Revealing the time course of laser-evoked potential habituation by high temporal resolution analysis

Dilara Kersebaum | Sophie-Charlotte Fabig | Manon Sendel | Alexandra Cristina Muntean | Ralf Baron | Philipp Hüllemann

Division of Neurological Pain Research and Therapy, Department of Neurology, University Clinic Schleswig-Holstein, Kiel, Germany

Correspondence
Dilara Kersebaum, University Hospital Schleswig-Holstein, Campus Kiel, Department of Neurology, Division of Neurological Pain Research and Therapy, Arnold-Heller-Straße 3, 24105 Kiel, Germany.
Email: dilara.kersebaum@uksh.de

Abstract

Background and Objectives: Reduced laser-evoked potential (LEP) habituation indicates abnormal central pain processing. But the paradigm (four stimulation blocks of 25 stimuli) is time consuming and potentially omits important information on the exact habituation time course. This study examined whether a high temporal resolution (HTR) analysis (dividing the four stimulation blocks into 12 analysis blocks) can answer the following questions: (a) After how many stimuli does LEP habituation occur? (b) Is there a difference in LEP habituation in younger versus older subjects? (c) Is HTR applicable on radiculopathy patients?

Methods: EEG data of 129 subjects were included. Thirty-four young healthy and 28 advanced-aged healthy subjects were tested with LEPs on the hand dorsum. Thirty-seven radiculopathy patients and 30 controls were tested with LEPs on the L3 dermatome. The EEG data of the hand dorsa have been analysed conventionally and with HTR analysis. The applicability of HTR has been tested on radiculopathy patients and respective controls.

Results: HTR was well feasible in young healthy subjects and revealed a strong habituation effect during the first 25 stimuli (i.e. within the first 5 min). After approximately 48 stimuli, no further significant habituation was detectable. LEP amplitudes were higher in young subjects. HTR was unsuitable for elderly subjects and middle-aged radiculopathy patients.

Conclusions: In young healthy subjects, HTR allows a shortening of the test protocol while providing a detailed information on the time course of LEP habituation. A shorter protocol might be useful for the applicability of the LEP paradigm for clinical and experimental settings as well as pharmacological studies.

Significance: The usage of high temporal resolution (HTR) analysis in young healthy subjects enables a short test protocol and provides the exact time course of laser-evoked potential habituation. This can be useful for the examination of neurological conditions affecting younger patients and for pharmacological studies. HTR was inapplicable in advanced-aged subjects and patients with radiculopathy.
1 | INTRODUCTION

As current pain research addresses a mechanism-based approach for the therapy of neuropathic pain (Baron et al., 2017; Rekatsina et al., 2020), it is obvious that the adequate diagnostic means are key to successful treatment. A decisive role is played by examination methods that generate objectifiable results (Finnerup et al., 2016). Among them, laser-evoked potentials (LEPs) are described to be “the easiest and most reliable of the neurophysiological methods for assessing function of nociceptive pathways” (Cruccu et al., 2010). The assessment of the spinothalamic tract and small-fibre integrity through the application of brief laser pulses to the skin has been well examined (Bromm & Lorenz, 1998; Treede et al., 2003) and rated to be of adequate sensitivity and specificity for clinical use to explore the presence of neuropathic pain (Garcia-Larrea & Hagiwara, 2019). So far, LEPs have been reported as a diagnostic procedure for numerous conditions such as diabetic polyneuropathy (Di Stefano et al., 2017), radiculopathy (Hüllemann et al., 2017b; Lorenz et al., 1996; Quante et al., 2007), central pain (Garcia-Larrea, 2002) and postherpetic neuralgia (Truini et al., 2003).

For diagnostic purposes, both LEP amplitudes and latencies are of interest. Seemingly abnormal amplitudes can be recorded when the physiological, so-called habituation effect which leads to a diminution of the LEP amplitude and laser pain rating over time does not occur (Hüllemann et al., 2013). LEP habituation has been suggested as a promising paradigm for the electrophysiological assessment of central pain processing by previous studies (De Tommaso, Libro, et al., 2005; Hüllemann et al., 2015, 2016; Valeriani et al., 2003), which is why age-dependent effects on LEP habituation appeared to be plausible.

4. Is the high temporal resolution (HTR)-habituation paradigm applicable to neuropathic pain patients and will it reveal additional information? Reduced LEP habituation in patients with painful radiculopathy has been reported previously by our group when using a $4 \times 25$ stimuli approach.

2 | METHODS

A total of 129 subjects were included in this study. They were distributed to four different groups (Figure 1).

- young healthy subjects (Experiment A, $n = 34$)
- old healthy subjects (Experiment B, $n = 28$)
- radiculopathy patients (Experiment C, $n = 37$)
- healthy, corresponding controls (Experiment D, $n = 30$)

While the EEG data for experiment B and partly D have been obtained especially for this study, the EEG data of experiment A, C and partly D have been collected during previously conducted studies. Each study was approved by the ethics committee of the University Hospital of Kiel and complied with the Declaration of Helsinki and all participants gave their written informed consent to participate.

2.1 | Experiment A – Healthy young subjects, right hand dorsum

Thirty-four subjects (age 24.9 ± 3.0 years, 14 males, 20 females) were tested with LEPs at the right hand dorsum. One hundred stimuli were applied in four blocks of 25 stimuli (overall duration approximately 20 min as the subjects were well introduced to the examination and the detection threshold was determined). The inter-block interval was ~10 s. Three seconds after the laser stimulus, the subjects were asked to report their laser pain sensation on a numeric rating scale (NRS, 0 = no pain, 10 = worst imaginable pain). The data were analysed in our group’s above-described conventional way sticking to the four stimulation blocks (which will be referred to as the conventional analysis throughout this study) and according to our below-described HTR criteria.

2.2 | Experiment B – Healthy subjects between the age of 50–70, right hand dorsum

Twenty-eight subjects (age 60.61 ± 5.6 years, 15 males, 13 females) were tested and analysed in analogy to experiment A.
2.3 | Experiment C – Patients with radiculopathy, L3 dermatome

The conventional analysis of LEP habituation in patients with painful radiculopathy has already been examined and published previously by our group (Hüllemann et al., 2017a).

To verify the new analysis approach in patients, EEG data from 37 radiculopathy patients affected in the L5 or S1 dermatome (age 50.29 ± 14.81 years, 11 males, 26 females), that had been obtained from the ventral medial thigh (L3) in analogy to experiment A and B were analysed for this study according to below-described high-resolution criteria.

2.4 | Experiment D – Healthy controls of radiculopathy patients, L3 dermatome

To verify the new analysis approach in the L3 dermatome, EEG data from 30 healthy subjects (age 48.73 ± 12.38 years 12 males, 18 females) from the ventral medical thigh (L3) were analysed for this study according to below-described high-resolution criteria.

2.5 | LEP recording

The recording took place in a constant tempered room with the subjects positioned on a comfortable stretcher. They were asked not to blink or move their eyes while the stimulus was being applied and 3 s until a ping tone emerged. Setup and methodology to elicit A-delta fibre mediated LEPs have been conducted in analogy to previously published setups of our group:

A Nd:YAP 1340 Stimul Laser (neodymium: yttrium-aluminium-perovskite, DEKA Lasertechnologie GmbH, Mainburg) with a beam diameter of 5 mm and a stimulus duration of 5 ms was used. The subject’s detection threshold was determined by up-regulating the energy stepwise (beginning with 0.5 J and the stepwise by 0.5-Joule increases) until a sensation was felt. Beginning from the detection threshold, the energy was increased further until the subjects reported a distinct pinprick pain sensation between 3 and 6 on the numerical pain-rating scale, which should be equal to a twofold detection threshold of the laser energy density (Hüllemann et al., 2013, 2016). The laser stimulator hand piece was relocated within the testing area after each stimulus in order to avoid receptor fatigue (Greffrath et al., 2007) or sensitization (Price et al., 1977). Stimuli were applied in blocks of 25 (5 min). The inter-stimulus intervals were randomized from 8 to 12 s (mean 10 s).”
10–20 system: Fz, Cz, PZ, C3, C4 with linked earlobes as reference for the recording of the N2/P2 component, T3 and T4 with Fz as reference (Cruccu & Truini, 2010). An EOG was attached for the detection of artefacts” as well as a grounding electrode. “The subjects reported the pain intensity of the perceived laser stimuli on a numerical rating scale (0 = no pain, 10 = most imaginable pain) after hearing the ping tone. The EEG was recorded with Brain vision recorder 1.2 using the BrainAmp MR plus EEG amplifier (Brain products GmbH) and analysed with Brain Vision Analyser 2.0 (Brain Products GmbH, Version 2.0.3.6367). All frames which contained artefacts 0.5 s before the laser stimulus and 1.5 s afterwards due to movement or blinking were excluded from analysis during visual inspection. The EEG was band-pass filtered with 0.3–35 Hz and the sampling rate was 1,000 Hz. The N2P2 amplitude was measured from the most negative to the most positive peak. The latency of each A-delta component was measured from the stimulus onset (0 ms) to the peak of the averaged potentials (N2 latency).” The N1 amplitude was not within the focus of this study and was thus omitted from the EP analyses. Furthermore, N1 appears inconsistently in elderly subjects, especially when the stimulated area is the thigh (Hüllemann et al., 2017b).

In case, a 25-stimulus trial included more than seven EEG frames with analysis-hindering artefacts, alpha-EEG or unfavourable signal-to-noise ratio, the subjects were excluded from the statistical analysis.

### 2.6 LEP analysis

#### 2.6.1 Conventional LEP analysis

For the conventional analysis, LEP latencies and N2P2 amplitudes were analysed according to the stimulation blocks (Table 1), keeping all artefact-free EEG frames of a 25-stimulus trial (25-frame analysis).

#### 2.6.2 High temporal resolution analysis

Through an increased temporal resolution, the exact time course of habituation was intended to be identified. For HTR analysis, each stimulation block (25 frames) was further divided into three blocks of eight laser stimuli (8-frame analysis). To ensure equal numbers of laser stimuli, every first frame of a 25-stimulus block was discarded. Consequently, the EEG segments from block 1 in the conventional analysis corresponded to those of blocks I, II and III in the HTR analysis and the segments of block 2 from the conventional analysis corresponded to those of blocks IV, V and VI in the HTR analysis, etc. (Table 1).

The inclusion criterion for the 8-frame analysis was having at least five out of eight artefact-free frames. If this criterion was not met, the subject was excluded from the statistical analysis. For a more precise analysis, the average of the corresponding artefact-free 25-stimulus trial was laid over the high-resolution segments for a better identification of the high-resolution potentials. EEG data of the young, healthy population were the first to be analysed in a high resolution way. The method was then applied to the data of the old healthy population. Subsequently, the practicability of this approach was examined in patients with painful radiculopathy and their age-matched controls.
2.7 Statistical analysis

LEP data and pain ratings were analysed descriptively with means, standard deviation and lower and upper limits of a 95% confidence interval. A \( p < 0.05 \) was determined to be statistically significant. The time course of habituation was analysed with a repeated measures ANOVA and post-hoc Bonferroni correction for pair-wise comparisons within one group, including N2, P2 latencies, N2P2 amplitudes and subjective pain rating. For HTR analysis, the data have been analysed with two methods, (a) calculating with the entirety of 12 blocks and (b) following a parsimonious approach. In general, each block has been compared with consecutive blocks, respectively. Method 1 included the entirety of 12 blocks and method 2 step-wise compared each block with the following in order to minimize possible effects of multiple testing. As method 2 did not deliver additional information in the meaning of significant results, we have decided to present only method 1 in the study for reasons of clarity and thoroughness.

When comparing the time course of LEP habituation within two groups, we used a two-way ANOVA using time as within and group as between-subjects factor. With this calculation, it was possible to learn whether there was an overall effect of the main factors independently of each other (i.e. time and group) and an effect of the time \( \times \) group interaction. For the two-way ANOVA, we used Mann–Whitney U as a post-hoc test, in order to compare block-wise LEP latency, amplitude and pain-rating differences. This statistical approach was applied for all experiments (A–D).

Furthermore, the percentual decrease of amplitude and laser pain ratings were compared between Experiment A and B descriptively. Figures were prepared using means and standard error of the mean.

3 RESULTS

Patient characteristics are displayed in Table 2. The EEG analysis of 90 out of 129 subjects complied with our inclusion criteria for statistical analysis (Figure 1).

3.1 Experiment A – Young healthy subjects, hand dorsum \( (n = 34) \)

LEP latencies, amplitudes and pain ratings are displayed in Table 3 (conventional analysis) and Table 4 (HTR analysis). Results for statistical analysis are shown in Appendix 1.

3.1.1 Conventional analysis \( (n = 34) \)

The conventional EEG analysis sticking to the four stimulation blocks per 25 segments (c-blocks) showed a significant N2P2 amplitude decrease over time \( (F_{2.390,78.857} = 44.22; p < 0.001) \). Neither N2 nor P2 latencies showed significant alterations over time. Pain ratings decreased significantly
over time ($F_{2.004;66.142} = 41.927; p < 0.001$), see also Table 3 and Appendix 1.

3.1.2 | HTR analysis ($n = 34$ for 12 and 6 blocks, respectively)

In the HTR analysis (12 blocks, 8 frames each), the course of the highly significant decrease of the amplitude could be well displayed ($F_{5.976;197.210} = 21.872; p < 0.01$). A significant amplitude decrease in comparison with consecutive blocks was observable from block I until block VI (see Appendix 1). After block VI, there was no further significant decrease of the N2/P2 amplitude, neither of the pain rating. At first glance, Figures 4 and 5 imply a further decrease from block VII to VIII (N2/P2) and VI to VII (NRS). Due to a notable inter-individual variability and after correction for multiple testing, these changes did not reach significance. The grand averages of the N2P2 potentials from block I to XII are shown in Figure 2a. For better visualization of the habituation effect, Figure 2b shows blocks I and XII only. The N2 and P2 latencies remained stable. There was a significant decrease of laser pain ratings over time ($F_{5.399;178.166} = 21.625; p < 0.001$). From block I to block XII, the pain ratings decreased by 47.97% (see also Table 4 and Appendix 1).

As the significant amplitude decrease in comparison with consecutive blocks was identifiable until block VI in the HTR analysis, we added a statistical analysis which only included the first six HTR blocks (corresponding to two conventional blocks while the first frame of each c-block was omitted). Here, the significant amplitude decrease ($F_{5.165} = 18.469; p < 0.001$) was observable until block II in the post-hoc analysis. The latencies remained stable. Pain ratings decreased significantly over time ($F_{3.479;114.819} = 19.398; p < 0.001$), see also Table 4 and Appendix 1.

3.2 | Experiment B – Old healthy subjects, hand dorsum ($n = 28$)

LEP latencies, amplitudes and pain ratings are displayed in Table 3 (conventional analysis) and Table 4 (HTR analysis). Results for the statistical analysis are shown in Appendix 2.

3.2.1 | Conventional analysis ($n = 24$)

Out of 28 subjects tested, four were excluded from the statistical analysis due to insufficient EEG quality caused by artefacts (movement, blinking, dozing, etc.), unfavourable signal-to-noise ratio or alpha waves. The conventional EEG analysis in the remaining 24 showed a significant decrease of amplitudes from block 1 to 4 ($F_{5.69} = 19.912; p < 0.01$). The N2 and P2 latencies showed no significant change over time. The pain ratings decreased significantly over time ($F_{1.923;44.219} = 14.569; p < 0.001$), see also Table 3 and Appendix 2.

3.2.2 | HTR analysis ($n = 15$ for 12 blocks, $n = 17$ for 6 blocks)

HTR was associated with difficulties identifying the potential for certain subjects in the course of analysis and was thus not applicable on every subject. It was possible to apply this evaluation method to 15 out of 28 subjects. Here, a significant decrease of the N2P2 amplitude in comparison with consecutive blocks was shown until block VI ($F_{11.154} = 7.870; p < 0.01$), see Table 4 and Appendix 2). The latencies showed no differences. There was a significant decrease of laser pain ratings over time ($F_{3.301;46.215} = 4.315; p = 0.007$) although post hoc comparisons showed no significant differences between the
blocks. From block I to block XII, the pain ratings decreased by 27.9% (see also Table 4 and Appendix 2).

A statistical analysis for only the first six blocks (again, corresponding to two c-blocks per 25 frames while the first frame of each block was omitted) was conducted in this group as well and was applicable on 17 subjects. There was a significant decrease of amplitudes ($F_{5.80} = 4.244; p = 0.002$) without significant differences in the blocks in the post-hoc analysis. The latencies remained unchanged. Pain ratings decreased significantly over time ($F_{2.181;34.895} = 5.159, p = 0.009$), see also Table 4 and Appendix 2.

### 3.3 Young versus old subjects, hand dorsum

#### 3.3.1 Conventional analysis

There was a significant difference for the time course of LEP habituation of young versus old subjects in the ANOVA. A significant effect could be shown for all of the main factors, i.e., ‘time’ ($F_{2.502;140.120} = 49.9; p < 0.001$), ‘group’ ($F_{1.56} = 33.5; p < 0.001$) and interaction of time × group ($F_{2.502;140.120} = 11.524; p < 0.01$), meaning that (a) there were different amplitudes in the four blocks, (b) different amplitudes in the groups young versus old and finally (c) there was an effect of the time × group interaction. Comparing each block separately, the post-hoc Mann–Whitney U-test indicates a significant difference between each block with higher amplitudes in the young group ($p < 0.001$ for blocks 1–4 of young versus old subjects, respectively). There was no significant difference between the young and the old group concerning the time course of the latencies in the ANOVA, although the factor ‘group’ had a significant effect on both N2 ($F_{1.56} = 23.25; p < 0.001$) and P2 latencies ($F_{1.56} = 4.535; p = 0.038$). Post-hoc Mann–Whitney U-test revealed a significant difference in the N2 latency between the groups for blocks 1, 2, 3 and 4, with the N2 appearing later in the older group (i.e. $p < 0.01$ for blocks 1–4 of young vs old subjects, respectively). For the P2 latency, blocks 2 ($p = 0.032$) and 4 ($p = 0.035$) of young versus old subjects showed a significant difference, with the P2 appearing later in the younger group. See Figure 3 for the LEP grand averages of both the young and the old cohort from stimulation block 1–4. The ANOVA revealed no significant differences in the course of laser pain ratings between the two groups (no effect of time × group interaction). The factor ‘time’ though had a significant effect ($F_{2.118;118.614} = 49.87; p < 0.001$), so did the factor ‘group’ ($F_{1.56} = 4.229; p = 0.044$). For blocks 1–4 of young versus old subjects, there was a significant difference in the pain ratings in the post-hoc Mann–Whitney U-test with higher pain ratings in the old group (blocks 1 and 2 $p < 0.05$; blocks 3 and 4 $p < 0.001$).

#### 3.3.2 HTR analysis

Again, there was a significant difference for the time course of LEP habituation of young versus old subjects in the ANOVA,
The values are presented in mean ± standard deviation with the lower and upper 95% confidence limit in brackets.

Abbreviation: HTR, high temporal resolution.

TABLE 4

| Block VII | Block VIII | Block IX | Block X | Block XI | Block XII |
|-----------|------------|----------|---------|----------|----------|
| 215 ± 23.1 [207; 223.1] | 213.5 ± 21.0 [206.1; 220.8] | 218.2 ± 28.9 [208.2; 228.3] | 216.9 ± 25.1 [208.2; 225.7] | 215.4 ± 21.2 [208; 222.8] | 221.8 ± 31.9 [210.7; 232.9] |
| 341.7 ± 40.2 [327.7; 355.8] | 344.1 ± 31 [333.2; 354.9] | 352.2 ± 39.2 [338.6; 365.9] | 344.7 ± 38.3 [331.3; 358.1] | 352.9 ± 29.8 [342.4; 363.3] | 349.3 ± 39.8 [335.4; 363.2] |
| 32.5 ± 22.2 [24.7; 40.2] | 26.7 ± 16.3 [21.1; 32.4] | 25.6 ± 13.8 [20.8; 30.4] | 24.4 ± 13.1 [19.8; 29] | 25.1 ± 14.8 [20; 30.3] | 23.8 ± 14.5 [18.7; 28.8] |
| 2.1 ± 1.3 [1.6; 2.6] | 2.2 ± 1.3 [1.7; 2.6] | 2 ± 1.3 [1.5; 2.4] | 2 ± 1.3 [1.5; 2.5] | 2 ± 1.3 [1.5; 2.4] | 1.9 ± 1.3 [1.4; 2.4] |

| Block VII | Block VIII | Block IX | Block X | Block XI | Block XII |
|-----------|------------|----------|---------|----------|----------|
| 234.9 ± 11.5 [228.5; 241.2] | 234.3 ± 15.5 [225.8; 242.9] | 234.3 ± 17.7 [224.6; 244.1] | 236.4 ± 18.8 [226; 246.8] | 233.1 ± 17.8 [223.3; 243] | 234.7 ± 21 [223.1; 246.3] |
| 337.9 ± 22.8 [325.3; 350.6] | 330 ± 28.8 [314.4; 345.6] | 328.1 ± 28.5 [312.3; 343.8] | 326.4 ± 36 [306.45; 346.4] | 329.9 ± 32.5 [311.9; 347.9] | 329.9 ± 29.8 [313.4; 346.5] |
| 14.1 ± 5.7 [10.9; 17.3] | 13.4 ± 5.8 [10.1; 16.6] | 11.6 ± 5.6 [8.5; 14.7] | 12.2 ± 5.9 [8.9; 15.5] | 10.5 ± 5.4 [7.5; 13.5] | 9.3 ± 4 [7.1; 11.6] |
| 2.6 ± 1.2 [2; 3.3] | 2.8 ± 1.3 [2; 3.5] | 2.8 ± 1.3 [2.1; 3.5] | 2.8 ± 1.4 [2; 3.5] | 2.6 ± 1.3 [1.9; 3.3] | 2.6 ± 1.4 [1.8; 3.4] |

The significant effect being present for all of the main factors (‘time’ $F_{6,38,300.036} = 16.85$; $p < 0.001$, ‘group’ $F_{1,47} = 18.2$; $p < 0.001$ and time x group interaction $F_{6,384,260.036} = 3.627$; $p = 0.001$), see Figure 4. Post-hoc Mann–Whitney U was highly significant for every block with $p < 0.001$ and higher amplitudes in the young group). The amplitudes of the young group decreased by 46.4% and those of the older group by 52.7%. In the ANOVA, the course of the latencies showed no differences. Again, the factor ‘group’ had a significant effect on N2 latencies ($F_{1,47} = 10.534$; $p = 0.002$) but not on P2 latencies, although very close ($F_{1,47} = 4.06$; $p = 0.05$). When looking at each stimulation block separately, the Mann–Whitney U-test revealed significant differences of N2 latencies for each block except for blocks VI and XII, with the N2 appearing later in the older group (all significant comparisons: $p < 0.01$; except for block IV: $p = 0.016$). Significant differences for P2 latencies have been found for block VI ($p = 0.03$), IX ($p = 0.041$) and XI ($p = 0.023$) with the P2 appearing later in the younger group. The course of laser pain ratings was not significantly different between the groups (see Figure 5), even though there was a significant effect of ‘time’ ($F_{5,535,260.132} = 17.281$; $p < 0.001$) and post-hoc Mann–Whitney U showed significant differences for blocks VI, IX and X of young versus old subjects ($p < 0.05$, higher pain ratings in older group).

In the comparison of the first six blocks, the directions of the significant results remained stable and again an effect of each main factor was shown for the LEP habituation

**FIGURE 3** Grand Averages of the four stimulation blocks for both Experiment A (young healthy hand dorsum) and B (old healthy hand dorsum)
### TABLE 5  
N2/P2 latencies, N2P2 amplitudes and pain ratings of Experiment C (radiculopathy patients) and D (radiculopathy controls) according to HTR analysis

| Block I | Block II | Block III | Block IV | Block V | Block VI |
|---------|----------|-----------|----------|---------|----------|
| Radiculopathy | | | | | |
| N2 (ms) | 227.4 ± 24.3 | 230.9 ± 24.9 | 234.9 ± 27.9 | 226.1 ± 27.3 | 231.9 ± 25.5 | 232.8 ± 29.9 |
| [213.4; 241.4] | [216.6; 245.3] | [218.8; 251.0] | [210.3; 241.9] | [215.5; 246.6] | [215.5; 250.0] |
| P2 (ms) | 339.3 ± 29.8 | 338.3 ± 23.8 | 337.0 ± 35.6 | 332.6 ± 26.2 | 336.1 ± 31.5 | 342.4 ± 31.2 |
| [322.1; 356.5] | [324.5; 357.5] | [317.4; 347.7] | [324.4; 360.4] | [354.3] | [354.3] | [354.3] |
| N2P2 (µV) | 26.0 ± 12.1 | 25.0 ± 12.7 | 21.4 ± 10.8 | 21.7 ± 12.3 | 19.4 ± 8.1 | 18.6 ± 8.1 |
| [19.0; 32.9] | [17.6; 32.3] | [15.2; 27.6] | [14.7; 28.8] | [14.7; 28.8] | [14.7; 28.8] | [14.7; 28.8] |
| Pain (NRS) | 5.3 ± 2.3 | 5.4 ± 2.0 | 5.2 ± 2.1 | 5.0 ± 1.8 | 5.0 ± 2.1 | 5.0 ± 2.1 |
| [3.9; 6.6] | [4.2; 6.5] | [4.0; 6.5] | [3.8; 6.3] | [3.8; 6.3] | [3.8; 6.3] | [3.8; 6.3] |

| Block I | Block II | Block III | Block IV | Block V | Block VI |
|---------|----------|-----------|----------|---------|----------|
| Radiculopathy control | | | | | |
| N2 (ms) | 223.3 ± 18.8 | 218 ± 14.4 | 228.7 ± 20.3 | 218.4 ± 22.1 | 230.2 ± 19.3 | 233.6 ± 22.1 |
| [209.9; 236.7] | [207.7; 228.3] | [214.2; 243.2] | [202.6; 243.2] | [216.4; 244.0] | [217.8; 249.4] |
| P2 (ms) | 317.2 ± 35.8 | 319 ± 33.0 | 327.2 ± 36.0 | 319.3 ± 38.8 | 329.2 ± 35.9 | 329.3 ± 39.6 |
| [291.6; 342.8] | [295.4; 342.6] | [301.5; 353.0] | [291.5; 347.1] | [303.5; 354.9] | [301.0; 357.6] |
| N2P2 (µV) | 22.5 ± 14.4 | 20.0 ± 12.7 | 17.9 ± 8.3 | 16.2 ± 6.4 | 18.0 ± 8.4 | 13.8 ± 5.5 |
| [12.2; 32.8] | [10.9; 29.1] | [12.0; 23.8] | [11.6; 20.8] | [11.9; 24.0] | [9.9; 17.8] |
| Pain (NRS) | 3.6 ± 1.1 | 3.6 ± 1.3 | 3.9 ± 1.4 | 3.4 ± 1.4 | 3.6 ± 1.4 | 3.1 ± 1.6 |
| [2.8; 4.4] | [2.7; 4.6] | [2.9; 4.9] | [2.4; 4.4] | [2.6; 4.5] | [2.0; 4.2] |

Abbreviation: HTR, high temporal resolution.

The values are presented in mean ± standard deviation with the lower and upper 95% confidence limit in brackets.

(time’ $F_{5,245}$ = 14.246; $p < 0.001$, ‘group’ $F_{1,49} = 22.675$; $p < 0.001$ and time × group interaction $F_{5,245} = 3.925$; $p < 0.001$), the Mann–Whitney U-test revealed a significant difference for each block ($p < 0.001$). Both N2 and P2 latencies showed no differences in the time × group interaction. Still, there was a significant effect of the factor ‘group’ both on the N2 ($F_{1,49} = 11.427$; $p = 0.001$) and the P2 latencies ($F_{1,49} = 21.798$; $p < 0.001$). Looking at each block separately, the Mann–Whitney U-test revealed a significant difference of the N2 latency in blocks I–V of young versus old subjects ($p < 0.01$, respectively) and a significant difference of the P2 latency in each block ($p < 0.001$). The time course of laser pain ratings differed significantly. While both ‘time’ ($F_{5,245} = 3.246$; $p < 0.001$) and the time × group interaction ($F_{5,245} = 2.696$; $p = 0.046$) had a significant effect, this was not the case for the effect of ‘group’ independently of ‘time’. The comparison of each block young versus old subjects in the post-hoc Mann–Whitney U resulted in a significant difference between the groups for blocks II ($p = 0.019$) and VI ($p = 0.01$).

### HTR course of N2P2-habituation young vs. old cohort

**Figure 4**  
N2P2 amplitudes of HTR analysis in Experiment A (young healthy hand dorsum) versus B (old healthy hand dorsum); **p < 0.001, *p < 0.01. HTR, High temporal resolution; SE, standard error**

**Figure 5**  
Pain ratings of HTR analysis in Experiment A (young healthy hand dorsum) versus B (old healthy hand dorsum); *p < 0.05. HTR, High temporal resolution, SE, standard error
| Block VII | Block VIII | Block IX | Block X | Block XI | Block XII |
|-----------|------------|----------|---------|----------|-----------|
| 228.7 ± 26.5 [213.4; 244.0] | 240.6 ± 23.9 [226.9; 254.4] | 231.0 ± 23.6 [217.4; 244.6] | 234.7 ± 24.9 [220.4; 249.1] | 237.1 ± 22.2 [224.3; 250.0] | 236.4 ± 23.5 [222.9; 250.0] |
| 349.7 ± 30.9 [331.9; 367.5] | 344.4 ± 32.9 [325.4; 363.4] | 352.9 ± 35.5 [332.4; 373.4] | 338.6 ± 29.0 [321.9; 355.4] | 336.3 ± 28.8 [319.7; 352.9] | 341.4 ± 32.1 [322.9; 360.0] |
| 19.0 ± 7.6 [14.6; 23.3] | 16.4 ± 11.4 [9.8; 23.0] | 18.4 ± 11.1 [12.0; 24.8] | 16.8 ± 7.4 [12.5; 21.1] | 14.9 ± 6.8 [10.9; 18.8] | 17.2 ± 8.6 [12.2; 22.2] |
| 4.4 ± 2.0 [3.2; 5.5] | 5.1 ± 2.2 [3.8; 6.4] | 4.9 ± 2.2 [3.6; 6.2] | 4.4 ± 1.9 [3.3; 5.5] | 4.4 ± 1.9 [3.3; 5.5] | 4.8 ± 1.9 [3.7; 6.5] |
| 218.7 ± 12.0 [210.1; 227.3] | 277.3 ± 16.5 [215.5; 239.1] | 222.4 ± 12.6 [213.4; 231.4] | 223.7 ± 15.8 [212.4; 235.0] | 222.9 ± 16.7 [211.0; 234.8] | 224.7 ± 20.1 [210.3; 239.1] |
| 318.1 ± 36.0 [292.3; 343.9] | 318.6 ± 43.4 [287.6; 349.6] | 313.9 ± 35.1 [288.8; 339.0] | 312.5 ± 27.7 [292.7; 332.3] | 304.3 ± 25.5 [286.1; 322.5] | 321.2 ± 34.5 [296.5; 345.9] |
| 14.6 ± 6.8 [9.7; 19.5] | 11.1 ± 6.0 [6.8; 15.4] | 13.2 ± 5.9 [9.1; 17.4] | 13.8 ± 7.0 [8.8; 18.8] | 11.7 ± 4.0 [8.9; 14.5] | 11.2 ± 7.2 [6.1; 16.4] |
| 3.0 ± 1.0 [2.2; 3.7] | 3.1 ± 1.5 [2.0; 4.2] | 3.3 ± 1.5 [2.2; 4.3] | 3.1 ± 1.1 [2.3; 3.9] | 3.1 ± 1.3 [2.2; 4.1] | 2.9 ± 1.5 [1.9; 4.0] |

### 3.4 | Experiment C – Radiculopathy patients (L3 dermatome, n = 37)

LEP latencies, amplitudes and pain ratings are displayed in Table 5. Results for the statistical analysis are shown in Appendix 3.

#### 3.4.1 | HTR analysis (n = 14 for 12 blocks and n = 18 for 6 blocks)

Just like for some of the subjects from Experiment B, high resolution in radiculopathy patients was associated with difficulties identifying the potential. In 14 subjects, the EEG data complied with our inclusion criteria for HTR analysis. The amplitudes decreased significantly over time ($F_{11.143} = 6.698; p < 0.001$). The latencies showed no significant changes. Laser pain ratings showed a significant decrease over time ($F_{4.204;54.652} = 2.636, p = 0.041$) without significances in the post-hoc analysis, see also Table 5 and Appendix 3.

When we adhered to the analysis of six HTR blocks, we could include 18 subjects with visible potentials in the EEG data. The amplitudes decreased significantly over time ($F_{5.585} = 6.781, p < 0.001$). Neither the N2 nor the P2 latencies showed significant changes over time. Laser pain ratings remained stable in the course of six HTR blocks, see also Table 5 and Appendix 3.

### 3.5 | Experiment D – Radiculopathy controls (L3 dermatome, n = 30)

LEP latencies, amplitudes and pain ratings are displayed in Table 5.

#### 3.5.1 | HTR analysis (n = 10 for 12 blocks and n = 14 for 6 blocks)

Only the EEG data of 10 subjects complied with our inclusion criteria for high-resolution analysis. Unfortunately, this sample size is too small for a repeated measures ANOVA with 12 steps which is why this analysis was not feasible.

When we again adhered to an analysis of six HTR blocks, we could include 14 subjects into the analysis. The N2P2 amplitudes did not change significantly over time in the first six blocks, whereas the N2 latency showed a significant increase ($F_{5.65} = 3.178, p = 0.013$). There was no significant change of P2 latencies. Laser pain ratings showed no significant change over time.

### 4 | DISCUSSION

The LEP habituation paradigm is promoted as an instrument to detect central sensitization in chronic pain patients (Arendt-Nielsen et al., 2018; Hüllemann et al., 2017a).
Among other conditions, it has been utilized for the examination of migraine (Bassez et al., 2020; Valeriani et al., 2003), fibromyalgia (De Tommaso et al., 2011) and radiculopathy (Hüllemann et al., 2017a). It has also been used in studies assessing the efficacy of drugs (De Tommaso et al., 2016; Di Clemente et al., 2013). Bearing the quality to verify a pain as neuropathic and to assess the efficacy of drugs, LEP findings have the potential to drive therapeutic decisions.

But, with the conventional approach, time-sensitive changes of the N2P2 amplitudes may be obscured since epochs of 4 min are reported within a 25-stimulus average.

In general, the healthy study population of experiments A and B each displayed a significant amplitude and pain-rating decrease over time without latency changes. These observations are, in line, with former reports (De Tommaso, Lo Sito, et al., 2005; Hüllemann et al., 2013, 2016; Valeriani et al., 2003). The N2P2 amplitudes of the younger subjects were significantly higher than those of the older cohort. Although only blocks VI, IX and X revealed a statistical significance which might just be due to fluctuations, the percentual comparison of the course of pain-rating decrease remains interesting (48% vs 28%).

In the conventional analysis of the young, healthy subjects, the post-hoc comparisons of both the amplitudes and pain ratings (see Appendix 1) already revealed that block 4 delivered no additional information. In this group, HTR analysis was well feasible. LEPs were easily detectable using the above-mentioned criteria. Both in the young and older group, a strong habituation effect was observed during the first three blocks of the HTR analysis (i.e. within the first 4 min corresponding to the first 25 stimuli, see Figure 4), suggesting that a central, modifying reaction to pain occurs much earlier than displayable by the conventional analysis. After block VI of HTR analysis (i.e. after ~8 min, corresponding to 48 stimuli), no further significant habituation was detectable. The reduction of pain ratings over time developed accordingly.

These results have two implications: (a) the conventional analysis skips important information about the exact time course of LEP habituation within the first 5 min and (b) 40 instead of 100 stimuli might provide sufficient information with respect to central pain processing when using the LEP habituation paradigm. Thus, compared to the conventional analysis, HTR has the quality to provide additional information in an even shorter time. The shortening of the LEP-recording process is a gain for experimental approaches investigating central pain processing, studies assessing the efficacy of analgesic drugs (Schaffler et al., 2017, 2018; Truini et al., 2010) and clinical practice. The time-consuming character being one of the hurdles causal to the lacking establishment of LEP usage in clinical practice (Schlereth, 2020), a shorter test protocol providing more information in even shorter time seems very welcome.

Looking at the results of our older healthy subjects, the HTR analysis could only be implemented in 15 out of 28 subjects due to either a bad signal-to-noise ratio or an increased number of artefacts. Furthermore, older subjects often presented alpha waves during EEG recording even though their eyes remained open during stimulus application. This might indicate loss of attention (Chen et al., 2007). The repetitively observed alpha waves in the EEGs of our advanced-aged subjects imply that a mild alteration of vigilance or attention might have been influential on their EPs. Attention and vigilance both have previously been reported to have an effect on amplitudes (Beydoun et al., 1993; García-Larrea et al., 1997; Treede et al., 2003). Besides, alpha wave amplitudes ranging to up to 60 µV might have prevented the detection of the smaller LEPs.

While the EEGs were of sufficient quality for the conventional analysis and LEPs were identifiable (presumably due to the averaging of more potentials in one block), the HTR was clearly impeded. Although the time course of LEP habituation of our included elderly subjects was similar to the younger subjects within the first ~8 min, the HTR analysis appears to be inappropriate—considering almost half of the subjects had to be excluded from the analysis due to our strict inclusion criteria. It was somewhat unexpected that relevant numbers of older subjects had to be excluded, implying that they might have struggled to keep focus for several minutes.

The significant difference of amplitudes between the younger and older cohort shown in Figure 4 is, in line, with former reports on age-dependent changes of LEP amplitudes (Creac’h et al., 2015; Cruccu et al., 1999; Truini et al., 2005). The decrease of amplitudes in elderly subjects has been hypothesized to be associated with a dysfunction of the peripheral and/or the central nervous system (Gagliese & Melzack, 2000). Creac’h et al. also consider age-related changes in skin thickness which might cause a greater dispersion of thermal energy, thus reducing the intensity delivered to each nociceptor (Creac’h et al., 2015; Procacci et al., 1970). The lower starting point of the amplitudes of the elderly subjects in our study might also be an explanation as to why their identification through artefacts and bad signal-to-noise ratio was not possible in the course of analysis.

Performing LEP in various age groups and correlating age and LEP components, Truini et al. (2005) found an age-related decrease of amplitudes due to a reduced afferent input, whereas N2 latencies remained unchanged. They argue that a sole affection of the amplitude rather than the latencies might be the result of central changes. In contrast, an increased N1, N2 and P2 latency with aging has been shown by Creac’h et al. (2015) on the feet, possibly due to a distal loss of peripheral inputs and a length-dependent de-synchronization of the ascending nociceptive volley. Accordingly, we found an increased N2 latency within the older healthy subjects, which
points to subclinical changes within the peripheral and/or central nervous system around the age of 60.

A reduced LEP habituation in painful radiculopathy patients compared to healthy controls has been reported earlier (Hüllemann et al., 2017a). In analogy to above-mentioned hurdles in advanced-aged healthy subjects, HTR analysis was less favourable for the radiculopathy patients and their control group and was only applicable on approximately half of the subjects, leading to an impaired or limited applicability of our statistical measures. We presume the big amount of data loss to be responsible for false results such as the increased N2 latency over time in the 6-block HTR of Experiment D.

Indeed, a limitation of this study is that the data of HTR analysis of numerous subjects had to be excluded. This has implications for the applicability of HTR on older subjects and patients with painful radiculopathy.

In summary, the application of HTR in young, healthy subjects provided important insight on the exact time course of central pain adaption, bearing implications for practical application. Given an inter-stimulus interval of 8–12 s, the main effect of the laser pain habituation develops within 8 min and then reaches a plateau, whereupon the LEP amplitude and the subjective pain rating remain constant in young healthy subjects. While we believe our findings to be relevant for experimental/diagnostic purposes in younger subjects/patients (e.g. migraine patients), our data indeed suggest that HTR analysis has only limited use for older subjects within the 5th life decade (and later) and cannot be recommended for the assessment of central pain processing in radiculopathy patients.

5 | CONCLUSION

For young subjects, we propose a new, HTR analysis of EEGs which will allow a shortening of the habituation test protocol to two blocks (25 stimuli each). This time-saving approach might be helpful for clinical and experimental settings as well as pharmacological studies.

For elderly subjects and radiculopathy patients on the other side, this method turned out to be less favourable. We suggest testing the practicability of HTR in pain conditions that concern younger patient groups, e.g., migraine.

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AUTHOR CONTRIBUTIONS
DK, PH and RB: Conception and design; DK and ACM: Acquisition of data; DK, PH, SCF and MS: Analysis and interpretation of data; DK, SCF, MS, ACM, RB and PH: Drafting the manuscript and/or revising it critically for important intellectual content. DK, SCF, MS, ACM, RB and PH: Final approval of the version to be published. All authors discussed the results and commented on the manuscript.
REFERENCES

Arendt-Nielsen, L., Mortion, B., Perrot, S., Dahan, A., Dickenson, A., Kress, H. G., Wells, C., Bouhassira, D., & Mohr Drewes, A. (2018). Assessment and manifestation of central sensitisation across different chronic pain conditions. European Journal of Pain, 22, 216–241. https://doi.org/10.1002/ejp.1140

Baron, R., Maier, C., Attal, N., Binder, A., Bouhassira, D. I., Cruccu, G., Finnerup, N. B., Haanpää, M., Hansson, P., Hüllemann, P., Jensen, T. S., Freynhagen, R., Kennedy, J. D., Magehr, W., Mainka, T., Reimer, M., Rice, A. S. C., Segerdahl, M., Serra, J., ... Treede, R. D. (2017). Peripheral neuropathic pain: A mechanism-related organizing principle based on sensory profiles. Pain, 158, 261–272. https://doi.org/10.1097/j.pain.0000000000000753

Bassez, I., van de Steen, F., Ricci, K., Vecchio, E., Gentile, E., Marinazzo, D., & de Tommaso, M. (2020). Dynamic causal modeling of the reduced habituation to painful stimuli in migraine: An EEG study. Brain Sci, 10, 1–11. https://doi.org/10.3390/brainsci100712

Beydoun, A., Morrow, T. J., Shen, J. F., & Casey, K. L. (1993). Variability of laser-evoked potentials: Attention, arousal and lateralized differences. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 88, 173–181. https://doi.org/10.1016/0168-5597(93)90002-7

Bromb, B., & Lorenz, J. (1998). Neurophysiological evaluation of pain. Electroencephalography and Clinical Neurophysiology, 107, 227–253. https://doi.org/10.1016/S0013-4694(98)00075-3

Chen, Z., Ohara, S., Cao, J., Vialatte, F., Lenz, F. A., & Cichocki, A. (2007). Statistical modeling and analysis of laser-evoked potentials of electrocorticogram recordings from awake humans. Computational Intelligence and Neuroscience, 2007, 1–24.

Crea’H, C., Bertholon, A., Convers, P., Garcia-Larrea, L., & Peyron, R. (2015). Effects of aging on laser evoked potentials. Muscle and Nerve, 51, 736–742. https://doi.org/10.1010/mus.24458

Cruccu, G., Romaniello, A., Amantini, A., Lombardi, M., Innocenti, P., & Manfredi, M. (1999). Assessment of trigeminal small-fiber function: Brain and reflex responses evoked by CO2-laser stimulation. Muscle and Nerve, 22(4), 508–516. https://doi.org/10.1002/(SICI)1097-4598(199904)22:4<508::AID-MUS13>3.0.CO;2-B

Cruccu, G., Sommer, C., Anand, P., Attal, N., Baron, R., Garcia-Larrea, L., Haanpaa, M., Jensen, T. S., Serra, J., & Treede, R. D. (2010). EFNS guidelines on neuropathic pain assessment: Revised 2009. European Journal of Neurology, 17, 1010–1018. https://doi.org/10.1111/j.1468-1331.2010.02969.x

Cruccu, G., & Truini, A. (2010). Neuropathic pain and its assessment. Surgical Oncology, 19, 149–154. https://doi.org/10.1016/j.suronc.2009.11.012

De Tommaso, M., De Luca, M., Ricci, K., Montemurro, A., Carbone, I., & Vecchio, E. (2016). Effects of onabotulinumtoxina on habituation of laser evoked responses in chronic migraine. Toxins (Basel), 8(6), 163. https://doi.org/10.3390/toxins8060163

De Tommaso, M., Federici, A., Santostasi, R., Calabrese, R., Vecchio, E., Lapadula, G., Iannone, F., Lamberti, P., & Livrea, P. (2011). Laser-evoked potentials habituation in fibromyalgia. Journal of Pain, 12, 116–124. https://doi.org/10.1016/j.jpain.2010.06.004

De Tommaso, M., Libro, G., Guido, M., Losito, L., Lamberti, P., & Livrea, P. (2005). Habituation of single CO2 laser-evoked responses during interictal phase of migraine. The Journal of Headache and Pain, 6, 195–198. https://doi.org/10.1007/s10194-005-0183-0

De Tommaso, M., Lo Sito, L., Di Frusco, O., Sardaro, M., Prudenzano, M. P., Lamberti, P., & Livrea, P. (2005). Lack of habituation of nociceptive evoked responses and pain sensitivity during migraine attack. Clinical Neurophysiology, 116, 1254–1264. https://doi.org/10.1016/j.clinph.2005.02.018

Di Clemente, L., Puledda, F., Biasiotta, A., Viganò, A., Vicenzini, E., Truini, A., Cruccu, G., & Di Piero, V. (2013). Topiramate modulates habituation in migraine: Evidences from nociceptive responses elicited by laser evoked potentials. The Journal of Headache and Pain, 14, 1–8. https://doi.org/10.1186/1237-14-25

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. https://doi.org/10.1097/j.pain.0000000000000889

Finnerup, N. B., Haroutounian, S., Kamerman, P., Baron, R., Bennett, D. L. H., Bouhassira, D., Cruccu, G., Freeman, R., Hansson, P., Nurmikko, T., Raja, S. N., Rice, A. S. C., Serra, J., Smith, B. H., Treede, R. D., & Jensen, T. S. (2016). Neuropathic pain: An updated grading system for research and clinical practice. Pain, 157, 1599–1606. https://doi.org/10.1097/j.pain.0000000000000492

Gagliese, L., & Melzack, R. (2000). Age differences in nociception and pain behaviours in the rat. Neuroscience and Biobehavioral Reviews, 24, 843–854. https://doi.org/10.1016/S0149-7634(00)00041-5

Garcia-Larrea, L. (2002). Laser-evoked potential abnormalities in central pain patients: The influence of spontaneous and provoked pain. Brain, 125, 2766–2781. https://doi.org/10.1093/brain/awf275

Garcia-Larrea, L., & Hagiwara, K. (2019). Electrophysiology in diagnosis and management of neuropathic pain. Revue Neurologique, 175, 26–37. https://doi.org/10.1016/j.neurorl.2018.09.015

Garcia-Larrea, L., Peyron, R., Laurent, B., & Mauguière, F. (1997). Association and dissociation between laser-evoked potentials and pain perception. NeuroReport, 8, 3785–3789. https://doi.org/10.1097/00001756-199712010-00026

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

Hüllemann, P., Mahn, F., Shao, Y. Q., Watfeh, R., Wasner, G., Binder, A., & Baron, R. (2013). Repetitive ipsilateral painful A-delta fibre stimuli induce bilateral LEP amplitude habituation. European Journal of Pain, 17, 1483–1490. https://doi.org/10.1002/j.1532-2149.2013.00353.x

Hüllemann, P., Shao, Y. Q., Manthey, G., Binder, A., & Baron, R. (2016). Central habituation and distraction alter C-fibre-mediated laser-evoked potential amplitudes. European Journal of Pain, 20, 377–385. https://doi.org/10.1016/j.ejp.735

Hüllemann, P., von der Brelie, C., Manthey, G., Dürstehöft, J., Helmers, A. K., Synowitz, M., & Baron, R. (2017a). Reduced laser-evoked potential habituation detects abnormal central pain processing in painful radiculopathy patients. European Journal of Pain, 21, 918–926. https://doi.org/10.1002/j.epj.994

Hüllemann, P., von der Brelie, C., Manthey, G., Dürstehöft, J., Helmers, A. K., Synowitz, M., Gierthmüelen, J., & Baron, R. (2017b). Laser-evoked potentials in painful radiculopathy. Clinical Neurophysiology, 128, 2292–2299. https://doi.org/10.1016/j.clinph.2017.09.100
Hüllemann, P., Watfeh, R., Shao, Y. Q., Nerdal, A., Binder, A., & Baron, R. (2015). Peripheral sensitization reduces laser-evoked potential habituation. *Neurophysiologie Clinique, 45*, 457–467. https://doi.org/10.1016/j.neucli.2015.10.088

Lorenz, J., Hansen, H. C., Kunze, K., & Bromm, B. (1996). Sensory deficits of a nerve root lesion can be objectively documented by somatosensory evoked potentials elicited by painful infrared laser stimulations: A case study. *Journal of Neurology, Neurosurgery and Psychiatry, 61*, 107–110. https://doi.org/10.1136/jnnp.61.1.107

Price, D. D., Hu, J. W., Dubner, R., & Gracely, R. H. (1977). Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. *Pain, 3*, 57–68. https://doi.org/10.1016/0304-3959(77)90035-5

Procacci, P., Bozza, G., Buzzelli, G., & Della Corte, M. (1970). The cutaneous pricking pain threshold in old age. *Gerontologia Clinica, 12*, 213–218. https://doi.org/10.1159/000245281

Quante, M., Hauck, M., Gromoll, M., Hille, E., & Lorenz, J. (2007). Dermatomal laser-evoked potentials: A diagnostic approach to the dorsal root. Norm data in healthy volunteers and changes in patients with radiculopathy. *European Spine Journal, 16*, 943–952. https://doi.org/10.1007/s00586-006-0253-2

Rekatsina, M., Paladini, A., Piroli, A., Zis, P., Pergolizzi, J. V., & Varrassi, G. (2020). Pathophysiologic approach to pain therapy for complex pain entities: A narrative review. *Pain and Therapy, 9*, 7–21. https://doi.org/10.1007/s40122-019-00147-2

Scharffler, K., He, W., Passier, P., Tracy, K., Fakhoury, A., & Paul, J. (2018). A phase I, randomized, double-blind, laser-evoked potential study to evaluate the analgesic/anti-hyperalgesic effect of ASP9226, a state-dependent N-type voltage-gated calcium channel inhibitor, in healthy male subjects. *Pain Med (United States), 19*, 2246–2255. https://doi.org/10.1093/pm/pnx338

Scharffler, K., Nicolas, L. B., Borta, A., Brand, T., Reitmeir, P., Roebling, R., & Scholpp, J. (2017). Investigation of the predictive validity of laser-EPs in normal, UVB-inflamed and capsaicin-irritated skin with four analgesic compounds in healthy volunteers. *British Journal of Clinical Pharmacology, 83*, 1424–1435. https://doi.org/10.1111/bcp.13247

Schereth, T. (2020). S2k-Leitlinie: Diagnose und nicht interventionelle Therapie neuropathischer SchmerzenS2k Guidelines: Diagnosis and non-interventional treatment of neuropathic pain. *Dg neurologie, 3*, 21–40. https://doi.org/10.1007/s42451-019-00139-8

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

Truini, A., Galeotti, F., Romaniello, A., Virtuoso, M., Iannetti, G. D., & Cruccu, G. (2005). Laser-evoked potentials: Normative values. *Clinical Neurophysiology, 116*, 821–826. https://doi.org/10.1016/j.clinph.2004.10.004

Truini, A., Haanpää, M., Zucchi, R., Galeotti, F., Iannetti, G. D., Romaniello, A., & Cruccu, G. (2003). Laser-evoked potentials in post-herpetic neuralgia. *Clinical Neurophysiology, 114*, 702–709. https://doi.org/10.1016/S1388-2457(03)00009-9

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. *European Journal of Pain, 14*, 222–225. https://doi.org/10.1016/j.ejpain.2009.05.001

Valeriani, M., De Tommaso, M., Restuccia, D., Le Pera, D., Guido, M., Iannetti, G. D., Libro, G., Truini, A., Di Trapani, G., Puca, F., Tonali, P., & Cruccu, G. (2003). Reduced habituation to experimental pain in migraine patients: A CO₂ laser evoked potential study. *Pain, 105*, 57–64. https://doi.org/10.1016/S0304-3959(03)00137-4

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APPENDIX 1

Results of statistical analysis for Experiment A (young controls), course of latencies, amplitudes and pain ratings over time. Each block has been compared with consecutive blocks, respectively. Only significant results are shown.

|                      | Conventional analysis | HTR analysis | HTR analysis |
|----------------------|-----------------------|--------------|--------------|
|                      | −4 blocks-             | −12 blocks-  | −6 blocks-   |
|                      | n = 34                 | n = 34       | n = 34       |
| Experiment A         |                       |              |              |
| N2                   | ns^a                  | ns^a         | ns^a         |
| P2                   | ns^a                  |              |              |
| N2P2                 | $F_{2.390; 78.857} = 44.22; p < 0.001^a$ | $F_{3.976; 197.210} = 21.872; p < 0.001^a$ | $F_{3.165} = 18.469; p < 0.001^a$ |
| Post hoc             |                       |              |              |
| • p < 0.001 for 1 versus 2–4 resp. | • p = 0.002 for I versus III | • p < 0.001 for I versus III, IV, V and VI resp. | • p = 0.001 for I versus III, IV, V and VI resp. |
| • p < 0.01 for 2 versus 3 | • p = 0.009 for I versus VII | • p = 0.013 for II versus III | • p = 0.001 for II versus IV |
| • p < 0.001 for 2 versus 4 | • p = 0.010 for I versus VIII, IX, X, XI and XII resp. | • p < 0.001 for II versus IV | • p < 0.001 for II versus IV and VI resp. |
| Other comparisons ns | • p = 0.002 for II versus IV | • p < 0.001 for II versus V, VI, VIII-XI resp. | • p = 0.002 for II versus IV |
|                      | • p = 0.009 for III versus VIII | • p = 0.002 for III versus IX | • p < 0.001 for II versus IV |
|                      | • p < 0.001 for III versus X and XI resp. | • p = 0.001 for III versus X and XI resp. | • p = 0.001 for II versus V and VI resp. |
|                      | • p < 0.001 for III versus XII | • p = 0.002 for III versus XII | • Other comparisons ns |
|                      | • p = 0.008 for IV versus VIII | • p = 0.001 for IV versus VIII | • Other comparisons ns |
|                      | • p = 0.010 for IV versus IX | • p = 0.010 for IV versus IX | • Other comparisons ns |
|                      | • p = 0.014 for IV versus XI | • p = 0.014 for IV versus XI | • Other comparisons ns |
|                      | • p = 0.021 for V versus VIII | • p = 0.021 for V versus VIII | • Other comparisons ns |
|                      | • p = 0.026 for V versus IX | • p = 0.026 for V versus IX | • Other comparisons ns |
|                      | • p = 0.009 for V versus X | • p = 0.009 for V versus X | • Other comparisons ns |
|                      | • p = 0.033 for V versus XI | • p = 0.033 for V versus XI | • Other comparisons ns |
| Other comparisons ns | • Other comparisons ns | • Other comparisons ns | • Other comparisons ns |

Abbreviations: HTR, high temporal resolution; ns, not significant; resp., respectively.^a Results of repeated-measures ANOVA; post hoc being post-hoc Bonferroni correction for pair-wise comparisons.
APPENDIX 2

Results of statistical analysis for Experiment B (old controls), course of latencies, amplitudes and pain ratings over time. Each block has been compared with consecutive blocks, respectively. Only significant results are shown.

|               | Conventional analysis | HTR analysis       | HTR analysis       |
|---------------|-----------------------|--------------------|--------------------|
|               | −4 blocks-            | −12 blocks-        | −6 blocks-         |
|               | n = 24                | n = 15             | n = 17             |

**Experiment B**

| Component | Conventional analysis | HTR analysis       | HTR analysis       |
|-----------|-----------------------|--------------------|--------------------|
| N2        | ns<sup>a</sup>        | ns<sup>a</sup>     | ns<sup>a</sup>     |
| P2        | ns<sup>a</sup>        |                    |                    |
| N2P2      | $F_{3,69} = 19.912; p < 0.01<sup>a</sup>$ Decrease | $F_{11,154} = 7.870; p < 0.01<sup>a</sup>$ Decrease | $F_{5,80} = 4.244; p = 0.002<sup>a</sup>$ Decrease |
|           | Post hoc              | Post hoc           | Post hoc           |
|           | • p = 0.001 for 1 versus 3 | • p < 0.05 for I versus IX, X, XI and XII, resp. | Comparisons ns |
|           | • p < 0.001 2 versus 4 | • p = 0.001 for II versus IX | |
|           | • p < 0.001 for 1 versus 4 | • p < 0.05 for II versus X and XII resp. | |
|           | Other comparisons ns  | • p = 0.046 for IV versus XII | |
|           |                       | • p = 0.001 for V versus XII | |
|           |                       | Other comparisons ns | |

| Pain      | $F_{1.923; 44.219} = 14.569; p < 0.001<sup>a</sup>$ Decrease | $F_{3.301; 46.215} = 4.315, p = 0.007<sup>a</sup>$ Decrease | $F_{2.181; 34.895} = 5.159, p = 0.009^a$ Decrease |
|           | Post hoc              | Post hoc           | Post hoc           |
|           | • p = 0.019 for 1 versus 2 | • p = 0.019 for 1 versus 2 | • p = 0.036 for II versus IV |
|           | • p = 0.001 for 1 versus 3 | • p < 0.001 1 versus 4 | Other comparisons ns |
|           | • p < 0.001 for 1 versus 4 | • p = 0.001 2 versus 4 | |
|           | Other comparisons ns  | Other comparisons ns | |

Abbreviations: HTR, high temporal resolution; ns, not significant; resp., respectively.<sup>a</sup> Results of repeated-measures ANOVA; post hoc being post-hoc Bonferroni correction for pair-wise comparisons.
### APPENDIX 3

Results of statistical analysis for Experiment C (radiculopathy patients), course of latencies, amplitudes and pain ratings over time. Each block has been compared with consecutive blocks, respectively. Only significant results are shown.

|                | HTR analysis |            | HTR analysis |            |
|----------------|--------------|------------|--------------|------------|
|                | −12 blocks-  | n = 14     | −6 blocks-   | n = 18     |
| Experiment C   |              |            |              |            |
| N2             | ns\(^a\)     |            | ns\(^a\)     |            |
| P2             | ns\(^a\)     |            | ns\(^a\)     |            |
| N2P2           |               |            |              | F\(_{5.85}\) = 6.781, p < 0.001\(^a\) decrease | post hoc  |
|                | F\(_{11.143}\) = 6.698, p < 0.001\(^a\) decrease | post hoc  |
|                | ● p = 0.049 for I versus VIII | post hoc  |
|                | ● p = 0.008 for I versus XI and XII resp. | post hoc  |
|                | ● p = 0.007 for II versus VIII | post hoc  |
|                | ● p = 0.049 for II versus XI | post hoc  |
|                | Other comparisons ns            | post hoc  |
| Pain           | F\(_{4.204; 54.652}\) = 2.636, p = 0.041\(^a\) decrease | post hoc  |
|                | post hoc          | post hoc  |
|                | Comparisons ns    | post hoc  |

Abbreviations: HTR, high temporal resolution; ns, not significant; resp., respectively.\(^a\) Results of repeated-measures ANOVA; post hoc being post-hoc Bonferroni correction for pair-wise comparisons.