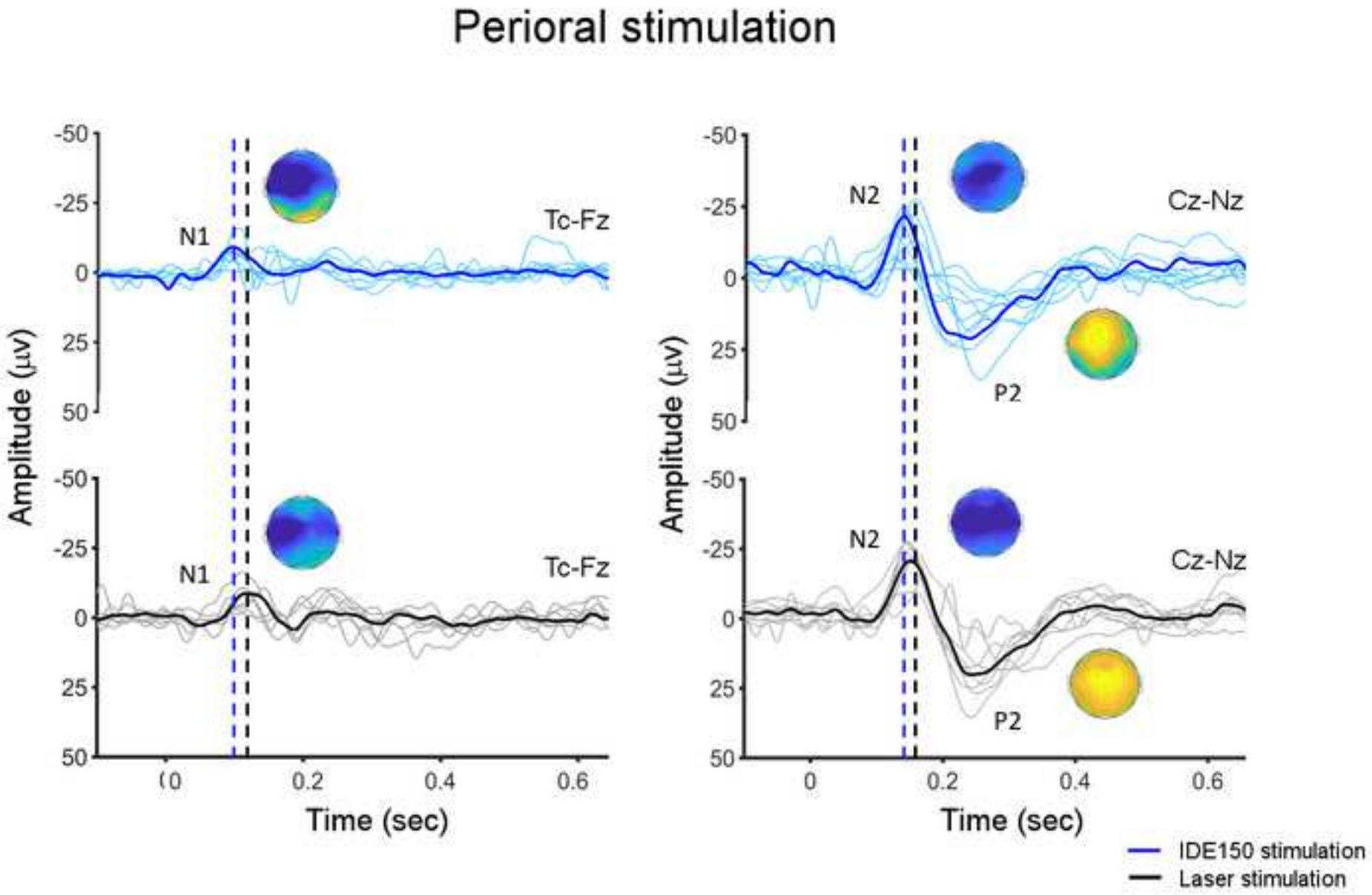

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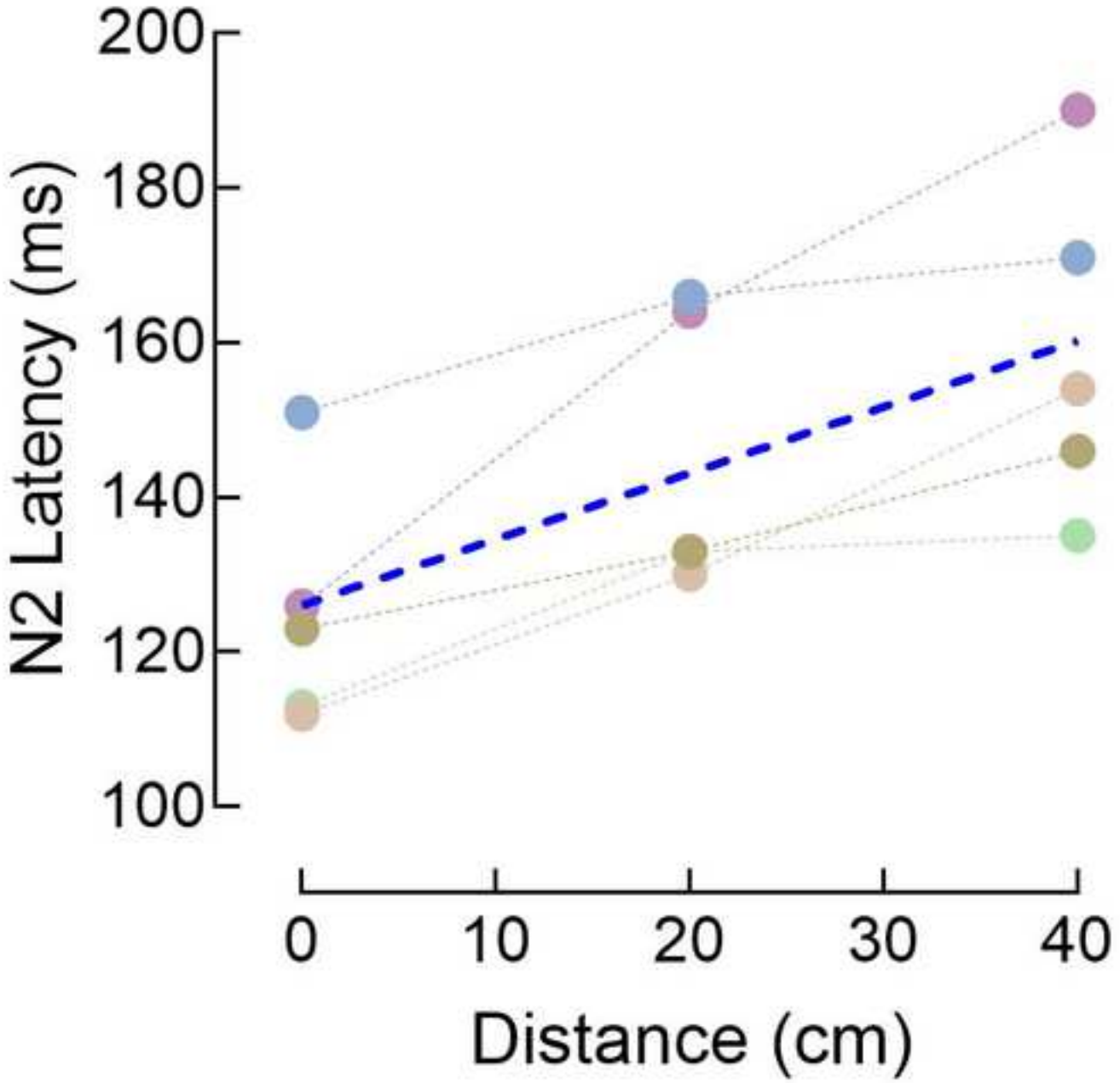

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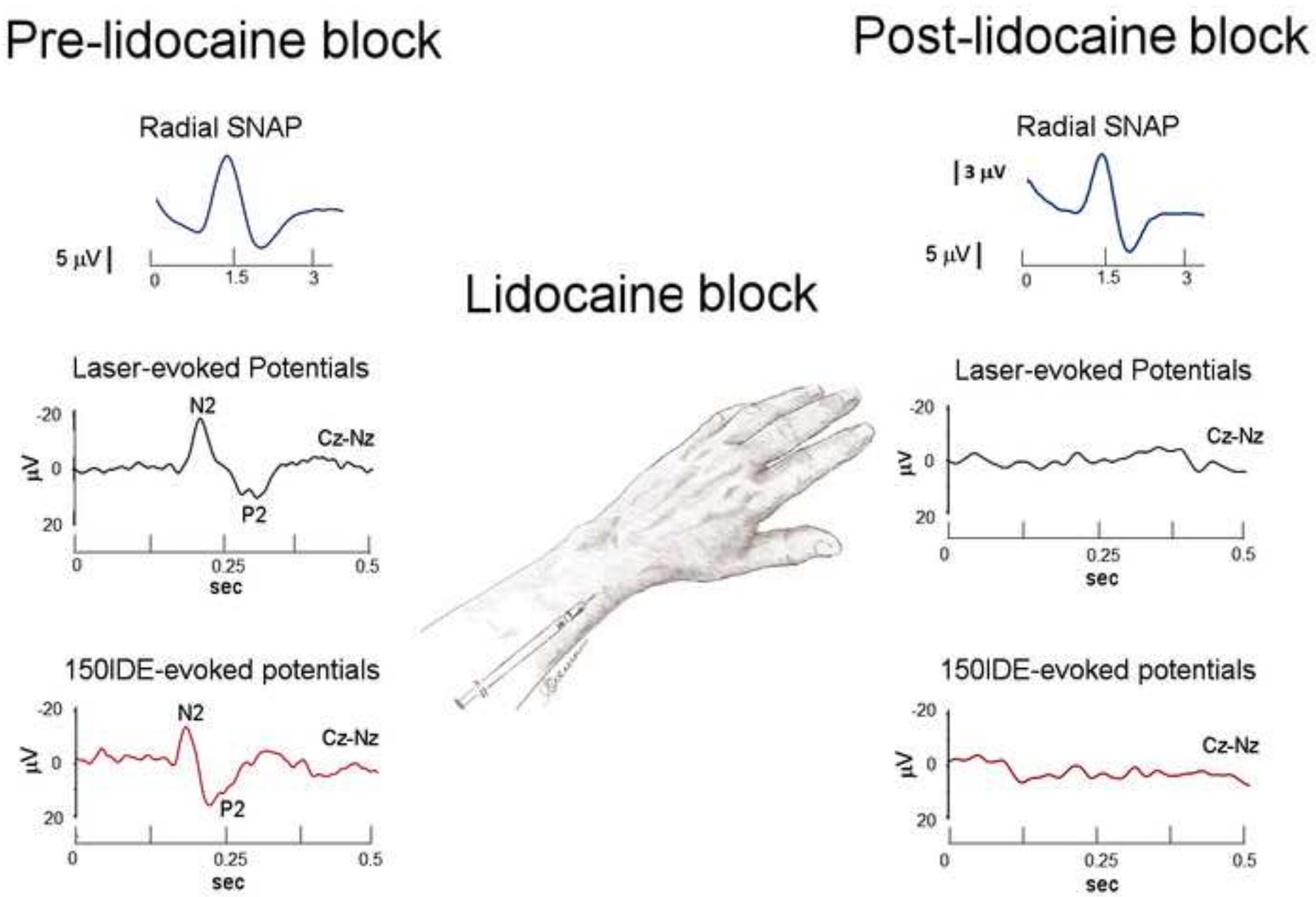

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