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The primary somatosensory cortex contributes to the latest part of the cortical response elicited by nociceptive somatosensory stimuli in humans

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ABSTRACT

Nociceptive laser pulses elicit temporally-distinct cortical responses (the N1, N2 and P2 waves of laser-evoked potentials, LEPs) mainly reflecting the activity of the primary somatosensory cortex (S1) contralateral to the stimulated side, and of the bilateral operculoinsular and cingulate cortices. Here, by performing two different EEG experiments and applying a range of analysis approaches (microstate analysis, scalp topography, single-trial estimation), we describe a distinct component in the last part of the human LEP response (P4 wave). We obtained three main results. First, the LEP is reliably decomposed in four main and distinct functional microstates, corresponding to the N1, N2, P2, and P4 waves, regardless of stimulus territory. Second, the scalp and source configurations of the P4 wave follow a clear somatotopical organization, indicating that this response is likely to be partly generated in contralateral S1. Third, single-trial latencies and amplitudes of the P4 are tightly coupled with those of the N1, and are similarly sensitive to experimental manipulations (e.g., to crossing the hands over the body midline), suggesting that the P4 and N1 may have common neural sources. These results indicate that the P4 wave is a clear and distinct LEP component, which should be considered in LEP studies to achieve a comprehensive understanding of the brain response to nociceptive stimulation.

Introduction

The electroencephalographic (EEG) responses elicited by intense laser heat pulses that selectively excite nociceptive free nerve endings in the epidermis (Bromm and Treede, 1984) are widely used to investigate the peripheral and central processing of nociceptive sensory input (Iannetti et al., 2003; Treede et al., 2003). Such laser-evoked potentials (LEPs) are mediated by the activation of type-II Aδ mechano-heat nociceptors (Treede et al., 1995) and spinothalamic neurons in the anterolateral quadrant of the spinal cord (Treede, 2003), and currently represent the best available tool to assess the spinothalamic function in patients (Haanpaa et al., 2011).

LEPs are composed of three main transient responses detected in the time domain (Carmon et al., 1976). The earliest response is a negative wave (N1) maximal over the central–temporal region contralateral to the stimulated side (Treede et al., 1988) and suggested to mainly reflect the activity of the operculoinsular (Garcia-Larrea et al., 2003) and the primary somatosensory cortices contralateral to the stimulated side (Tarkka and Treede, 1993; Valentini et al., 2012). The N1 wave is followed by a biphasic negative–positive complex (N2 and P2 waves) maximal at the scalp vertex (Bromm and Treede, 1984), and largely reflecting the activity of the bilateral operculoinsular and anterior cingulate cortices (Garcia-Larrea et al., 2003).

While the late N2–P2 waves are functionally similar to other vertex potentials elicited by intense stimuli belonging to non-nociceptive sensory modalities (Mouraux and Iannetti, 2009) and largely reflect saliency-related neural processes possibly related to the detection of relevant changes in the sensory environment (Downar et al., 2000), the early contralateral N1 wave reflects somatosensory-specific activity, more related to the magnitude of the incoming nociceptive input (Lee et al., 2009). Thus, the contribution of somatosensory-specific activities is predominant in the early part of the LEP waveform (i.e., the time interval corresponding to the N1 wave and the onset of the N2 wave; Mouraux and Iannetti, 2009). However, when examining carefully the time course of the respective contribution of somatosensory-specific and multimodal EEG activities to the LEP response (e.g., Fig. 3 in Mouraux and Iannetti, 2009), a small but clear contribution of somatosensory-specific activities to the very last part of the LEP is

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evident. Accordingly, a topographical lateralization of the last part of the LEP response is often anecdotally observed.

Here, using data from two different experiments conducted using multi-channel EEG on 32 healthy subjects (20 for Experiment 1 and 12 for Experiment 2), we describe a distinct component in the last part of the LEP waveform, which we labeled P4. The P4 was isolated as a distinct functional microstate in the LEP response elicited by both hand and foot stimulation. Both topographical distribution and source analysis indicate that primary somatosensory areas contribute at least partly to its generation (Experiment 1). Also, the P4 was not affected by the location of the stimulus in external space, but strongly depended on the somatotopical representation of the stimulated territory (Experiment 2). In addition, its latency and amplitude were significantly more related to the N1 than to the N2 and P2 waves (Experiment 1).

Altogether, both experiments provide compelling evidence of a late, somatosensory-specific component (P4 wave) in the human LEPs.

Materials and methods

Experiment 1

Subjects, experimental paradigm and EEG recording

EEG data were collected from 20 healthy subjects (9 females) aged 27.5 ± 4.4 years (mean ± SD). All subjects gave their written informed consent and were paid for their participation. The local ethics committee approved the procedures.

Nociceptive-specific radiant-heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Italy). At this wavelength the laser pulses activate directly nociceptive terminals in the most superficial skin layers (Baumgartner et al., 2005; Iannetti et al., 2006). Laser pulses were directed at the dorsum of both right and left hands and feet, on a squared area (5 × 5 cm) defined prior to the beginning of the experimental session. An He–Ne laser pointed to the area to be stimulated. The laser pulse was transmitted via an optic fiber and its diameter was set at approximately 5 mm (≈20 mm^2) by focusing lenses. The pulse duration was 3 ms. One energy of stimulation was used in each of the four stimulation sites. The group-average energy values were as follows: right and left hands, 2.35 ± 0.32 J and right and left feet, 2.43 ± 0.32 J. At these energies laser pulses elicited a clear pinprick pain, related to the activation of Aδ fibers. After each stimulus, the laser beam target was shifted by approximately 1 cm in a random direction, to avoid increases of baseline skin temperature, and nociceptor fatigue or sensitization.

Before the EEG recording session, the energy of the laser stimulus was individually adjusted using the method of limits (laser step size: 0.25 J), separately for each of the four stimulated territories (left hand, LH; right hand, RH; left foot, LF; right foot, RF), to ensure that the elicited sensation was in the painful range. During this procedure subjects were asked to report the quality and the intensity of the sensation elicited by each laser pulse using a numerical rating scale (0 = no sensation, 1 = low warmth, 2 = moderate warmth, 3 = high warmth, 4 = non-painful sensation, 5 = mild pain, 6 = moderate pain, 7 = high pain; 8 = unbearable pain; Valentini et al., 2012). The energy of laser stimulation needed to achieve a rating of 6 was used in the following Experiment 1.

Laser-evoked EEG responses were obtained following the stimulation of the dorsum of the right and left hands and feet in four separate blocks, on the same day. The order of the four blocks was balanced across subjects. In each block we delivered 30 laser pulses, using an inter-stimulus interval (ISI) ranging between 5 and 15 s. At the end of each block, subjects were asked to rate the intensity of the painful sensation elicited by the laser stimuli using a visual analog scale ranging from 0 (not painful) to 100 (extremely painful).

Subjects were seated in a comfortable chair in a silent, temperature-controlled room. They wore protective goggles and were asked to focus their attention on the stimuli and relax their muscles. The EEG was recorded using 64 Ag–AgCl scalp electrodes placed according to the International 10–20 system, referenced against the nose. Electro-oculographic (EOG) signals were simultaneously recorded using surface electrodes. Signals were amplified and digitized at a sampling rate of 1000 Hz.

EEG data analysis

EEG data were processed using EEGLAB (Delorme and Makeig, 2004), an open source toolbox running in the MATLAB environment. Continuous EEG data were band-pass filtered between 1 and 30 Hz. EEG epochs were extracted using a window analysis time of 1500 ms (500 ms pre-stimulus and 1000 ms post-stimulus) and baseline corrected using the pre-stimulus interval. Trials contaminated by eye-blinks and movements were corrected using an Independent Component Analysis (ICA) algorithm (Delorme and Makeig, 2004; Jung et al., 2001; Makeig et al., 1997). In all datasets, these independent components (ICs) had a large EOG channel contribution and a frontal scalp distribution. After ICA and an additional baseline correction, EEG epochs were re-referenced to a common average reference.

In each subject, epochs belonging to the same experimental condition were averaged, time-locked to the stimulus onset. This procedure yielded, in each subject, four average waveforms (one waveform for each experimental condition: LH, RH, LF, RF). Single-subject average waveforms were subsequently averaged to obtain group-level waveforms. Group-level scalp topographies were computed by spline interpolation.

For each of the four experimental conditions, group-level scalp topographies were parsed into functional microstates, defined as a temporally consecutive ERP topographies with quasi-stable potential landscape (Lehmann and Skrandies, 1980), using a statistical method based on a modified version of the classical k-means clustering analysis (Pascual-Marqui et al., 1995). The number of microstates was determined using a cross-validation criterion (see Pascual-Marqui et al., 1995 for technical details). Since the aim of the present study was to explore the Aδ-related brain responses, we considered the functional microstates that: (1) were observed within the time-interval from 100 to 500 ms; (2) within this time-interval showed a global field power (GFP) stronger than the GFP within the 0–100 ms time interval (i.e., when there was no LEP); and (3) were observed in all four experimental conditions.

To determine whether the elicited EEG responses were lateralized as a function of the stimulated side, single-subject LEP waveforms elicited by stimulation of the right and left territories were compared using the following procedure. First, for each condition and subject, LEP waveforms were normalized and expressed as z values, by subtracting from each time point the mean of the waveform, and then by dividing the resulting value by the standard deviation of the waveform. Second, a point-by-point paired sample t-test was used to assess the effects of stimulated side for hand and foot stimulation separately. This analysis yielded a time course of P values, representing the significant level of difference between LH and RH, or between LF and RF, for each electrode. Third, to account for multiple comparisons, the significance level (P value) was corrected using a false discovery rate (FDR) procedure (Durka et al., 2004). Fourth, single-subject difference LEP waveforms (LH–RH and LF–RF) were calculated to emphasize the difference of the stimulation in the right and left territories.

To display the differences between LEPs elicited by stimulation of the right and left sides, scalp topographies and corresponding statistical differences (P value) were plotted, in steps of 10 ms, from 390 to 410 ms for hand stimulation, and from 430 to 450 ms for foot stimulation. Scalp topographies of the earliest LEP activity (corresponding to the N1 wave) and their significant differences were also plotted, in steps of 10 ms, from 150 to 170 ms for hand stimulation, and from 190 to 210 ms for foot stimulation.

To define the best approach to identify such late activity as a separate peak in the LEP response elicited by hand stimulation, a bipolar montage using the contralateral central electrode referenced to Fz (Cc–Fz) was used and compared with the bipolar montage using ipsilateral central electrode referenced to Fz (Ci–Fz) (Hu et al., 2010). However, because the scalp distribution of the latest part of the LEP elicited by foot stimulation was not contralateral as that observed in the LEP elicited by hand stimulation, a bipolar montage using CPz referenced to Fz (CPz–Fz) was used.

For each subject, peak latencies and amplitudes of the N1, N2, P2, and P4 waves were measured from the average waveforms (at Cz–avg for N2 and P2, and at Cc–Fz [hand] or CPz–Fz [foot] for N1 and P4 waves). Single-trial peak latencies and amplitudes of the same four waves elicited by hand stimulation were estimated using the combination of wavelet filtering and multiple linear regression with dispersion term ($WF + MLR_d$, as detailed in our previous studies, Hu et al., 2010, 2011). Extracting such single-trial amplitude values allowed testing the trial-by-trial relationships between the P4 wave and the N1, N2, and P2 waves. To account for response variability across subjects, estimated single-trial peak latencies and amplitudes were normalized, in each subject, by subtracting the mean and dividing by the standard deviation (i.e., they were represented as z values). A kernel statistical independence measure, the Hilbert-Schmidt Independence Criterion (HSIC), was employed to evaluate the dependence between the latencies/amplitudes of the P4 wave and those of the N1, N2, and P2 waves (Gretton et al., 2007). Compared with the conventional linear correlation coefficient, the HSIC allows detecting not only linear dependencies but also non-linear dependencies between two sets of variables. The kernel size used in the HSIC tests was set to the median distance between samples of input variables, and the P values were calculated based on 10,000 permutations (Gretton et al., 2007).

To estimate the sources of the latest part of the laser-evoked brain activity, LEP scalp waveforms were projected in source space using LORETA (Pascual-Marqui et al., 1994). This yielded an estimated time course of neural activity sampled by each voxel within the brain. On the basis of the scalp distributions and the statistical maps of the difference between the right and left LEPs, we defined, using a data-driven approach, a seed region constituted by a sphere with a diameter of 30 mm, centered in the contralateral S1 (27, −29, 52 mm; −26, −27, 53 mm; −1, −42, 61 mm; 1, −37, 63 mm for LH, RH, LF, and RF, respectively; Valentini et al., 2012). The time course of each voxel within the seed region was extracted. A principal component analysis (PCA) with Varimax rotation was performed on the time courses of all voxels in the seed region, to select the voxels that mostly contributed to the generation of the latest LEP activities (Michel et al., 2004; Valdes-Sosa et al., 2009). Principal components (PCs) peaking at approximately 380 ms for hand stimulation and 420 ms for foot stimulation (i.e., in the last part of the LEP response) were extracted.

Experiment 2

Subjects, experimental paradigm and EEG recording

In Experiment 2 we analyzed part of a dataset collected in a previous study (Gallace et al., 2011). EEG data were collected from 12 healthy subjects (6 females) aged 31 ± 10.6 years (mean ± SD). All subjects gave their written informed consent and were paid for their participation. The local ethics committee approved the procedures.

Nociceptive stimulation was generated by an infrared Nd:YAP laser, as in Experiment 1. Subjects were comfortably seated in a silent, temperature-controlled room, and wore protective laser-proof goggles. Earplugs and headphones were worn to remove possible auditory cues arising from the operation of the devices. Two large wooden screens placed in front of the subjects occluded their view of both their arms and the experimenter. Before the beginning of the experiment subjects were familiarized with the nociceptive stimulation (5–10 stimuli). Laser pulses were delivered to the dorsum of the right and left hands. During the experiment the subjects were asked to keep their arms either uncrossed or crossed over the midline. The distance between the hands was the same (40 cm) in the 2 conditions. LEPs were recorded in four separate blocks (two crossed and two uncrossed), and their order balanced across subjects. In each block 15 stimuli were delivered to each hand in pseudorandom counterbalanced order, for a total of 30 stimuli per block. Full details of the experimental procedure are reported in Gallace et al. (2011).

The EEG was recorded using 21 Ag–AgCl scalp electrodes placed according to the International 10–20 system, referenced against the nose. The EOG was simultaneously recorded using surface electrodes. Signals were amplified and digitized at a sampling rate of 1024 Hz (SD128, Micromed, Italy).

EEG data analysis

The preprocessing of EEG data was identical to Experiment 1, except that EEG epochs were extracted using a time window of 1000 ms (from 200 ms pre-stimulus to 800 ms post-stimulus).

In each subject, epochs belonging to the same experimental condition were averaged, time-locked to the onset of the stimulus. This procedure yielded, in each subject, two average waveforms (one waveform for each experimental condition: crossed and uncrossed arms). Single-subject average waveforms were subsequently averaged to obtain group-level average waveforms. Group-level scalp topographies were computed by spline interpolation. Latency and baseline-to-peak amplitude of the latest LEP wave at crossed and uncrossed conditions were measured using the bipolar montage Cc–Fz (Hu et al., 2010), and compared using paired sample t-test.

Results

Experiment 1

During the EEG recording session of Experiment 1, all participants described the sensation elicited by the laser stimuli as clearly painful and pricking. The average ratings of the sensation elicited by the laser stimuli were as follows: LH, 66.8 ± 14.2; RH, 60.6 ± 16.1; LF, 66.3 ± 14.7; and RF, 63.7 ± 15.4.

LEP microstates

Fig. 1 shows the dissection of the LEP responses in functional microstates, for all four experimental conditions. The optimal numbers of microstates, estimated using a widely-accepted cross-validation criterion (Pascual-Marqui et al., 1995), were 6, 5, 8, and 9 for LH, RH, LF, and RF, respectively. Among these, four functional microstates were commonly observed in all experimental conditions (see Section 1 of the Supplementary materials for details on testing the correspondence of functional microstates in different experimental conditions). The first common microstate (in yellow, Fig. 1) was located at 123–173 ms, 129–182 ms, 162–229 ms, and 179–220 ms for LH, RH, LF, and RF, respectively ('N1 microstate'). The second common microstate (in blue, Fig. 1) was located at 174–260 ms, 183–256 ms, 230–294 ms, and 221–304 ms for LH, RH, LF, and RF, respectively ('N2 microstate'). The third common microstate (in red, Fig. 1) was located at 261–350 ms, 257–355 ms, 343–407 ms, and 345–410 ms for LH, RH, LF, and RF, respectively ('P2 microstate'). The fourth common microstate (in green, Fig. 1) was located at 351–473 ms, 356–457 ms, 408–497 ms, and 411–510 ms for LH, RH, LF, and RF, respectively ('P4 microstate'). N1 and P4 microstates showed a clear maximum (negative for N1 and positive for P4) on the central–parietal electrodes overlying the *contralateral* hemisphere for hand stimulation, but a clear central distribution (with a maximum between Cz and Pz) for foot stimulation (Fig. 1). N2 and P2 microstates were similar across the four experimental conditions: the N2 microstates extended bilaterally towards temporal regions and the P2 microstates were more centrally-distributed (Fig. 1).

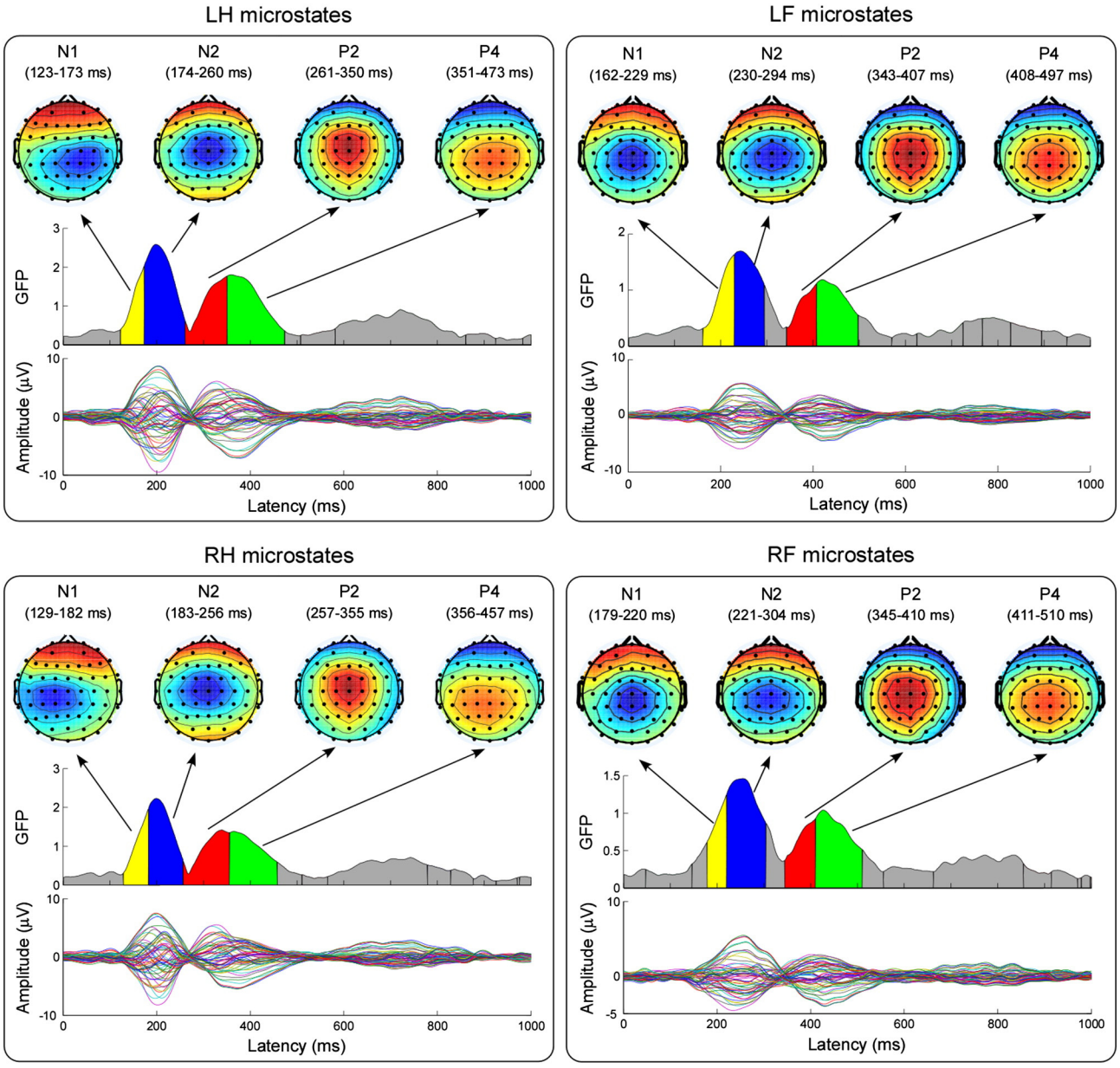

Fig. 1. Dissection of laser-evoked potentials (LEPs) into functional microstates. Group-level waveforms, global field power (GFP), and functional microstates of LEPs elicited by the stimulation of the hand dorsum (left panels) and the foot dorsum (right panels), on the left side (upper panels) and the right side (lower panels). Data were collected from 64 electrodes, in 20 subjects. Signals from different electrodes are plotted in different colors and superimposed. Four functional microstates (marked in yellow, blue, red, and green) were commonly observed in all experimental conditions, located at the time intervals corresponding to the N1, N2, P2, and P4 components. Note that N1 and P4 microstates showed a clear maximum (negative for N1 and positive for P4) on the central–parietal electrodes overlying the hemisphere *contralateral* to the stimulated hand. In contrast the N1 and P4 microstates were centrally-distributed, with a maximum between Cz and Pz for foot stimulation.

LEP time courses and topographies

Fig. 2 shows the grand average LEP waveforms from all 64 channels, together with the scalp topographies at the peak latency of the N1, N2, and P2 waves, and in the time window of the P4 wave (390–410 ms for hand stimulation; 430–450 ms for foot stimulation). Similarly to what observed in the N1 microstate, the scalp topography of N1 elicited by hand stimulation displayed a clear negative maximum on the central–parietal electrodes overlying the hemisphere contralateral to the stimulated side (Fig. 2, left panel). In contrast, and consistently with what we previously observed (Valentini et al., 2012), the scalp topography of the N1 activity elicited by foot stimulation was centrally-distributed, with a maximum between Cz and Pz (Fig. 2, right panel). Scalp topographies of the N2 and P2 peaks were remarkably similar across the four stimulation conditions. As previously described (Kunde and Treede, 1993; Mouraux and Iannetti, 2008), the N2 extended bilaterally towards temporal regions, whereas the P2 was more centrally distributed.

Similarly to what was observed in the topography of the P4 microstate, the last part of the LEP activity elicited by hand stimulation (390–410 ms) displayed a positive amplitude on the central–parietal electrodes overlying the hemisphere *contralateral* to the stimulated side (Fig. 2, left panel). In contrast, the last part of the LEP activity elicited by foot stimulation (430–450 ms) was centrally-distributed, with a maximum between Cz and Pz (Fig. 2, right panel).

Fig. 3 shows the group averages and GFP of difference waveforms obtained from the LEPs elicited by the stimulation of the hand dorsum (LH minus RH) and the foot dorsum (LF minus RF). Scalp maps are shown at both earliest and latest LEP time windows (150–170 ms and

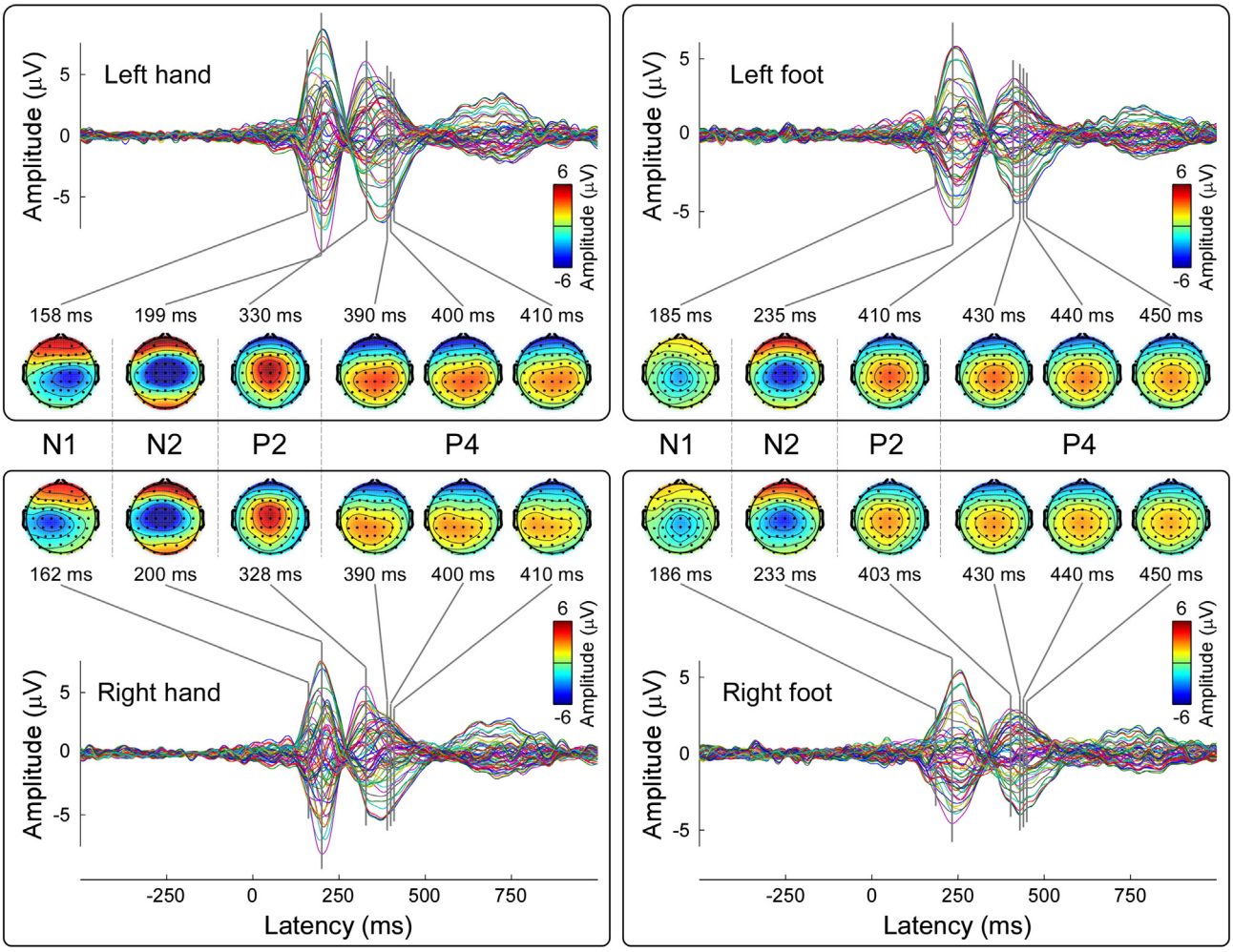

Fig. 2. Time courses and scalp topographies of laser-evoked potentials. Group averages and scalp topographies of LEPs elicited by the stimulation of the hand dorsum (left panels) and the foot dorsum (right panels), on the left side (upper panels) and the right side (lower panels). Data were collected from 64 electrodes, in 20 subjects. Signals from different electrodes are plotted in different colors and superimposed. Scalp topographies are displayed at the peak latency of the N1, N2 and P2 waves, and in the time window of the P4 wave. Note that the scalp topography of the latest part of the response elicited by the stimulation of the hand displays a positive maximum contralateral to the stimulated side, whereas the scalp topography of the response elicited by the stimulation of the foot is centrally distributed.

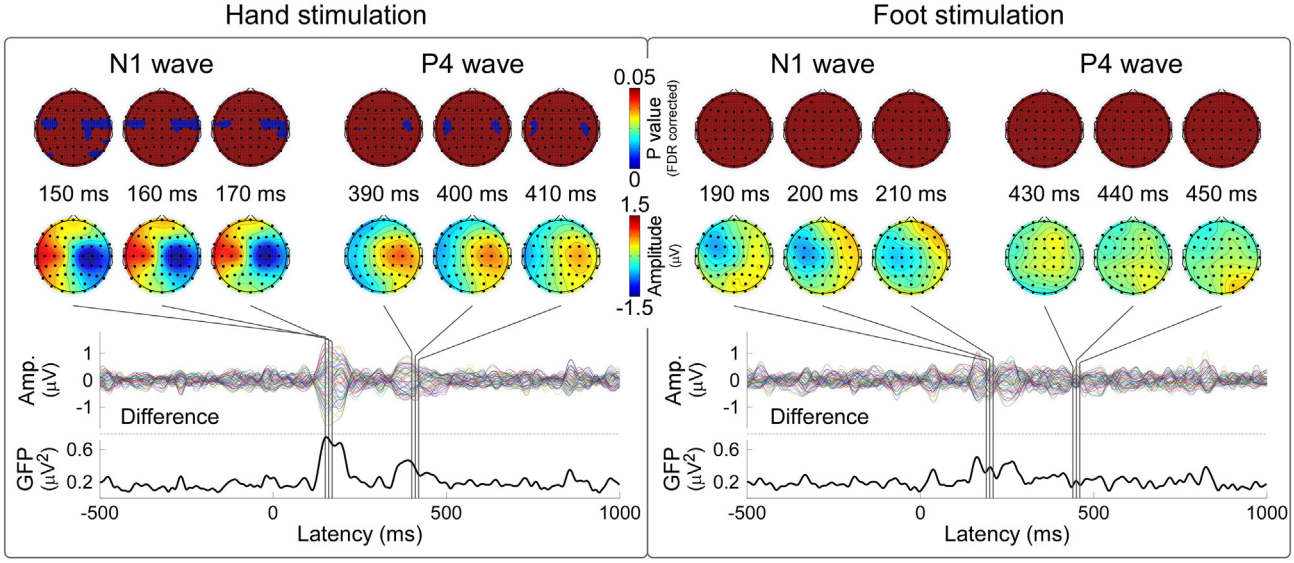

Fig. 3. Statistical comparison of early and late activities of laser-evoked potentials. Group-level waveforms, GFP and scalp topographies of the difference waveform between the right and left LEPs elicited by hand stimulation (left panel) and foot stimulation (right panel). The top row shows the scalp topographies of the statistical comparison between the right and left LEPs, at the latency of the N1 and P4 waves. Note that only LEPs elicited by the stimulation of the right and left hands showed significant differences ($P < 0.05$, FDR corrected), whose scalp topographies were similar in the early and the late part of the waveform. Significant differences were localized bilaterally, on the electrodes overlying the hand area in the somatosensory cortex.

390–410 ms for hand stimulation; 190–210 ms and 430–450 ms for foot stimulation). Whereas the scalp topographies of the earliest and latest part of the LEPs elicited by the stimulation of the right and left hands were significantly different (Fig. 3, left panel), the scalp topographies of the earliest and latest LEP peaks elicited by the stimulation of the right and left feet were not (Fig. 3, right panel). Importantly, the significant differences in both the earliest and latest part of the LEPs elicited by the right and left hand stimulation were bilaterally distributed on the electrodes overlying the primary somatosensory cortex.

Visualizing the latest part of the LEP: the P4 wave

Because of the contralateral scalp distribution of the latest part of the LEP elicited by hand stimulation, a bipolar montage using the contralateral central electrode referenced to Fz (Cc–Fz) was used to detect the lateralized latest peak of the LEP activity (387 ms for LH and 389 ms for RH), to which we refer to as "P4" (Fig. 4, left panel). This montage is also optimal to detect and measure the amplitude of the N1 wave (Hu et al., 2010; Valentini et al., 2012). The resulting waveform was compared with that obtained using the bipolar montage ipsilateral central electrode referenced to Fz (Ci–Fz) (Fig. 4, left panel). Because of the lack of a contralateral scalp distribution in the latest part of the LEP elicited by foot stimulation, a bipolar montage using the central posterior electrode referenced to Fz (CPz–Fz) was used to detect the latest peak of LEP activity (420 ms for LF and 424 ms for RF) (Fig. 4, right panel). Thus, two different bipolar montages are recommended to isolate the P4 wave from the preceding P2 wave: Cc–Fz when stimulating the hand and CPz–Fz when stimulating the foot. It is important to highlight, however, that because of the lack of lateralization of the source activity when the foot is stimulated (Fig. 2), the isolation of the P4 from the P2 is more easily achieved in hand LEP data.

Trial-by-trial relationship between P4 and the other LEP peaks

Single-trial amplitudes of the P4 wave were significantly dependent on all other LEP waves, and the strongest dependence was observed between the P4 and the N1 waves (P4 vs. N1: HSIC $= 15 \times 10^{-4}$, $P < 0.0001$; P4 vs. N2: HSIC $= 13 \times 10^{-4}$, $P < 0.0001$; P4 vs. P2: HSIC $= 12 \times 10^{-4}$, $P = 0.0001$). Single-trial latencies of the P4 wave were only significantly dependent on the latencies of the N1 wave (P4 vs. N1: HSIC $= 12 \times 10^{-4}$, $P = 0.0004$), and not on the latencies of the N2 and P2 waves (P4 vs. N2: HSIC $= 2.9 \times 10^{-4}$, $P = 0.32$; P4 vs. P2: HSIC $= 4.9 \times 10^{-4}$, $P = 0.06$).

Source activity contributing to the latest part of the LEP response

Fig. 5 shows the time course of the responses elicited in the primary somatosensory cortex by laser stimulation, in the four experimental conditions (LH, RH, LF, RF). In all conditions the time courses showed a peak with a latency approximately corresponding to the latest part of the LEP activity (369, 366, 412, and 413 ms for LH, RH, LF, and RF, respectively; Fig. 5, left panel). In addition, PCA with varimax rotation of these time courses yielded, for each stimulation site, one PC showing a clear peak in the latest part of the LEP waveform (378, 384, 420, and 420 ms for LH, RH, LF, and RF, respectively; Fig. 5, right panel). The extracted PCs explained the 14.9% (LH), 10.4% (RH), 16.5% (LF), and 16.0% (RF) of the total variance of the activity of the corresponding S1 source.

Experiment 2

Fig. 6 shows the effect of crossing the arms over the midline on the LEPs elicited by hand stimulation. In both the crossed and uncrossed conditions, the scalp topography of the P4 wave displayed a maximum on the central–parietal electrodes contralateral to the stimulated hand (Fig. 6). That is, when crossing the hands the location of the P4 wave did not change hemisphere. Furthermore, both peak latency (crossed: 388 ± 36 ms; uncrossed: 389 ± 38 ms; $P = 0.87$) and amplitude (crossed: 3.4 ± 2.7 μV; uncrossed: 3.7 ± 3 μV; $P = 0.53$) of the P4 wave were not significantly different in the two conditions. Since in a previous study we demonstrated that only the peak amplitude of the N2 and P2 waves was reduced by crossing the hands over the midline, whereas that of the early N1 wave was not (Gallace et al.,

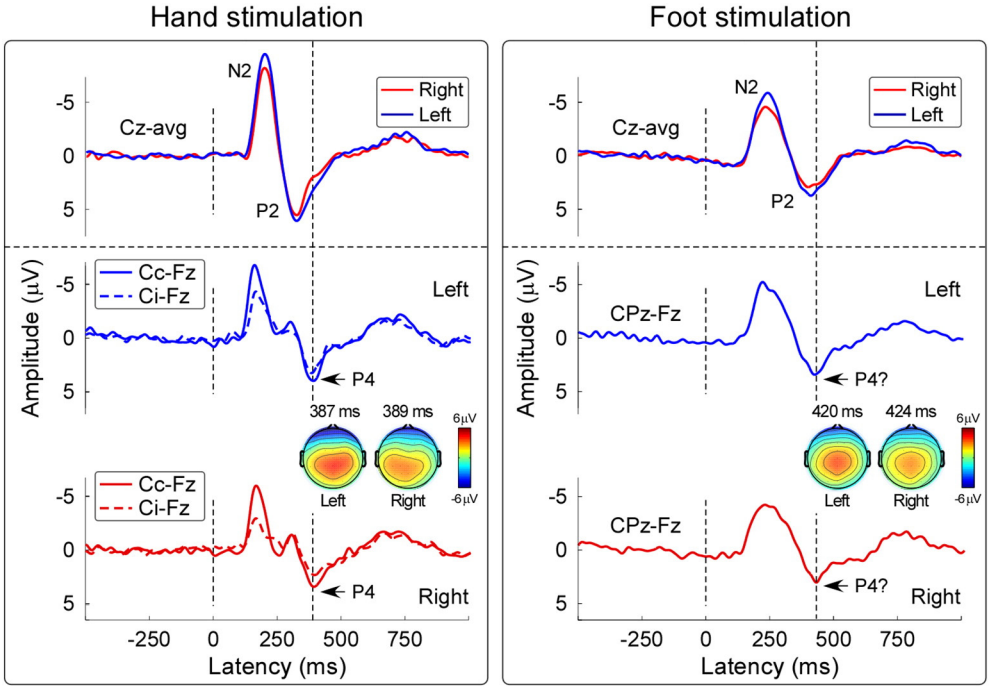

Fig. 4. Detection of the P4 wave of LEPs. Group-level waveforms and scalp topographies of the latest part of the LEPs elicited by stimulation of the hand (left panel) and the foot (right panel), on the left (blue waveforms) and the right side (red waveforms). The top panels show the N2 and P2 waves at electrode Cz. The bottom left panel shows the P4 wave using both the Cc–Fz montage (full line) and the Ci–Fz montage (dashed line). The bottom right panel shows the LEP waveform using the CPz–Fz montage. Note that a distinct P4 wave can be only detected in the LEP elicited by hand stimulation. Note also that the P4 wave is larger on the electrodes contralateral to the stimulated hand.

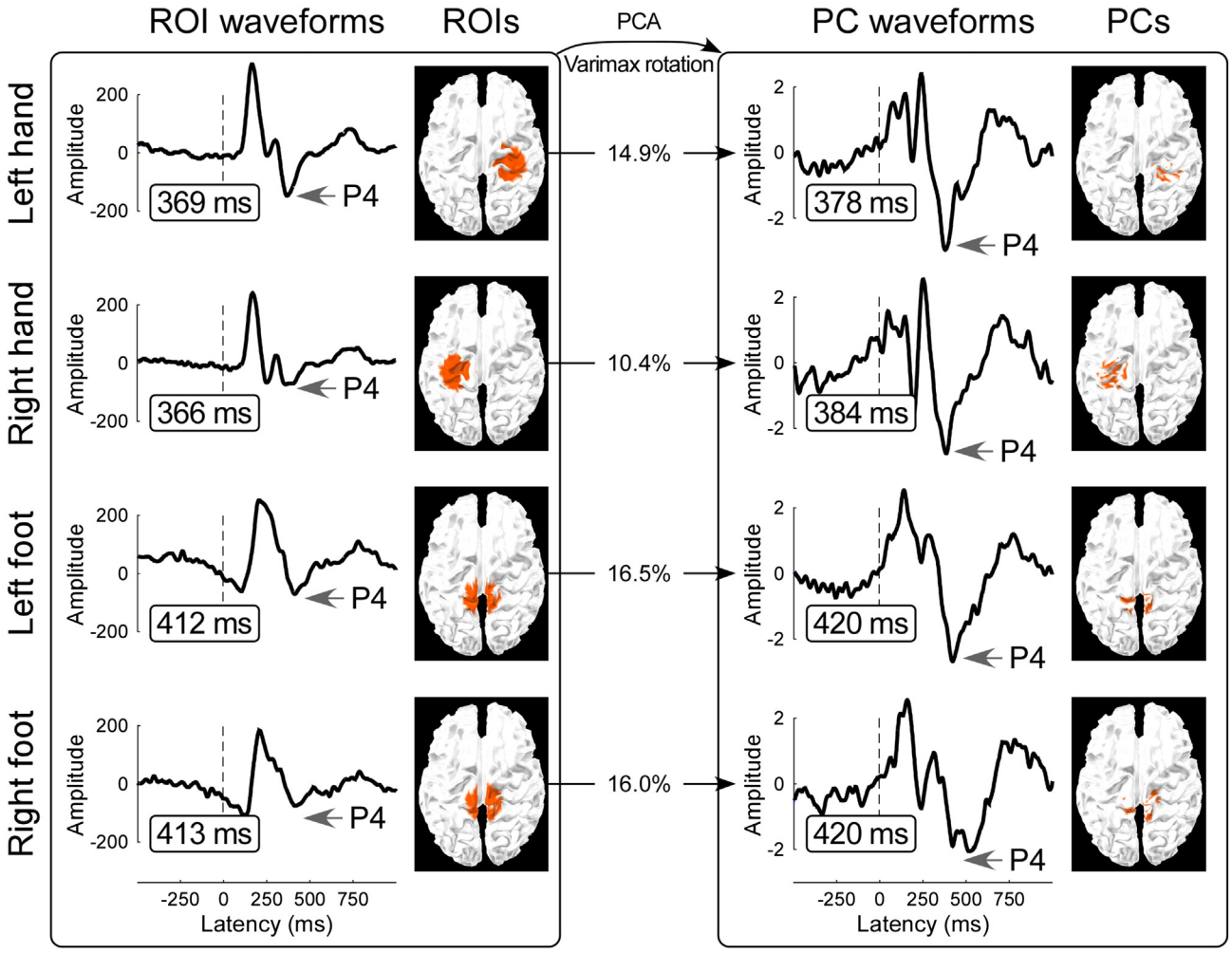

Fig. 5. Time course of the S1 activity elicited by nociceptive laser stimuli. Left panel: time course of the S1 activity elicited by laser stimuli. The peak of the S1 activity corresponding to the latency of the P4 wave is highlighted (LH: 369 ms; RH: 366 ms; LF: 412 ms; RF: 413 ms). Right panel: S1 time courses were decomposed using PCA with varimax rotation. PCs peaking at a latency corresponding to the P4 wave are displayed (LH: 378 ms; RH: 384 ms; LF: 420 ms; RF: 420 ms), together with their location (LH: 24, −36, 56 mm; RH: −27, −31, 56 mm; LF: −1, −46, 60 mm; RF: 3, −39, 62 mm). These PCs explained the 14.9%, 10.4%, 16.5%, and 16.0% of the variance of the S1 source activity.

2011), the result of Experiment 2 shows that (1) the P4 is generated in somatotopically arranged cortical areas (i.e., not in areas coding for the position of stimuli in external space) and (2) similarly to what was observed in the N1 wave, which is partly generated in the primary somatosensory cortex (Frot et al., 2012; Valentini et al., 2012), the P4 is not modulated by the change of stimuli in external space.

Discussion

In this paper we describe a distinct component (P4) in the latest part of the human LEP response. Using datasets from two different EEG experiments we obtained three main results.

First, besides the three main LEP components usually detected in the time domain (N1, N2, and P2), functional microstate analysis reliably identified a clear P4 component, represented as a distinct functional microstate. Such P4 component could be isolated in the human LEP response regardless of stimulus territory (Experiment 1, Fig. 1).

Second, when the LEP was elicited by hand stimulation, the P4 wave was maximal on the central–parietal electrodes contralateral to the stimulated side, whereas the P4 elicited by foot stimulation was centrally-distributed, with a maximum between Cz and Pz (Experiment 1, Fig. 2). This mediolateral somatotopy is compatible with the functional organization of S1, suggesting that this cortical areas contributes to the generation of the P4 wave (Experiment 1, Figs. 1–3). Furthermore, the scalp distribution of the P4 was not modulated by crossing the hands over the midline and thus changing the location of the stimulus in external space (Experiment 2, Fig. 6). Taken together, these observations indicate that the neural activities generating the P4 wave follow a clear somatotopical organization compatible with a source in the S1 contralateral to the stimulated side.

Third, trial-by-trial analysis revealed that N1 and P4 latencies were strongly coupled; in contrast, N1 and P4 latencies were less coupled with the latency of the N2 and P2 (Experiment 1). Furthermore, when the N2 and P2 amplitudes were selectively reduced by crossing the hands over the midline, both N1 and P4 amplitudes were not (Experiment 2, Fig. 6). Taken together, these observations suggest that N1 and P4 may share common neural sources, seem to be similarly sensitive to experimental manipulations, and their trial-by-trial variability is tightly coupled, and rather independent of that of the N2 and P2 sources.

Early somatosensory-specific components in the LEP response

In 1976 Carmon et al. provided the first report of laser-evoked EEG responses in the time domain (i.e., the laser-evoked potentials, LEPs). This was achieved by delivering laser pulses to the hand dorsum while the EEG was recorded using two EEG montages, each with three scalp electrodes (Cz, Cc and Pc; or Cz, Cc and Ci). They observed that “contralateral and ipsilateral responses had amplitudes smaller by 20–40% but shape-identical to the vertex response”, and that the

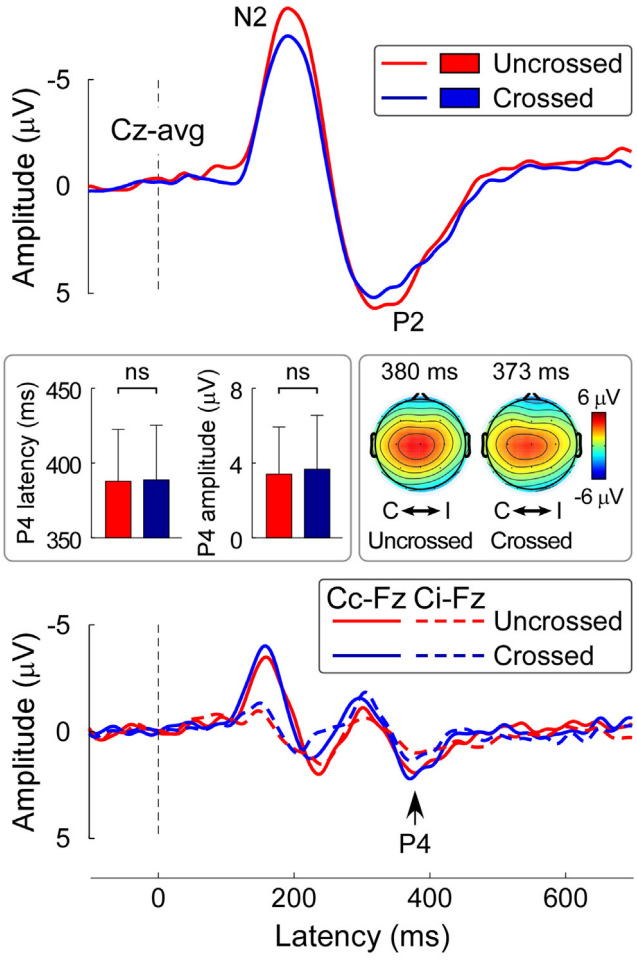

Fig. 6. Effect of crossing the arms on the LEP P4 wave. Group-level waveforms and scalp topographies of the latest part of the LEPs elicited by hand stimulation while arms were crossed over the body midline (blue waveforms) or uncrossed (red waveforms). Data were collected from 21 electrodes, in 12 subjects. The N2 and P2 waves are displayed from the vertex (Cz, top panel), while the latest activity is displayed from the contralateral and ipsilateral central electrodes (Cc–Fz and Ci–Fz, bottom panel). Note that the P4 wave is always maximal on the hemisphere contralateral to the stimulated side, regardless of whether the hands were crossed over the body midline.

amplitude of the biphasic vertex potential (which is now commonly referred to as N2–P2 complex; Treede et al., 2003) recorded at Cc was "slightly smaller" than the responses recorded at Ci (Carmon et al., 1976). Thus, although it is not clear whether the amplitude measures were obtained at the peak of the N2 and P2 waves, or in a time interval capturing part or the whole of the N2–P2 complex, they already suggested that, when stimulating the hand, certain parts of the evoked response were contralateral to the stimulation side. The later introduction of multi-channel EEG and source analysis has allowed describing the detailed scalp topography of the N2 and P2 waves (centrally-distributed and maximal at the vertex; Treede et al., 2003). In a seminal description of the scalp topography of the LEPs elicited by hand and foot stimulation, Treede et al. (1988) showed that when laser stimuli are delivered to the hand, they elicit an early-latency negative wave with a scalp distribution maximal on the contralateral central–parietal electrodes, but that this early contralateral peak is only present when the laser stimulus was delivered on the hand, and not when it was delivered on the foot. This observation was interpreted as indicating a contribution of the primary somatosensory cortex to the early part of the electrocortical response elicited by selective nociceptive stimuli (Treede et al., 1988). This finding was later repeatedly confirmed using higher-density EEG (Baumgartner et al., 2011; Valentini et al., 2012), and the interpretation proven to be correct by different source analysis approaches (Apkarian et al., 2005; Baumgartner et al., 2011; Ploner et al., 1999; Tarkka and Treede, 1993; Valentini et al., 2012).

Previous hints and current evidence for late somatosensory-specific ERP components

Two studies recorded high-density ERPs elicited by stimuli of different sensory modalities (touch, pain, vision and audition) and used probabilistic ICA to dissect the contribution of multimodal neural activities (i.e., activities also elicited by stimuli of other sensory modalities) and somatosensory-specific neural activities (i.e., activities elicited by both nociceptive and non-nociceptive somatosensory stimuli) (Liang et al., 2010; Mouraux and Iannetti, 2009). Both studies observed that the early lateralized activity of the laser-evoked response was *somatosensory-specific*, i.e., only present in the response elicited by either nociceptive or non-nociceptive somatosensory stimuli (Liang et al., 2010; Mouraux and Iannetti, 2009). Such somatosensory-specific activities predominantly contributed to the early part of the LEP waveform, while the later part of the response (N2–P2 complex: maximal at the vertex and centrally-distributed) was largely contributed by multimodal activities (i.e., activities similarly elicited by visual and auditory stimuli). However, when examining carefully the time course of somatosensory-specific and multimodal EEG activities to the LEP and SEP responses, it is possible to notice a small but clear contribution of somatosensory-specific activities to the very last part of the waveform (see Fig. 3 in Mouraux and Iannetti, 2009 and Fig. 6 in Liang et al., 2010).

This glimpse of a possible late somatosensory-specific activity in the data reported by Mouraux and Iannetti (2009) and Liang et al. (2010) prompted us to collect high-density EEG from a larger cohort of subjects, to test whether a similar activity could be detected in standard scalp LEP recordings. The present results show that the late somatosensory-specific neural activity previously observed is likely to correspond to the cortical source of the P4 component described here. Importantly, while the observations of Mouraux and Iannetti (2009) and Liang et al. (2010) rely on a complex signal decomposition analysis (probabilistic ICA), here we show that late somatosensory neural activities can be isolated and measured in standard ERP recordings in the time-domain as a clear and distinct positive wave (P4 component).

Why was such a clear and distinct component appearing in the latest part of the LEP (Figs. 2–4) overlooked by the numerous previous investigations of LEP waveforms and topographies (Bromm et al., 1984; Carmon et al., 1976; Garcia-Larrea et al., 2003; Kakigi et al., 1989; Tarkka and Treede, 1993; Treede et al., 2003)? Few reasons may explain this. First, the signal-to-noise ratio of the P4 wave is low, both in absolute terms and compared to all preceding LEP waves (see Section 2 of the Supplementary materials for details). Second, the P4 wave has (i) the same polarity, (ii) a similar scalp distribution, and (iii) a largely overlapping time course with the preceding P2 wave — which is a particularly relevant issue given its much larger magnitude. Finally, putting forward the hypothesis that late somatosensory-specific neural activities exist in somatosensory-evoked potentials might have been discouraged by the established notion that more "exogenous" sensory-specific responses should precede "endogenous" multimodal responses (Desmedt and Robertson, 1977).

We provide convincing evidence that the neural processes reflected in the P4 wave are different from those reflected in the P2 wave. Indeed, the P2 and P4 scalp topographies can be clearly dissociated, depending on the stimulated side and body territory. Importantly, P2 and P4 were reliably separated into two distinct functional microstates in the time domain, regardless of stimulus territory (Fig. 1). Whereas the scalp topography of both the P2 peak and the P2 microstate was always centrally distributed and maximal at Cz, regardless of the stimulated limb (Treede et al., 1988; Valentini et al., 2012), that of the P4 peak and the P4 microstate were maximal on the central–parietal electrodes

contralateral to the stimulated side when laser stimuli were delivered to the hand, but they were centrally-distributed when laser stimuli were delivered to either foot (Fig. 2), an observation corroborated by strict statistical comparison across all scalp electrodes (Fig. 3). Such mediolateral topographical shift is compatible with the somatotopic arrangement of the body in the sensorimotor cortex (Penfield and Boldrey, 1937). Accordingly, source analysis results provided evidence that the primary somatosensory cortex contributed to the generation of the latest part of the LEP response (Fig. 5). It should be noted that the observation that the extracted four PCs explained the 14.9% (LH), 10.4% (RH), 16.5% (LF), and 16.0% (RF) of the total variance of the activity in the corresponding S1 source indicated that only a relatively small part of the overall S1 activity contributed to the generation of P4 wave. This observation does not suggest that P4 wave was largely generated from other neural sources (not from S1), or the contrary. In other words, the P4 wave may be largely generated from S1, but it only contributed to a small part of the total S1 activity, due to its low amplitude. However, the limited spatial resolution of this approach (Michel et al., 2004) does not allow drawing firm conclusions about the relative contribution of possible postcentral (somatosensory) and precentral (motor) sources to the P4 wave. In addition, our results do not exclude a possible contribution of other neural sources (e.g., operculo-insular cortex) to the generation of the P4 wave, given the prior assumptions that must be made when defining the sources of the response recorded on the scalp (Michel et al., 2004).

That the P4 wave reflects the activity of low-level primary sensorimotor cortices and not of higher-level posterior parietal structures is also demonstrated by the observation that its scalp topography is entirely unaffected when the position of the stimulated hand is altered within the egocentric frame of reference, by crossing the arms over the body midline (Fig. 6). Had the P4 being generated by multimodal posterior parietal areas, coding, for example, for the position of the limbs in egocentric space, its topographical distribution would have been changed when LEPs were recorded with the hand crossed over the midline.

Here we confirm that the initial part of the LEP response displayed a scalp topography compatible with activity in the primary somatosensory cortex. Following this initial somatosensory-specific activity, the recorded response is dominated by multimodal activities whose scalp distribution is central and maximal at the vertex, and constitutes a biphasic "vertex potential" (Mouraux and Iannetti, 2009). However, we observed that when such a large multimodal activity fades away, the distribution of the electrical potential in the latest part of the LEPs resembles that recorded at the early latencies, and it is compatible with activity in the primary somatosensory cortex.

At least two possible alternative physiological phenomena can explain this observation. Either the primary somatosensory cortex responds transiently at the beginning and at the end of the LEP time window, or they are tonically active throughout this time window, but their scalp expression is masked in the middle part of the waveform by the large multimodal activity. The time course of the S1 source reported in our previous study (see Fig. 5 in Valentini et al., 2012) suggests the first explanation. Indeed it shows two distinct peaks: a large and positive peak at the latency of the N1 wave, followed by a small negative peak at the latency of the P4 wave. Both the latency and the polarity difference suggest that the functional significance of the early and late activation of the primary somatosensory cortices in response to transient stimuli is different. The recent observation that laser stimuli induce a transient and short-lasting time-frequency increase in gamma band oscillations (GBOs) in the primary somatosensory cortex (Gross et al., 2007; Zhang et al., 2012), and that these GBOs are only concomitant to the earliest part of the LEP waveform indicates that, within the LEP time window, the S1 activity is functionally heterogeneous.

The possibility of exploring both the early and the late S1 activities through the detection and the measurement of the N1 and the P4 waves may allow clarifying these physiological questions.

Trial-to-trial correlation of earliest and latest LEP components

The recent development of robust and unbiased analytical approaches to estimate the latency and amplitude of ERP waves at the level of single trials has dramatically increased the amount of physiological information that can be extracted from EEG and magnetoencephalographic (MEG) experiments (Hu et al., 2010, 2011; Mohseni et al., 2009; Spencer, 2005). We have capitalized on these analysis approaches and estimated the latency and amplitude of the four main LEP waves (N1, N2, P2, and P4) in single trials of Experiment 1. We observed a significant dependency between the peak latencies and amplitudes of the P4 waves and those of N1 wave within each trial. Crucially, the single-trial dependency between P4 and N1 amplitudes was higher than that between P4 and N2 amplitudes and between P4 and P2 amplitudes. The single-trial analysis of latency variability yielded a remarkable result — that there was no significant single-trial dependency either between P4 and N2 latencies or between P4 and P2 latencies.

Both findings indicate a tight physiological link between the neural activities subserving the N1 and the P4 waves. The clear decoupling between trial-to-trial variability of N1 and P4 on one side, and N2 and P2 on the other also parallels the source analysis results of the LEP response. Indeed, the N1 (Valentini et al., 2012) and the P4 (Figs. 1–3) may both be generated in the primary somatosensory cortex, while the N2 and P2 waves are mostly generated from the operculoinsular and anterior cingulate cortices (Garcia-Larrea et al., 2003).

The strong decoupling between single-trial N1/P4 latencies and N2/P2 latencies is peculiar and of great interest. This indicates that the latency of the different LEP peaks is remarkably independent within the same trial. That is, even if in a given trial the N1 and P4 peak latencies fall in the bottom 20% of their respective distributions, in that same trial the N2 and P2 peak latencies can fall in the top 20% of their respective distributions. And the inverse of such a latency pattern can be observed in a successive trial.

What can be the neurophysiological substrate of this observation? One possibility is that the primary somatosensory cortex (generating the N1 and the P4) and the operculoinsular and cingulate cortices (generating the N2 and P2 waves) are activated in parallel, through two distinct thalamocortical pathways whose functional properties are relatively independent. Indeed, it has been recently suggested that the detection of salient information in the bilateral insula and cingulate cortices (i.e., the generators of the N2 and P2 waves) is at least partly mediated by direct thalamocortical projections bypassing the primary sensory cortices (like the primary somatosensory cortex, i.e., the generator of the N1 and P4 waves) (Liang et al., 2013). Thus, these two parallel thalamo-cortical pathways might represent the functional mechanisms underlying the decoupling between the latency of N1/P4 and N2/P2 single-trial responses.

The laser P4 wave is different from P3a or P3b waves

A typical P300 wave (later renamed P3b) can be elicited by laser stimuli, when they are rare and task-relevant (e.g., Legrain et al., 2002, 2003). Importantly, given the conduction velocity of Aδ fibers, such laser P3b should not be expected before 500 ms post-stimulus when the hand is stimulated. Indeed, the laser P2 wave and the laser P3b can be clearly separated based on both latency and scalp topography (Kanda et al., 1996; Legrain et al., 2002, 2003, 2009; Lorenz and Garcia-Larrea, 2003). In contrast, the laser P2 wave can be partly overlapping with a P3a wave elicited by novel stimuli outside the attentional focus (this P3a wave has been sometimes labeled as P400; see, for example, Legrain et al., 2002). However, several observations suggest that the P4 is fundamentally different from a P3a wave. First, a P3a is elicited by intense and novel stimuli happening outside the attentional focus, which was not consistent with the current experimental design, in which stimuli were delivered to the same district

within each recording block and subjects were not required to perform a cognitive task besides intensity rating. Second, the P4 wave was maximal on the central–parietal electrodes contralateral to the stimulated side when laser pulses were delivered on the hand, and centrally-distributed, with a maximum between Cz and Pz, when laser pulses were delivered to the foot. In contrast, the P3a does not follow such mediolateral somatotopy (Polich, 2007). Third, as discussed in the Trial-to-trial correlation of earliest and latest LEP components section, trial-by-trial variability of the latency of the P4 wave was strongly coupled with the trial-by-trial variability of the latency of the N1 wave, which has been shown to be the most "exogenous" component of the LEPs, the most related to the incoming somatosensory input (Lee et al., 2009; Mouraux and Iannetti, 2009). Thus, the P4 wave described in this report is unlikely reflecting neural activities underlying the P3a or P3b waves.

Practical implications and conclusion

The P4 wave is a distinct component detectable in the latest part of the LEP response, and it reflects the activity of the primary somatosensory cortex. Although of small amplitude, the P4 wave can be isolated and measured in single subjects. The compelling evidence that the neural activity underlying the P4 wave arises from S1 (Fig. 4) has practical implications for its detection. Indeed, similarly to what was recommended for the N1 wave, when the LEP is elicited by hand stimulation the latest activity displays a positive maximum contralateral to the stimulation side, the P4 wave is optimally detected at the temporal or the central electrode contralateral to the stimulated hand (Tc or Cc) re-referenced to Fz (Hu et al., 2010; Valentini et al., 2012). Importantly, this montage does not allow detecting the P4 when the LEP is elicited by foot stimulation (Fig. 4), because the foot area in S1 is located close to the hemispheric midline (Penfield and Jasper, 1954). Thus, because of their midline generators when the foot is stimulated, the early and late primary somatosensory activities are difficult to be isolated as a separate component on the LEP waveform.

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Conflict of interest

The authors declare no competing financial interests.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.08.057.

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