Somatosensory spatial attention modulates amplitudes, latencies, and latency jitter of laser-evoked brain potentials

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Submitted 23 January 2015; accepted in final form 10 February 2015

Franz M, Nickel MM, Ritter A, Miltner WH, Weiss T. Somatosensory spatial attention modulates amplitudes, latencies, and latency jitter of laser-evoked brain potentials. J Neurophysiol 113: 2760–2768, 2015. First published February 11, 2015; doi:10.1152/jn.00070.2015.—Several studies provided evidence that the amplitudes of laser-evoked potentials (LEPs) are modulated by attention. However, previous reports were based on across-trial averaging of LEP responses at the expense of losing information about intertrial variability related to attentional modulation. The aim of this study was to investigate the effects of somatosensory spatial attention on single-trial parameters (i.e., amplitudes, latencies, and latency jitter) of LEP components (N2 and P2). Twelve subjects participated in a sustained spatial attention paradigm while noxious laser stimuli (left hand) and noxious electrical stimuli (right hand) were sequentially delivered to the dorsum of the respective hand with noxious air puffs randomly interspersed within the sequence of noxious stimuli. Participants were instructed to mentally count all stimuli (i.e., noxious and nonnoxious) applied to the attended body location. Laser stimuli, presented to the attended hand (ALS), elicited larger single-trial amplitudes of the N2 component compared with unattended laser stimuli (ULS). In contrast, single-trial amplitudes of the P2 component were not significantly affected by spatial attention. Single-trial latencies of the N2 and P2 were significantly smaller for ALS vs. ULS. Additionally, the across-trial latency jitter of the N2 component was reduced for ALS. Conversely, the latency jitter of the P2 component was smaller for ULS compared with ALS. With the use of single-trial analysis, the study provided new insights into brain dynamics of LEPs related to spatial attention. Our results indicate that single-trial parameters of LEP components are differentially modulated by spatial attention.

spatial attention; laser-evoked potentials; single-trial analysis; latency jitter

IT IS WELL KNOWN THAT COGNITIVE factors, such as emotion, expectation, hypnotic suggestions, and attention, modify the processing of pain (Villemure and Bushnell 2002). Directing attention toward or away from a painful stimulus modifies both the subjective intensity of pain and the cortical processing of noxious stimuli (Miltner et al. 1989; Peyron et al. 1999). The attentional modulation of cortical nociceptive processing has been extensively studied, e.g., by means of laser-evoked potentials (LEPs) (for review, see Lorenz and Garcia-Larrea 1997; Siedenberg and Treede 1996; Yamashiki et al. 1999). Since these studies compared LEPs of attention vs. distraction conditions, the observed results can be accounted for by an intermodal attention effect which modifies both the N2 and P2 amplitudes of the LEP responses (Legrain et al. 2002; Lorenz and Garcia-Larrea 2003). Furthermore, the N1 and N2 waves were shown to be sensitive to spatial attention within the same sensory modality (intramodal attention effect), such that laser stimuli, delivered to the spatially attended body location, elicited larger N1 and N2 amplitudes relative to laser stimuli applied to the unattended body location (Legrain et al. 2002). Attentional modulations of the amplitudes of sensory-evoked brain potentials have also been described in other sensory modalities, such as visual, auditory, and somatosensory (for reviews, see Desimone and Duncan 1995; Eimer and Driver 2001; Hillyard et al. 1998). Hence, this amplitude modulation was suggested as a relevant mechanism of selective attention (Hillyard et al. 1998). In particular, spatial attention may operate by controlling the gain of sensory-evoked responses (Hillyard et al. 1998).

A typical feature of event-related brain signals is trial-to-trial variability in amplitude and latency (i.e., latency jitter), which is especially high in LEP responses (Iannetti et al. 2005). This variability was shown to contain physiologically relevant information on brain dynamics (Jung et al. 2001). It was pointed out that trial-to-trial variability of event-related potentials (ERP) may be modified when cognitive tasks, including selective attention, are involved (Gasser et al. 1983; Trucco et al. 2002). Thus, if intertrial latency jitter of ERP components is systematically modified by experimental conditions, then across-trial averaging may be inadequate and leads to important distortions (i.e., biased amplitudes, morphology) of the averaged ERP (Mouraux and Iannetti 2008); see Fig. 1 for illustration. Previous reports on attentional modulation of LEPs are based on across-trial averaging of LEP responses at the expense of losing information about single-trial brain dynamics. In particular, the spatial attention effect, leading to higher N1 and N2 amplitudes of the averaged LEP responses (Legrain et al. 2002), may be partially attributable to a smaller latency jitter of single-trial LEP responses under attentional focus.

In the present study, we investigated the effects of spatial selective attention on single-trial parameters (i.e., amplitudes,
latencies, and latency jitter) of the LEP vertex complex (N2-P2).
Specifically, we addressed the question of whether spatial selective attention reduces the intertrial latency jitter of LEP components and, if so, whether the difference in the amplitude of averaged LEPs is (partly) due to a difference in latency jitter between the two attentional conditions and not solely due to differences in single-trial amplitudes of attended (ALS) vs. unattended laser stimuli (ULS).

MATERIALS AND METHODS

Subjects. Twelve healthy right-handed volunteers (11 women, mean age ± SD: 24.9 ± 6.4 yr, range: 20–40 yr) were recruited from the Friedrich Schiller University and participated in the study after giving written, informed consent. One subject was excluded from analysis due to poor data quality and a small signal-to-noise ratio. Hence, the presented results are based on 11 subjects (10 women, mean age: 23.5 ± 4.6 yr, range: 20–32 yr). The study was approved by the ethics committee of the Friedrich Schiller University Jena.

Stimuli. Subjects participated in a sustained spatial attention task including noxious laser heat pulses, noxious electrical stimuli and nonnoxious air puffs, which were administered sequentially to the dorsum of both hands (Fig. 2).

Laser pulses were generated by a thulium YAG laser device (pulse duration: 1.0 ms; wavelength: 1.96 μm; beam diameter: 6 mm; Themis laser device, Starmedtec, Erlangen, Germany). Painful laser heat stimuli were applied to the dorsum of the left hand with a mean intensity of 500 mJ (SD = 71.9), ensuring a pinprick-like sensation. To minimize the risk of sensitization and habituation of nociceptors, the laser beam was slightly moved after each laser stimulus. The location of the stimulation site was indicated by a He-Ne pilot laser.

Electrical stimuli consisted of biphasic constant-current square-wave pulses, each lasting 5 ms (DS5, Digitimer, Hertfordshire, UK). Stimuli were delivered by a concentric surface electrode (CE; K2 stimulation electrode, Walter Graphtek, Lübeck, Germany). The CE (central cathode Ø: 0.5 mm; insolation insert Ø: 5 mm; external anode ring Ø: 6 mm) is designed to gain a high-current field density at low-current intensities which confines the depolarization of nerve fibers predominantly to nociceptive nerve terminals within the epidermis (Kaube et al. 2000). The CE was placed centrally on the dorsum of the right hand. Before attaching the electrode, the skin was cleaned with alcohol and gently abraded to reduce impedance level. Electrical stimuli were applied with a mean intensity of 2.9 mA (SD = 2.5). The stimulation with a CE was used, providing a noxious stimulation comparable to the laser stimulation in terms of intensity and quality (pinprick sensation) of pain without requiring a second laser device. However, in our experiment, the CE stimulation may not have been nociceptive specific due to high intensities of stimulation which are known to co-excite large myelinated Aβ fibers (de Tommaso et al. 2011; Kaube et al. 2000; Perchet et al. 2012), unless electrical stimuli are applied with a maximal intensity of twice the perception threshold (Mouraux et al. 2010).

Nonnoxious stimulation was carried out by a computer-controlled air puff stimulator (custom-built) that delivered brief somatosensory air puffs (Hashimoto et al. 1992) via plastic air tubes ending in nozzles. Tactile air puffs were applied to the dorsum of both hands innervated by the radial and ulnar nerve. For each hand, two nozzles were positioned over the prolonged course of the index finger and the little finger centered with regard to the length of the dorsum (Fig. 2). All four nozzles (inner diameter: 4 mm) were placed about 1 cm above the skin surface to avoid direct contact. Stimulus duration was ~300 ms, and air puffs were delivered at a constant pressure of 0.1 bar, eliciting a discernible sensation in every subject. At such intensities, subjects were required to shift attention to the respective hand to clearly detect the occurrence of air puffs.

Fig. 1. Variability of the event-related potential (ERP) component. Differences between experimental conditions in the amplitude of averaged ERP components can be caused by several factors, such as amplitude variation of the ERP component (left), intertrial latency jitter of the ERP component (middle), or a combination of both amplitude variation and intertrial latency jitter (right). When latency jitter is substantial, the mean value of single-trial amplitudes is larger than the peak amplitude of the average ERP. Hence, it is inadequate to compare amplitudes of ERPs averaged over sets of trials with varying degrees of latency jitter (Spencer 2005). The simulated ERP component of single trials (n = 7) is depicted by gray lines, and the corresponding average by a black line.

Fig. 2. A: stimulation sites on the dorsum of each hand. Laser stimuli and electrical stimuli were delivered to the left (area marked in gray) and right hand dorsum, respectively, in pseudorandomized stimulus order. Between two noxious stimuli, a varying interval (1–5) of air puffs was pseudorandomly presented to one or both hands. B: schematic of the stimulus events. The exemplary sequence shows the “attention on left hand” condition, as indicated by the white arrow pointing to the left (bottom), which was presented via computer screen throughout the block. Subjects were instructed to silently count all stimuli at the attended dorsum of the hand. AL, attentional instruction at the start of each block; R, at the end of each block, subjects were requested to report the number of counted targets, as well as to rate the average pain intensity elicited by laser heat stimuli and electrical stimuli, respectively. ISI, interstimulus interval.
Procedure. Participants were seated in a comfortable chair with both forearms placed on arm rests. The distance between the arm rests was 58 cm. To minimize involuntary movements during the experiment, hands were loosely fixated by strips being placed over the proximal phalanges. Both forearms and stimulation hardware were occluded from subject’s view by a screen made of opaque cloth. Acoustic isolation was ensured using earplugs and diffusing white noise through speaker. For safety reasons, participants and experimenters wore laser protective goggle during the experiment (for further safety constraints regarding laser stimulation, see Weiss et al. 1997).

Individual pain thresholds were separately assessed for laser stimuli and electrical stimuli by the method of limits using three ascending and descending series of stimulus intensities. After each stimulus, subjects were requested to provide a rating of their subjective sensation. Perceived intensity was judged on the Ellermeier scale (Ellermeier et al. 1991), ranging from 0 (“no pain”) to 50 (“very severe pain”). For the main experiment, the mean intensity of stimuli (i.e., laser stimuli and electrical stimuli, respectively) was used that elicited pain ratings between 21 and 30 (“medium pain”). Thus the intensities of both types of stimulation (laser and electrical) were matched to produce a similar sensation, saliency, and pain intensity. Subjects were familiarized with the experimental setup during an exemplary stimulus sequence prior to the experiment (10 laser stimuli and 10 electrical stimuli pseudorandomly intermitted by 1 to 5 air puffs).

Each subject participated in one session, consisting of eight blocks with breaks of 90 s after the second, the fourth and the sixth block, amounting to a total session time of ~55 min. In each block, 40 noxious stimuli (20 laser stimuli and 20 electrical stimuli) were applied in a pseudorandomized order with the constraint that, when taking into account only noxious stimuli, no more than 3 noxious stimuli of a given modality (laser vs. electrical) were applied consecutively. Following each noxious stimulus, a varying number of air puffs was pseudorandomly presented (range: 1–5 with probabilities of 0.2, 0.33, 0.33, 0.07, and 0.07, respectively). In contrast to laser stimuli and electrical stimuli, air puffs were applied to both hands using all possible combinations of the four different stimulation sites (n = 2^4 = 16), i.e., simultaneous stimulation of both hands as well as stimulation of a single hand was possible. In each block, the total number of nonoxious air puffs ranged from 90 to 107 (98.8 ± 6.1). The total amount of target stimuli at the attended hand (noxious and nonnoxious stimuli) was ranging from 81 to 111 (100.6 ± 10.6). The experimental conditions (ALS vs. ULS) varied randomly across the blocks. All stimuli were presented with randomly varying interstimulus intervals between 1,000 and 3,000 ms. A schematic illustration of the stimulus sequence is outlined in Fig. 2.

At the beginning of each experimental block, subjects were instructed to focus attention either on the left hand (ALS) or the right hand (ULS). To ensure that the attentional focus was maintained, participants were requested to mentally count all stimuli being perceived at the attended dorsum of the hand (left hand: laser stimuli and air puffs; right hand: electrical stimuli and air puffs). A left- or right-pointing arrow (gray against black background) was displayed at the center of the screen throughout the block, indicating the side (left or right dorsum of the hand) to which attention had to be directed. Participants were instructed to maintain gaze on the arrow to minimize ocular movements. After each block, participants were asked to report the counted number of target stimuli, and to verbally rate the average pain intensity inflicted by laser and electrical stimulation, respectively.

EEG data preprocessing. EEG data were preprocessed using EEGlab 10.2.2.4b, an open source toolbox, running in the MATLAB environment (Mathworks, Natick, MA). Data preprocessing included the following steps. Imported data sets were re-referenced to linked mastoid. Portions of the data contaminated by eye-blinks or ocular movements were corrected using independent component analysis (Jung et al. 2000). Independent components representing ocular artifacts were identified semiautomatically using CORRMAP (Viola et al. 2009) and subtracted from EEG signals. Data segments contaminated by muscle artifacts, linear trends or discontinuities were automatically marked for rejection, applying higher order statistics (Delorme et al. 2007) and visual inspection. Subsequently, continuous EEG data were band-pass filtered between 1 and 30 Hz using finite impulse response filters and down-sampled to 1,000 Hz. Epochs of 1,500 ms were extracted (from −500 to 1,000 ms relative to laser onset), and contaminated data segments were discarded from further analysis. A baseline correction was performed using the prestimulus interval from −200 to 0 ms. Finally, two separate datasets of laser epochs were generated (i.e., ALS vs. ULS) and subjected to single-trial analysis.

Single-trial analysis. Preprocessed epochs (ALS and ULS) were further analyzed to obtain reliable estimates of single-trial latency and amplitude of LEPs (N2 and P2 wave). We performed an automated single-trial analysis using the open source single-trial toolbox “STEP1” (http://www.iannettelab.net/1measure/), running inside the MATLAB environment. This method of analysis is based on two consecutive processing steps: 1) time-frequency wavelet filtering to enhance signal-to-noise ratio of LEPs both in single trials and averages (Hu et al. 2010); and 2) multiple linear regression (MLR) to provide reliable and unbiased single-trial estimates of latency and amplitude of LEP components, as shown previously (Hu et al. 2011). For detailed information, please refer to Hu et al. (2010). Single-trial analysis of LEP components (N2, P2) was confined to data recorded at electrode Cz.

Statistical analysis. Error rate (ER), expressed as percentage, was defined as the modulus of the difference between the reported number of counted stimuli (NC) and the total number of stimuli applied to the attended dorsum of the hand (NA) relative to NA:

\[
ER(\%) = \left| \frac{\text{NC} - \text{NA}}{\text{NA}} \right| \times 100
\]

Two-tailed t-test for paired samples was applied to compare ERs between experimental conditions (attention on left hand vs. attention on right hand). Attentional effects on the perceived intensity of nociceptive stimuli were evaluated by two-way repeated measures analyses of variance (ANOVA) with factors attentional CONDITION (attention on left hand vs. attention on right hand) and STIMULUS TYPE (laser vs. electric stimuli). Normal distribution of variables was checked using Q-Q-plots. One-tailed t-tests for paired samples were conducted for statistical comparison (attended vs. unattended laser stimuli) of LEP parameters obtained by the single-subject average LEP waveforms (amplitude and latency of N2 and P2) and by single-trial LEP waveforms (N2 and P2 amplitude, latency, and latency jitter). Wilcoxon signed-rank tests were applied when variables were not normally distributed. Latency jitter values, as measured by within-subject standard deviation (SD) of single-trial latencies (Kramer et al. 2013) of the respective LEP component, were subjected to log transformation (base 10) prior to statistical testing, since SD parameters usually do not follow a Gaussian distribution. Linear regression analysis was performed to assess the extent to which the spatial attention effect (i.e., enhanced amplitudes of single-subject LEP averages) can be accounted for by single-trial amplitudes, latency jitter or a combination of both. Presence of multicollinearity among predictor variables was assessed using tolerance statistics.

RESULTS

Behavioral data. Subjects performed the counting task with high accuracy, as indicated by the mean ERs of 3.51 ± 5.50%
(attention on left hand) and 4.45 ± 3.58% (attention on right hand) within the experimental conditions which did not significantly differ from each other ($t_{10} = -0.49$, $P = 0.64$). ANOVA for pain intensity ratings of valid blocks revealed no significant main effects of CONDITION and STIMULUS TYPE as well as no interaction effect. On average, neither the pain intensity ratings of ALS (21.73 ± 6.7) and of ULS (22.54 ± 8.86), nor the pain intensity ratings of attended electrocutaneous stimuli (20.32 ± 5.16) and unattended electrocutaneous stimuli (20.83 ± 6.60) differed between conditions. Additionally, overall intensity ratings did not vary between laser stimuli (22.36 ± 7.53) and electrocutaneous stimuli (20.40 ± 5.78). Similar results were revealed by the ANOVA based on intensity ratings of all blocks (see Table 1 for values) by showing no significant main effects of CONDITION and STIMULUS TYPE as well as no significant interaction.

**Effects of spatial attention on individual LEP averages.** The grand average LEP waveforms ($n = 11$) of spatially ALS and ULS are depicted in Fig. 3. We first tested whether spatial attention modulated peak amplitudes and peak latencies (N2 and P2 component) of the individual LEP averages of ALS and ULS at electrode Cz. Results for the LEP components obtained by standard averaging and by averaging of MLR-fitted single trials are summarized in Table 2. Applying standard averaging, spatial attention significantly modulated the N2 amplitude, that is, ALS elicited a larger N2 amplitude than ULS ($-10.4 ± 5.2 \mu V$ vs. $-7.9 ± 4.2 \mu V$; $t_{10} = -2.86$, $P = 0.017$), while the magnitude of P2 amplitudes was not significantly different between ALS and ULS (12.0 ± 5.4 $\mu V$ vs. 10.0 ± 3.4 $\mu V$). There was no significant effect of spatial attention on peak latencies of the N2 (178 ± 16.1 ms vs. 179 ± 8.6 ms) and P2 (290 ± 23.1 ms vs. 296 ± 24.8 ms) between ALS and ULS.

Since testing of our main hypothesis is based on estimated single-trial parameters (peak amplitude and peak latency) of the LEP components, we also investigated whether the spatial attention effect was still present in the individual LEP averages of MLR-fitted single trials, which is a necessary precondition for single-trial analysis (Table 2). Again, we found significantly higher N2 amplitudes for the individual LEP averages of ALS compared with ULS ($-10.2 ± 5.9 \mu V$ vs. $-7.1 ± 3.8 \mu V$; $t_{10} = -3.41$, $P = 0.007$). There was no spatial attention effect for the P2 amplitude ($8.3 ± 4.6 \mu V$ vs. 6.9 ± 3.1 $\mu V$). N2 latencies of the individual averages of the MLR-fitted single trials did not significantly vary between ALS and ULS (180 ± 18.4 ms vs. 183 ± 11.8 ms). However, P2 latency was significantly increased for ULS compared with ALS (288 ± 25.3 ms vs. 303 ± 27.8 ms; $t_{10} = 2.51$, $P = 0.031$).

### Table 1. Task performance and mean pain intensity of ALS and ULS as well as AE and UE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Attention on Left Hand</th>
<th>Attention on Right Hand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALS</td>
<td>UE</td>
</tr>
<tr>
<td>Task performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error rate, %</td>
<td>3.51 ± 5.50</td>
<td>4.45 ± 3.58</td>
</tr>
<tr>
<td>No. of valid blocks*, x4</td>
<td>3.64 ± 0.92</td>
<td>2.03 ± 6.60</td>
</tr>
<tr>
<td>Mean pain intensity (Ellermeier, 0–50)</td>
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<td></td>
</tr>
<tr>
<td>Valid blocks</td>
<td>21.73 ± 6.77</td>
<td>20.83 ± 6.60</td>
</tr>
<tr>
<td>All blocks</td>
<td>22.00 ± 6.87</td>
<td>20.86 ± 6.62</td>
</tr>
</tbody>
</table>

Values are mean ± SD. ALS, attended laser stimuli; ULS, unattended laser stimuli; AE, attended electric stimuli; UE, unattended electric stimuli. *A block is considered valid when the respective error rate was <10%.

**Effects of somatosensory-spatial attention on wavelet filtered single-trial LEPs.** To obtain reliable single-trial estimates of the LEP components, we applied a single-trial detection method based on MLR which allows for estimation of single-trial variability of latency, amplitude, and morphology of ERP.

Group data of single-trial LEP estimates (amplitude, latency and latency jitter) for ALS and ULS are summarized in Table 3. Since ERs of the counting task were slightly higher in the “attention on right hand” condition (Table 1), the single-trial analysis of ULS included a smaller number of valid trials compared with ALS (669 (76%) vs. 737 (84%)). However, the difference in the number of valid trials between ALS and ULS was not significant (Table 2). Single-subject values of the mean amplitude, mean latency, and trial-by-trial variability (amplitude variation and latency jitter, expressed as SD) of the N2 and P2 components are displayed in Fig. 4. Estimated single-trial N2 amplitudes were significantly modulated by spatial attention, with higher amplitudes for ALS compared with ULS ($-12.5 ± 6.6 \mu V$ vs. $-9.4 ± 4.7 \mu V$; $t_{10} = -3.25$, $P = 0.009$, Table 3). In contrast, single-trial P2 amplitudes were not significantly different between ALS and ULS (10.6 ± 5.6 $\mu V$ vs. 8.7 ± 3.8 $\mu V$; Table 3). Both N2 and P2 component showed shorter mean latencies for ALS compared with ULS (21.0 ± 5.5 SD vs. 23.1 ± 4.7 SD; $t_{10} = -2.55$, $P = 0.029$). Unexpectedly, we obtained a converse result for the P2 latency jitter which was significantly larger for ALS compared with ULS (26.3 ± 3.6 SD vs. 23.6 ± 2.3 SD; $t_{10} = 2.48$, $P = 0.033$). Figure 5 depicts the frequency distribution of single-trial latencies plotted against the corresponding amplitudes (N2, top row; P2, bottom row) for ALS ($n = 737$) and ULS ($n = 669$).

Furthermore, we conducted three separate linear regression analyses to determine to what extent the difference in amplitude of the averaged N2 component was due to a difference in latency jitter, single-trial amplitudes, or a combination of both, between the two experimental conditions (ALS vs. ULS) (Table 4). Model 1 assessed the amount of variance that could be accounted for by a difference in N2 latency jitter (ALS vs. ULS). When entered as a single predictor, the difference in single-trial latency jitter (ALS vs. ULS) predicted nearly 43% of the variance in the N2 amplitude differences between the averaged LEPs of ALS and ULS ($R^2 = 0.426$, $P = 0.029$). Differences in single-trial N2 amplitudes (ALS vs. ULS) accounted for 96.3% of the variance on its own ($Model 2, R^2 = 0.963$).

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Finally, both variables were entered simultaneously into the regression analysis as predictors (Model 3). The tolerance statistic was 0.58, indicating no signs of serious multicollinearity between the two regressors. Results from the final regression analysis indicate that almost all of the variance is in fact shared with differences in single-trial amplitude, while latency jitter did not account for unique predictive power ($\beta = 0.961$, $P < 0.001$ vs. $\beta = 0.032$, $P = 0.724$, $R^2 = 0.964$).

**DISCUSSION**

In this study, we explored the effects of sustained somatosensory spatial attention on single-trial brain dynamics related to pain processing. 

Fig. 3. Grand average laser-evoked potential waveforms across 11 subjects of spatially attended (ALS; black lines) and unattended (gray lines) laser stimuli (ULS) at midline (Fz, Cz, Pz) and lateral electrodes (C3, C4). Spatial attention to the location of painful laser stimuli significantly enhanced the N2 amplitude at electrode Cz, while no significant difference was observed for the P2 wave (see Table 2).

### Table 2. Peak amplitudes and latencies of the LEP components (N2 and P2) of ALS and ULS obtained by standard averaging and averaging of wavelet filtered single trials fitted by MLR

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALS</th>
<th>ULS</th>
<th>$P$ value</th>
<th>ALS</th>
<th>ULS</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of valid single trials* (%)</td>
<td>67.6 ± 16.9 (84.5)</td>
<td>63.1 ± 18.9 (78.9)</td>
<td>0.824$^A$</td>
<td>67.0 ± 16.7 (83.8)</td>
<td>61.7 ± 20.0 (77.2)</td>
<td>0.764</td>
</tr>
<tr>
<td>Amplitude, $\mu V$</td>
<td>N2 (Cz)</td>
<td>-10.4 ± 5.2</td>
<td>-7.9 ± 4.2</td>
<td>0.017</td>
<td>-10.2 ± 5.9</td>
<td>-7.1 ± 3.8</td>
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<tr>
<td></td>
<td>P2 (Cz)</td>
<td>12.0 ± 5.4</td>
<td>10.0 ± 3.4</td>
<td>0.075$^A$</td>
<td>8.3 ± 4.6</td>
<td>6.9 ± 3.1</td>
</tr>
<tr>
<td>Latency, ms</td>
<td>N2 (Cz)</td>
<td>178 ± 16.1</td>
<td>179 ± 8.6</td>
<td>0.895</td>
<td>180 ± 18.4</td>
<td>183 ± 11.8</td>
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<tr>
<td></td>
<td>P2 (Cz)</td>
<td>290 ± 23.1</td>
<td>296 ± 24.8</td>
<td>0.168</td>
<td>288 ± 25.3</td>
<td>303 ± 27.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. LEP, laser-evoked potential. *For no. of valid single trials, only artifact-free trials of valid blocks (error rate <10%) were included in analysis. Nos. in parentheses are percentage. The mean number of valid single-trials was slightly smaller for the average of multiple linear regression (MLR)-fitted single trials compared with standard averaging, since single-trial estimates could not be obtained for some trials due to poor signal-to-noise ratio, and hence were discarded from further analysis. $^+$Wilcoxon signed-rank test.

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to the cortical processing of noxious laser stimuli. Using a novel method of single-trial analysis that takes into account the across-trial variability of LEPs, we compared single-trial amplitudes and trial-to-trial latency jitter of the N2 and P2 component of spatially ALS and ULS. We observed five main findings. First, ALS elicited significantly larger single-trial amplitudes of the N2 component than ULS. In contrast, the increase of single-trial P2 amplitudes by spatial attention did not reach significance. Second, single-trial latencies of the N2 and P2 were significantly smaller for ALS vs. ULS. Third, the N2 latency jitter was significantly smaller for ALS compared with ULS, whereas the P2 latency jitter was reduced for ULS compared with ALS. Fourth, multiple regression analysis indicated that differences in amplitude of the averaged N2 component between ALS and ULS were mainly explained by the differences in single-trial amplitudes, while differences in latency jitter did not uniquely contribute. Fifth, behavioral results reveal that the intensity of perceived pain was not significantly modulated by somatosensory spatial attention.

The results of the experiment show that allocation of attention to a body location, in this case the dorsum of the hand, evoked enlarged N2 amplitudes relative to ULS. This was substantiated both by standard across-trial averaging and by single-trial analysis. In contrast, while there was a tendency toward higher P2 amplitudes, the effect of spatial attention on the P2 amplitudes did not reach significance using both standard averaging and single-trial analysis. These results are in line with a previous study (Legrain et al. 2002) showing that the N1 and N2 components, but not the P2 component, of averaged LEP responses are sensitive to voluntary shifts of spatial attention within the same sensory modality. Likewise, experiments in both the visual (Hillyard and Munte 1984) and auditory (Hansen and Hillyard 1980) modality revealed that early components of transient sensory-evoked brain potentials are strongly modulated by spatial attention. For instance, studies in the visual domain have shown that amplitudes of early ERP components (P1, peaking between 90 and 140 ms, and the N1, 160–190 ms), recorded over the posterior visual cortex, are enhanced when visual-spatial attention is directed toward the location of the stimulus (Mangun et al. 1993). Additionally, enhanced ERP (P1-N1) amplitudes of attended visual stimuli revealed the same scalp topography and phasic waveform as those of unattended stimuli. These effects were interpreted in the sense that spatial attention acts by controlling the gain of the sensory-evoked neural responses within a particular brain area without altering the spatial pattern or time course of the neural response (Hillyard et al. 1998). Likewise, in our study, the grand average LEP components (N2-P2) of ALS and ULS

Table 3.  Group data of single-trial parameters (amplitude, latency, latency jitter) of the LEP components (N2 and P2) for spatially ALS and ULS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ALS</th>
<th>ULS</th>
<th>P value</th>
<th>ALS</th>
<th>ULS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, μV</td>
<td>−12.5 ± 6.6</td>
<td>−9.4 ± 4.7</td>
<td>0.009</td>
<td>10.4 ± 5.7</td>
<td>8.8 ± 4.4</td>
<td>0.051</td>
</tr>
<tr>
<td>Latency, ms</td>
<td>180 ± 14.9</td>
<td>186 ± 12.7</td>
<td>0.027</td>
<td>287 ± 22.2</td>
<td>297 ± 27.8</td>
<td>0.010</td>
</tr>
<tr>
<td>Latency jitter (SD)†</td>
<td>21.0 ± 5.5</td>
<td>23.1 ± 4.7</td>
<td>0.029</td>
<td>26.3 ± 3.6</td>
<td>23.6 ± 2.3</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Values are mean ± SD; ALS, n = 737 trials (84%); ULS, n = 679 trials (77%). Estimated parameters are based on a MLR method that was applied to the wavelet filtered single trials. †SD were log transformed before statistical testing.

Fig. 4. Mean single-trial latencies (top) and amplitudes (bottom) of the N2 (left) and P2 (right) component of ALS (black squares) and ULS (white squares) for each subject. Square symbols represent individual means. Error bars represent trial-to-trial variation (latency jitter and amplitude variation), expressed as SD. Subjects were sorted according to their mean single-trial N2 latency in ascending order.
intervening nonnoxious stimuli (air puffs). Thus it is unlikely pseudorandom sequence of noxious (electrical and laser) and short interstimulus intervals between 1 and 3 s) by using a could potentially account for differences in amplitude of LEP modality switching and task switching (Spence et al. 2002) that Legrain et al. (2002), we eliminated possible confounds like attended dorsum of the hand. Hence, similar to the study of stimuli (noxious and nonnoxious) that were applied to theously focused on the somatosensory modality and counted all changes in task-related arousal (Legrain et al. 2002; Lorenz and Garcia-Larrea 2003), these experiments compared LEPs of attended vs. distracted stimulus conditions, i.e., when attention was either directed to the laser stimulus or distracted toward other sensory modalities or tasks. Therefore, modulation of the N2-P2 amplitude was related to an inter-
distracted toward other sensory modalities or tasks. Therefore,

had the same scalp topography (maximal at Cz) and the same phasic waveform, as can be seen in Fig. 3. Taken together, one
equation of spatial attention seems to amplify the LEP responses of the N1 (Legrain et al. 2002) and N2 components. In contrast, the first studies investigating the attentional effects on nociceptive processing reported enhanced LEP amplitudes of the N2-P2 complex (Beydoun et al. 1993; Friederich et al. 2001; Garcia-Larrea et al. 1997; Siedenberg and Treede 1996; Yamasaki et al. 1999). As pointed out by Legrain et al. (2002) and Lorenz and Garcia-Larrea (2003), these experiments compared LEPs of attended vs. distracted stimulus conditions, i.e., when attention was either directed to the laser stimulus or distracted toward other sensory modalities or tasks. Therefore, modulation of the N2-P2 amplitude was related to an inter-modal attentional effect, differences in task difficulty, or changes in task-related arousal (Legrain et al. 2002; Lorenz and Garcia-Larrea 2003). In contrast to these interpretations, there are several reasons to suggest that the findings of the current study are due to shifts in spatial attention. First, in both experimental conditions (ALS vs. ULS), participants continuously focused on the somatosensory modality and counted all stimuli (noxious and nonnoxious) that were applied to the attended dorsum of the hand. Hence, similar to the study of Legrain et al. (2002), we eliminated possible confounds like modality switching and task switching (Spence et al. 2002) that could potentially account for differences in amplitude of LEP components. Second, stimuli were delivered at a high rate (i.e., short interstimulus intervals between 1 and 3 s) by using a pseudorandom sequence of noxious (electrical and laser) and intervening nonnoxious stimuli (air puffs). Thus it is unlikely that subjects were able to attend to both hands and to correctly count the number of stimuli presented at the requested hand, albeit we did not test this separately. Third, both types of noxious stimuli were matched with regard to sensation (pin-pricking) and pain intensity. Taken together, both experimental conditions (ALS vs. ULS) were the same, except for the focus of spatial attention.

Conversely to standard averaging, single-trial analysis disclosed an accelerated processing of ALS, which was indicated by shorter mean latencies of the N2 and P2 component. This is in line with the literature showing shorter reaction times when spatial attention was allocated toward noxious stimuli (Van Damme and Legrain 2012; Van Ryckeghem et al. 2011). Furthermore, single-trial analysis indicates that spatial attention also modulated the latency jitter of the LEP components (N2, P2). Accordingly, the latency jitter of the N2 was smaller for ALS vs. ULS while the opposite was observed for the P2 component with a smaller latency jitter for ULS vs. ALS. A typical feature of long-latency ERP components is across-trial variability in amplitude and latency (i.e., latency jitter) (Mouraux and Iannetti 2008), which is especially high in LEP responses (Hu et al. 2011; Iannetti et al. 2005). As pointed out by several authors, across-trial variability of ERP may be modified when cognitive tasks or selective attention are involved (Gasser et al. 1983; Truccolo et al. 2002) and may also represent physiologically relevant information. To study the degree of latency jitter, we used the mean SD of single-trial latencies which has previously been shown to be a sensitive measure of across-trial variability (Kramer et al. 2013). Our findings suggest that spatial attention differentially affects the timing consistency of neural population responses related to the N2 and P2 component. At present, we can only speculate about the mechanisms leading to a modulation of the latency jitter and its physiological relevance. The changes in latency jitter may be due to modulations in neural excitability or synaptic efficacy. In support of this view, it was demonstrated that tactile spatial attention enhances stimulus-induced gamma-band activity (60–95 Hz) in the primary sensory cortex (Bauer et al. 2006). Similarly, gamma-band oscillations have also been observed in response to painful stimuli (Gross et al. 2007), were sensitive to attention (Hauk et al. 2007; Tiemann et al. 2010), and a direct correlate of subjective pain intensity (Zhang

![Figure 5](http://example.com/figure5.png)

**Fig. 5.** Two-dimensional histograms depicting the frequency distribution (absolute values) of single-trial latencies (x-axis) and amplitudes (y-axis) of the N2 wave (top) and P2 wave (bottom) for ALS (left) and ULS (right). Histograms contain all artifact-free trials of the valid blocks: ALS, 737 trials (84%); ULS, 679 trials (77%). Since mean single-trial latencies of the N2 and P2 component differed between subjects (see Fig. 5), we employed mean-corrected single-trial latencies for illustration purposes. For each subject, single-trial latencies were adjusted by subtracting the subject’s mean latency and adding the group mean latency of the respective component. Note N2 single-trial latencies of ALS are more densely distributed (smaller latency jitter) compared with those of ULS. All histograms have a bin width of 5 ms (x-axis) and 4.5 μV (y-axis).
significant smaller for ALS vs. ULS, while the P2 latency smaller for ALS vs. ULS. Moreover, the N2 latency jitter was smaller for ULS vs. ALS. The results indicate that directing attention toward a body site, in this case the dorsum of the hand, differentially affects single-trial parameters (i.e., amplitude, latency, and latency jitter) of the LEP components (N2, P2).

GRANTS
The work reported in this article was supported by funding from the BMBF (German Federal Ministry of Education and Research: 01EC1003B).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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J Neurophysiol • doi:10.1152/jn.00070.2015 • www.jn.org