Dishabituation of Laser-evoked EEG Responses: Dissecting the Effect of Certain and Uncertain Changes in Stimulus Modality

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Abstract

■ The repetition of nociceptive stimuli of identical modality, intensity, and location at short and constant interstimulus intervals (ISIs) determines a strong habituation of the corresponding EEG responses, without affecting the subjective perception of pain. To understand what determines this response habituation, we (i) examined the effect of introducing a change in the modality of the repeated stimulus, and (ii) dissected the relative contribution of bottom–up, stimulus-driven changes in modality and top–down, cognitive expectations of such a change, on both laser-evoked and auditory-evoked EEG responses. Multichannel EEG was recorded while participants received trains of three stimuli (S1–S2–S3, a triplet) delivered to the hand dorsum at 1-sec ISI. S3 belonged either to the same modality as S1 and S2 or to the other modality. In addition, participants were either explicitly informed or not informed of the modality of S3. We found that introducing a change in stimulus modality produced a significant dishabituation of the laser-evoked N1, N2, and P2 waves; the auditory N1 and P2 waves; and the laser- and auditory-induced event-related synchronization and desynchronization. In contrast, the lack of explicit knowledge of a possible change in the sensory modality of the stimulus (i.e., uncertainty) only increased the ascending portion of the laser-evoked and auditory-evoked P2 wave. Altogether, these results indicate that bottom–up novelty resulting from the change of stimulus modality, and not top–down cognitive expectations, plays a major role in determining the habituation of these brain responses. ■

INTRODUCTION

Brief radiant heat pulses generated by infrared laser stimulators selectively activate Aδ and C skin nociceptors in the most superficial skin layers (Bromm & Treede, 1984, 1991). Such stimuli elicit a number of transient brain responses (laser-evoked potentials, LEPs) in the ongoing electroencephalogram (EEG) (Carmon, Mor, & Goldberg, 1976). These responses are mediated by the activation of type II Aδ mechano-heat nociceptors (AMH) and spinothalamic neurons in the dorsal horn of the spinal cord (Treede, 2003). Aδ-related LEPs have been extensively used to investigate the peripheral and central processing of nociceptive sensory input, both in physiological (e.g., Iannetti et al., 2003) and in pathophysiological studies (reviewed in Treede, Lorenz, & Baumgartner, 2003), and are currently considered the best available diagnostic tool to assess the function of Aδ nociceptive pathways in patients (Cruccu et al., 2004).

The largest LEP response is a biphasic negative–positive complex (N2–P2) maximal at the scalp vertex, peaking at approximately 200–350 msec after the stimulation of the hand dorsum (Bromm & Treede, 1984). The N2–P2 response is preceded by a smaller negative deflection (N1 wave) maximal over the temporal–central region contralateral to the stimulated side, peaking at approximately 160 msec after the stimulation of the hand dorsum (Hu, Mouraux, Hu, & Iannetti, 2010; Treede, Kief, Holzer, & Bromm, 1988). LEPs represent the sum of neural activities arising from several cortical generators, which have been partly localized using dipole modeling of scalp and subdural recordings and direct intracranial recordings (for a review, see Garcia-Larrea, Frot, & Valeriani, 2003). LEPs seem to result from sources in bilateral operculo-insular cortices, anterior cingulate cortex, and, possibly, contralateral primary sensory cortex. LEPs are known to be significantly modulated by attentional factors (reviewed in Lorenz & Garcia-Larrea, 2003). In particular, Legrain, Perchet, and Garcia-Larrea (2009), Legrain, Bruyer, Guerit, and Plaghki (2003), and Legrain, Guerit, Bruyer, and Plaghki (2002, 2003) showed that the laser-evoked N1, N2, and P2 waves are modulated by manipulating spatial attention, suggesting that their sources are sensitive to "top–down" attentional mechanisms. Moreover, the laser-evoked P2 wave is enhanced by the probability of stimulus occurrence, suggesting that its sources also reflect brain processes involved in "bottom–up" stimulus-driven mechanisms of arousal or attentional orientation.

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Sensory stimuli do not only elicit time-locked deflections in the EEG (i.e., ERPs), but also induce transient modulations of the ongoing oscillatory EEG activity (Mouraux, Guerit, & Plaghki, 2003). These modulations may appear either as a transient increase (event-related synchronization, ERS) or as a transient decrease (event-related desynchronization, ERD) of EEG power, usually confined to a specific frequency band. The functional significance of ERS and ERD is thought to differ according to the frequency band within which they occur. ERS in the alpha band (frequencies ranging from 8 to 12 Hz) has been hypothesized to reflect cortical deactivation or inhibition, whereas ERD in the same frequency band has been hypothesized to reflect cortical activation or disinhibition (reviewed in Pfurtscheller, & Lopes da Silva, 1999). In contrast, ERS in the gamma band (frequencies >40 Hz) has been hypothesized to reflect the formation of transient cortical assemblies, and thus, plays a role in cortical integration (Rodriguez et al., 1999; Tallon-Baudry, Bertrand, Wienbruch, Ross, & Pantev, 1997). By performing a time–frequency analysis of the EEG signals elicited by nociceptive laser stimuli, two novel electrophysiological responses related to the activation of Aδ fibers have been disclosed (Ploner, Gross, Timmermann, Pollok, & Schnitzler, 2006; Ohara, Crone, Weiss, & Lenz, 2004; Mouraux et al., 2003): a short-lasting ERS, starting about 160 msec after stimulus onset, followed by a long-lasting ERD, starting about 500 msec after stimulus onset. The frequency of both responses is centered around 10 Hz. The neural generators and the functional significance of these two responses remain largely unknown.

Although LEPs are increasingly used to investigate nociceptive pathways, a full understanding of their functional significance remains to be achieved. Because LEPs are elicited by stimuli entirely selective for nociceptive peripheral afferents, and because in most experimental conditions they correlate with the amount of perceived pain, LEPs are sometimes regarded as nociceptive-specific responses, and referred to as "pain-evoked potentials" (e.g., Edwards, Inui, Ring, Wang, & Kakigi, 2008; Schmidt et al., 2007; Kakigi, Watanabe, & Yamasaki, 2000). However, as already pointed out by Stowell (1984) and Carmon et al. (1976) in their seminal work, LEPs could also reflect stimulus-triggered brain processes that are entirely unspecific for nociception. We recently investigated the functional significance of LEPs by recording the psychophysical and EEG responses elicited by short trains of three consecutive nociceptive stimuli of identical energy (S1–S2–S3, a triplet), delivered at a short (1 sec) and constant interstimulus interval (ISI) to the hand dorsum (Iannetti, Hughes, Lee, & Mouraux, 2008). Using this paradigm, we showed that the well-known positive correlation between the intensity of perceived pain and the magnitude of both LEPs and laser-induced ERD and ERS (e.g., Iannetti, Zambreanu, Cruccu, & Tracey, 2005; Mouraux et al., 2003; Beydoun, Morrow, Shen, & Casey, 1993; Bromm & Treede, 1991) can be significantly disrupted, thus providing evidence that a large part of the laser-evoked EEG responses do not reflect brain processes directly related to pain perception. Specifically, we found that stimulus repetition did not affect the subjective perception of pain, whereas the magnitude of the ERP response was strongly decreased after the first stimulus repetition.

What determined this observed response reduction induced by stimulus repetition? To address this question, it is important to consider that the four basic types of information defining a sensory stimulus (modality, location, intensity, and timing; Gardner, Martin, & Jessell, 2000) were identical for each of the three stimuli (S1–S2–S3) constituting the triplet. Thus, the observed response reduction could be explained by a lack of change in the sensory information contained in S2 and S3 as compared to S1, that is, by the fact that S2 and S3 were identical to S1 in terms of modality, location, intensity, and timing, hence, that S2 and S3 were less novel than S1 (bottom–up stimulus-driven changes). Furthermore, the observed response reduction could be explained by the fact that S2 and S3 were also more certain than S1, that is, by the fact that subjects were aware that the information contained in S2 and S3 would be the same than that contained in S1 (top–down cognitive expectations). However, with the paradigm used by Iannetti et al. (2008), we were not able to determine (1) which type of sensory information is important in determining the habituation of the ERP response, and (2) the respective contribution of stimulus-driven changes (i.e., the occurrence of a change in sensory information) and cognitive expectations (i.e., the uncertainty of such a change).

In the present study, we aimed to (1) examine the effect of introducing a selective change in the modality of the repeated stimulus (i.e., without altering its location, intensity and timing) on the ERP response habituation, and (2) dissect the relative contribution of stimulus-driven changes in stimulus modality and the cognitive expectations of such a change in determining response habituation.

METHODS

Subjects

Twelve healthy subjects (7 women) aged 22–35 years (mean ± *SD* = 26.2 ± 4.2) participated in this study. All participants gave their written informed consent. The study conformed to the standards required by the Declaration of Helsinki and was approved by the local ethics committee.

Nociceptive and Auditory Stimulation

Noxious radiant stimuli were generated by an infrared neodymium yttrium aluminium perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Florence, Italy). At this wavelength, the laser pulses activate directly the Aδ and C-fiber nociceptive terminals located in the superficial layers of the skin (Bromm &

Treede, 1984). The laser beam was transmitted via an optic fiber and its diameter was set at approximately 8 mm (50 mm^2) by focusing lenses. The duration of the laser pulses was 4 msec. Laser pulses were directed at the dorsum of the right hand, on a squared area (5 × 5 cm) defined prior to the beginning of the experimental session. The spot location was automatically controlled by a computer that used two servo-motors (HS-422; Hitec RCD; angular speed, 60°/160 msec) to orient the laser beam along two perpendicular axes (see Lee, Mouraux, & Iannetti, 2009 for details). To familiarize subjects with the nociceptive stimulus, a small number of low-energy laser pulses were delivered to the right-hand dorsum. The energy of the laser stimulus was then adjusted individually using the method of limits, in order to elicit a clear pricking pain sensation (3.1 ± 0.3 J), related to the activation of Aδ nociceptors (Treede, 1995).

Auditory stimuli were brief 800-Hz tones (50 msec duration; 5 msec rise and fall times) delivered through a speaker (VE100AO, Audax, France) placed in front of the right hand (~55 cm from the subject and ~50 cm from the midline). At the beginning of the experiment, the intensity of auditory stimulation was adjusted in order to match the intensity of the laser stimulation. The intensity-matching procedure consisted of two steps. First, immediately after setting the intensity of the nociceptive stimulus, a series of tones of increasing loudness was presented to the subjects in order to familiarize them with the auditory sensation. Second, subjects were asked to match the perceived intensity of the auditory sensation to the perceived intensity of the nociceptive sensation by self-adjusting the intensity of the auditory stimulation. Laser pulses were delivered both shortly before and shortly after the auditory stimulus to make sure that the matching was not related to the order of occurrence of the two stimuli. This matching procedure was repeated at the end of each recording block to ensure that the perceived intensity of the auditory and nociceptive sensations remained matched throughout the experiment. The average intensity of auditory stimulation was 85 ± 5 dB.

Experimental Design

A schematic illustration of the experimental design is shown in Figure 1. Four different blocks of stimulation were counterbalanced across subjects. In each block, trains of both laser and auditory stimuli were presented. Each train consisted of three stimuli (S1–S2–S3, a triplet) delivered to the hand dorsum at a constant ISI of 1 sec. The time interval between each triplet ranged between 8 and 12 sec (rectangular distribution). S1 and S2 always belonged to the same sensory modality (nociceptive or auditory), whereas S3 belonged either to the same modality as S1 and S2 (triplet *same*) or to the other modality (triplet *different*). Approximately 3 sec before the onset of each triplet, subjects were verbally informed of the sensory modality of S1 and S2. In two out of the four blocks, participants were also informed of the sensory modality of S3 (condition *certain*), whereas in the remaining two blocks they were not (condition *uncertain*). Within each uncertain block, the occurrence of same and different triplets was balanced and pseudorandomized. The maximum number of consecutive triplets belonging to the same condition (i.e., same or different) was three. Before starting the recording, subjects were instructed to relax and equally attend all the stimuli of each triplet, independently of experimental condition and sensory modality.

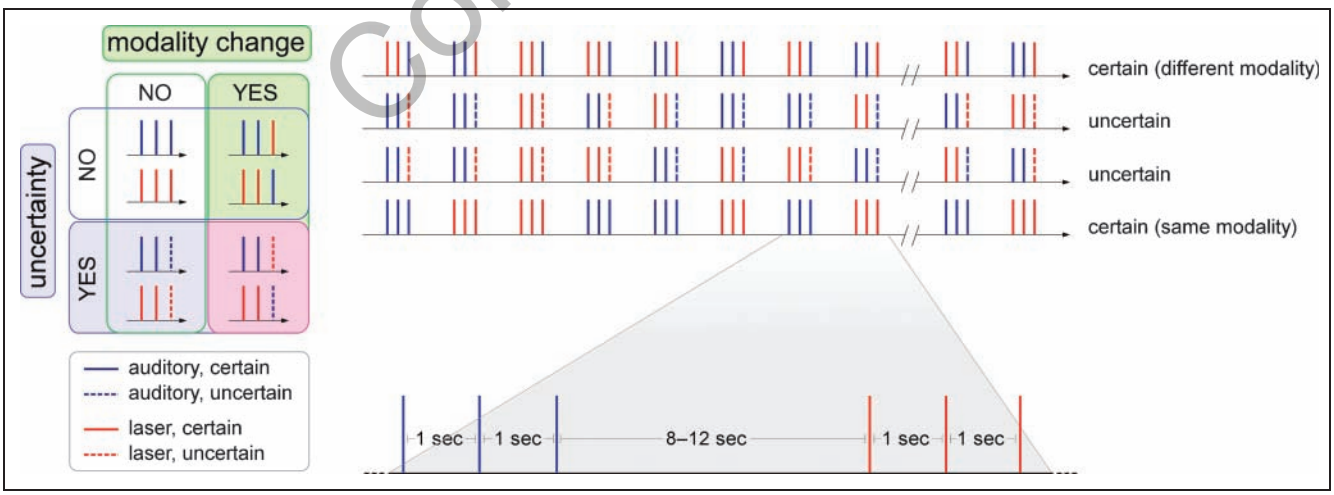


Figure 1. Experimental design. Event-related potentials (ERPs) were recorded in four blocks, whose order was counterbalanced across subjects (top right). In each block trains of laser (red) and auditory (blue) stimuli were delivered to or around the hand dorsum. Each train consisted of three stimuli (S1–S2–S3, a triplet) delivered at a constant ISI of 1 sec (bottom right). S1 and S2 always belonged to the same sensory modality, whereas S3 belonged either to the same modality as S1 and S2 (triplet same) or to the other modality (triplet different). In two out of four blocks the participants were also informed of the sensory modality of S3 (condition certain), whereas in the remaining two blocks they were not (condition uncertain). This design allowed us to dissect the selective effect of "modality change" from the effect of the "uncertainty" of such a change in determining the ERP response habituation (left).

In each block we delivered 40 triplets, for a total of 160 triplets in the whole experiment. To avoid nociceptor fatigue or sensitization, between each laser pulse of a given triplet, the target of the laser beam was displaced by approximately 1 cm along a proximal–distal axis on the hand dorsum. The direction of this displacement was balanced in each block (20 stimuli in the proximal direction and 20 stimuli in the distal direction). This procedure aimed to minimize differences due to the variation in thickness and innervation of the irradiated skin and, consequently, the variability in the intensity of the somatosensory nociceptive input (Schlereth, Magerl, & Treede, 2001). Because variations in baseline skin temperature could bias results (Baumgartner, Cruccu, Iannetti, & Treede, 2005), an infrared thermometer was used to ensure that baseline skin temperatures were similar at the beginning of each block.

EEG Recording

Participants were seated on a comfortable chair in a silent, temperature-controlled room. They were asked to place their hands on a desk, and to keep their eyes open and gaze slightly downward. A screen was placed ~30 cm in front of the participants, in order to block the view of the hands without being interposed between the speaker and the participants' right ear. EEG was recorded using 20 Ag–AgCl electrodes placed on the scalp according the International 10–20 system and referenced to the nose. The EOG was recorded from two surface electrodes, one placed over the right lower eyelid, the other placed lateral to the outer canthus of the right eye. Signals were amplified and digitized at a sampling rate of 1024 Hz and a conversion of 12 bit, giving a resolution of 0.195 μV (SD32; Micromed, Treviso, Italy).

EEG Analysis

Preprocessing

EEG data were preprocessed and analyzed using Letswave (http://amouraux.webnode.com) (Mouraux & Iannetti, 2008b) and EEGLAB (Delorme & Makeig, 2004). EEG data were segmented into epochs using a time window ranging from 1 sec before the first stimulus (S1) to 1 sec after the third stimulus (S3) of each triplet (total epoch duration: 4 sec). Each epoch was baseline-corrected using the interval from −0.5 to 0 sec as reference. EEG epochs were band-pass filtered from 1 to 40 Hz using a fast Fourier transform filter. EOG artifacts were subtracted using a validated method based on independent component analysis (ICA; Jung et al., 2000). In all data sets, independent components (ICs) related to eye movements had a large EOG channel contribution and a frontal scalp distribution. Because frequency filtering and ICA changed the EEG signals, a second baseline correction was performed using the same −0.5 to 0 sec reference interval, thus ensuring that the average prestimulus amplitude was equal to zero. Finally, epochs with amplitude values exceeding ±65 μV (i.e., epochs likely to be contaminated by an artifact) were excluded from further analyses. These epochs constituted 6 ± 0.2% of the total number of epochs.

Analysis in the Time Domain

Epochs belonging to the same experimental condition were averaged and time-locked to the onset of the first stimulus of each triplet. This procedure yielded, in each subject, eight average waveforms (one waveform for each experimental condition and sensory modality: certain/same, certain/different, uncertain/same, and uncertain/different). For each average waveform, the latency and the baseline-to-peak amplitude of the ERP elicited by each stimulus of the triplet were measured (see Figure 2). For LEPs, N1, N2 and P2 waves were measured as follows. The N1 wave was measured at the temporal electrode contralateral to the stimulated side (T3), referenced to Fz. It was defined as the negative deflection preceding the N2 wave, which appears as a positive deflection in this montage. The N2 and P2 waves were measured at the vertex (Cz) referenced to the nose. The N2 wave was defined as the most negative deflection after stimulus onset. The P2 wave was defined as the most positive deflection after stimulus onset. For AEPs, N1 and P2 waves were measured at the vertex (Cz) referenced to the nose. The N1 wave was defined as the most negative deflection after stimulus onset. The P2 wave was defined as the most positive deflection after stimulus onset.

Analysis in the Time–Frequency Domain

An estimate of the amplitude of oscillatory activity as a function of time and frequency was obtained for each EEG epoch. Because this estimate is a time-varying expression of oscillation amplitude regardless of its phase, averaging these estimates across trials discloses both phase-locked and non-phase-locked modulations of signal amplitude, provided that these modulations are both time-locked to the onset of the event and consistent in frequency (i.e., the latency and frequency at which they occur are reproducible across trials). To obtain this estimate, we used the continuous wavelet transform, which adapts the width of its window of analysis as a function of frequency, and thereby offers an optimal compromise for time–frequency resolution (Mouraux & Iannetti, 2008b). We used a Morlet wavelet consisting in a complex exponential function localized in time by a Gaussian envelope. The initial spread of the Gaussian envelope was set to $2.5/\pi\omega_0$ (ω_0 being the central frequency of the wavelet; for details of the method, see Mouraux & Iannetti, 2008b; Mouraux et al., 2003). Across-trial averaging of these time–frequency representations produced a spectrogram of the average EEG oscillation amplitude as a function of time and frequency. This time–frequency map was used

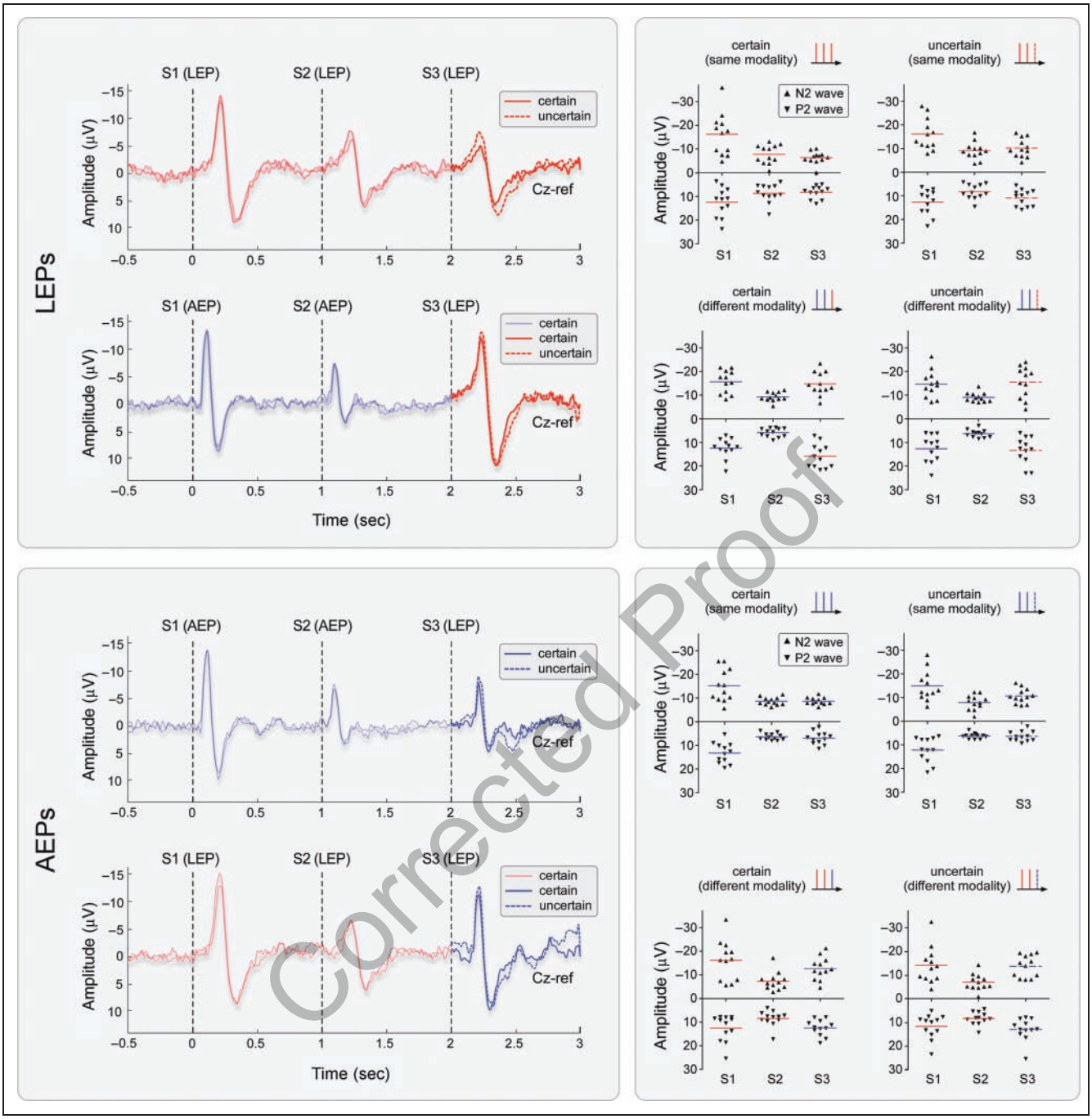


Figure 2. Left panels: Group-level average ERPs elicited by laser stimuli (red) and auditory stimuli (blue). S1 and S2 always belonged to the same sensory modality, whereas S3 belonged either to the same modality as S1 and S2 (triplet same, top waveforms of each panel) or to the other modality (triplet different, bottom waveforms of each panel). The change of modality of S3 was either certain (full line) or uncertain (dashed line). Displayed signals were recorded at electrode Cz (nose reference). *x*-axis, time (sec); *y*-axis, amplitude (μV). The vertical dashed lines mark the onset of the three stimuli. Right panels: Single-subject and group-level average peak amplitudes of the N2 and P2 waves elicited by S1, S2, and S3. *x*-axis, stimulus number; *y*-axis, amplitude (μV). Colored horizontal lines represent the group averages (red: LEPs; blue: AEPs; full lines: ERPs elicited by certain stimuli; dashed lines: ERPs elicited by uncertain stimuli). Note the significant amplitude reduction between S1-ERP and S2-ERP. Note also the larger amplitude of S3-ERP in triplets where there was a change of modality between S2 and S3.

to identify non-phase-locked, laser- and auditory-induced modulations of ongoing EEG rhythms (ERS and ERD). For each estimated frequency, results were displayed as an increase or decrease of oscillation amplitude relative to a prestimulus reference interval (−0.5 to −0.1 sec before the onset of S1), according to the following formula: $ER_{t,f}\% [A_{t,f} - R_f]/R_f$, where $A_{t,f}$ is the signal amplitude at a given time t and at a given frequency f, and R_f is the signal amplitude averaged within the reference interval (Pfurtscheller & Lopes da Silva, 1999).

Quantitative Analysis of Time–Frequency Spectrograms

To explore the differences between the brain responses elicited in the four different experimental conditions, three time–frequency regions of interest (ROIs) were defined in the spectrograms obtained at Cz. For laser-induced brain related activity, the time–frequency limits were defined based on previous work from our group: LEP (1–8 Hz and 100–500 msec), ERS (10–20 Hz and 100–500 msec), and ERD (7–13 Hz and 400–900 msec) (Iannetti et al., 2008). For auditory-induced brain activity, the time–frequency limits were derived from Mayhew, Dirckx, Niazy, Iannetti, and Wise (2010) and centered around the locations of the main foci of activity: AEP (1–10 Hz and 0–500 msec), ERS (10–25 Hz and 0–500 msec), and ERD (10–15 Hz and 400–900 msec). Within each time–frequency ROI, ER% values were extracted to compute the mean of the 20% of points displaying the highest increase (LEP/AEP and ERS) or decrease (ERD). This "top 20%" summary measure reflects the higher ER% values within each window of interest, with the aim of reducing the noise introduced by including all points of the spectrogram, some of which may display little or no response. This approach, which has already been used successfully to analyze both ERPs (Iannetti et al., 2008) and blood oxygen level dependent fMRI data (Mitsis, Iannetti, Smart, Tracey, & Wise, 2008; Iannetti et al., 2005), offers several advantages for disclosing condition-specific effects (for a review, see Mouraux & Iannetti, 2008b).

Statistical Analyses

A two-way repeated measures ANOVA was used to explore the effect of "modality change" (two levels: "same," "different") and "uncertainty" (two levels: "certain," "uncertain"), as well as the possible interaction between these two factors, on the following electrophysiological responses: (1) N1, N2, and P2 peak amplitudes of the LEP elicited by S3; (2) N1 and P2 peak amplitudes of the AEP elicited by S3; (3) ER% summary measure of each ROI of the response elicited at Cz by S3 laser stimuli; (4) ER% summary measure of each ROI of the response elicited at Cz by S3 auditory stimuli. When a main effect or the interaction were significant, post hoc Tukey's tests were used to perform pairwise comparisons. These statistical comparisons were performed using Statistica 8.0 (Statsoft Inc. 2001).

Furthermore, to disclose the time course of the effects of "modality change" and "uncertainty" on the ERP response in the time domain, we performed the same repeated measures ANOVA, but using each time point of the averaged ERP waveforms, as implemented in LetsWave (http://amouraux.webnode.com). This analysis yielded two waveforms expressing the significance of the effect of each of the two experimental factors across time. A consecutivity threshold of 50 msec was chosen to account for multiple comparisons.

RESULTS

Laser-evoked Brain Potentials

Grand-average waveforms of LEPs elicited by S3 in the four different conditions are shown in Figure 3 (top). Significances of ANOVA main effects and interaction are summarized in Table 1.

Effect of Modality Change

There was a significant main effect of the factor modality change on the amplitude of the LEP elicited by S3 (Figure 4, top). The magnitude of N1, N2, and P2 waves was significantly larger when the S3 laser stimulus was preceded by auditory S1 and S2 stimuli (triplet different) as compared to when it was preceded by laser S1 and S2 stimuli (triplet same) [N1: $F(1, 11) = 12.268, p = .005$; N2: $F(1, 11) = 56.456, p < .001$; P2: $F(1, 11) = 16.964, p = .002$] (Figure 4, top).

Effect of Uncertainty

In contrast, there was a weak main effect of uncertainty only on the amplitude of the N2 wave [$F(1, 11) = 6.230$, $p = .03$], but not on the amplitude of the N1 and P2 waves [N1: $F(1, 11) = .007, p = .93$; P2: $F(1, 11) = 1.528, p = .24$]. Post hoc comparisons revealed that the magnitude of the N2 wave was significantly larger when the laser S3 stimulus was uncertain, independently of the change of its modality (Figure 4, top).

Interaction between Modality Change and Uncertainty

Finally, there was a significant interaction between the factors modality change and uncertainty only on the amplitude of the P2 wave [$F(1, 11) = 16.216, p = .002$], but not on the amplitude of the N1 [$F(1, 11) = 0.969, p = .35$] and N2 [$F(1, 11) = 2.953, p = .11$] waves elicited by S3. Post hoc comparisons revealed that the change of sensory modality induced a significantly larger increase in P2 wave magnitude when S3 was certain than when S3 was uncertain ($p = .001$).

Time Course of the Effect of Modality Change and Uncertainty

To follow the effect of these two experimental factors across time, in addition to peak amplitude analysis, we computed a two-way repeated measures ANOVA for each time point of the obtained averaged LEP waveforms (Figure 5). At electrode Cz, the factor modality change was a significant source of variance within two different time intervals: 208–242 msec (coinciding with the latency of the N2 wave) and 275–394 msec (coinciding with the latency of the P2 wave) (Figure 5, top left). The factor uncertainty was a significant source of variance in the time interval 340–500 msec (coinciding with the latency of the second half of the P2 wave). The interaction between modality change and uncertainty across time was not significant.

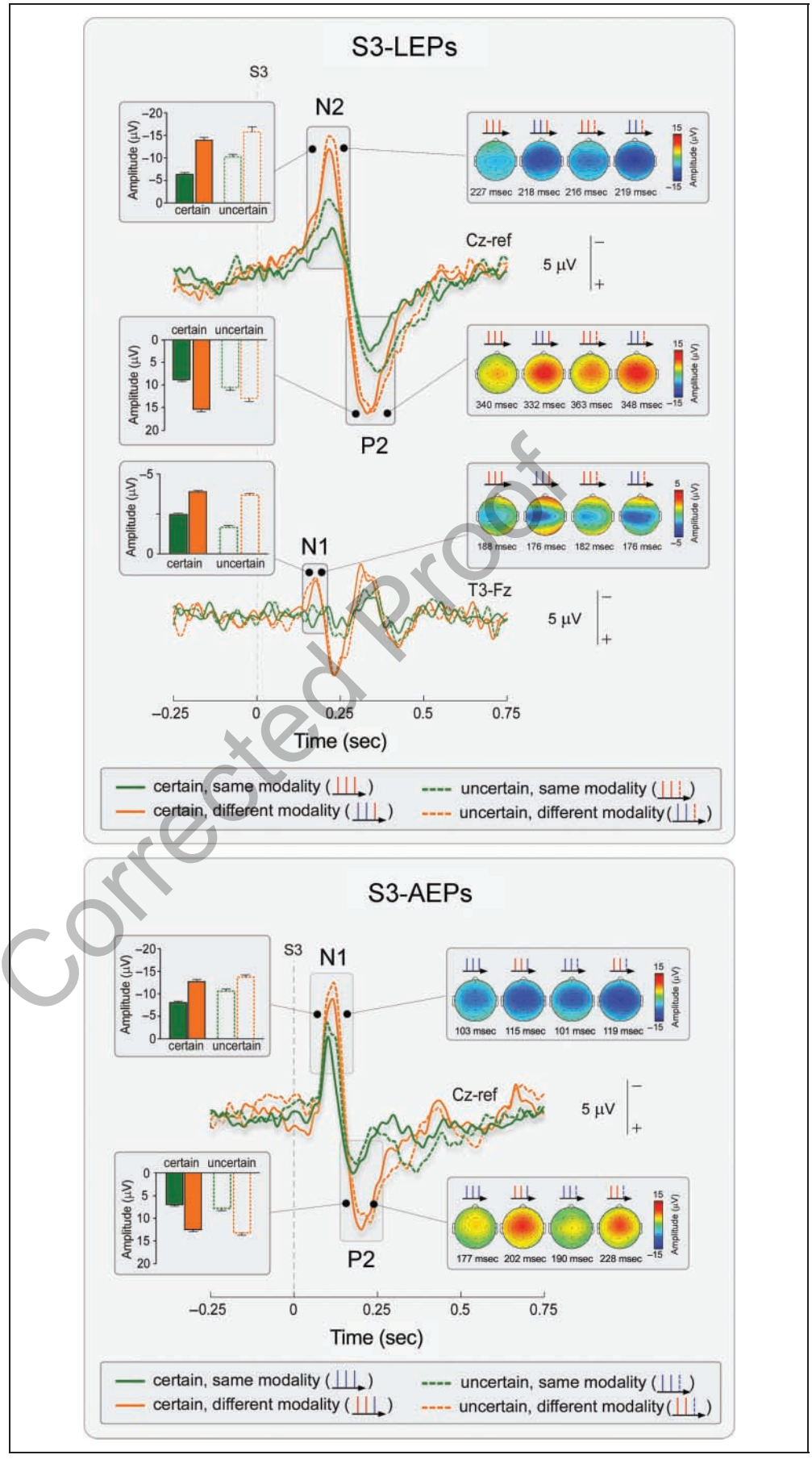


Figure 3. Group-level average LEP (top) and AEP (bottom) waveforms elicited by S3 in the four experimental conditions. Orange waveforms represent the S3-ERPs when there was a change of modality (triplets different). Green waveforms represent the S3-ERPs when there was not a change of modality (triplets same). Displayed signals were recorded at electrode Cz (AEPs; N2–P2 waves of LEPs) and T3 (N1 wave of LEPs). *x*-axis, time (sec); *y*-axis, amplitude (μV). Full and dashed lines represent the S3-ERPs elicited by certain and uncertain stimuli, respectively. The vertical dashed lines mark the onset of S3. Average peak amplitudes and scalp maps are shown in the insets. Note the significant increase in ERP amplitude when a change in modality of S3 took place.

Table 1. Summary of ANOVA Results: Laser-evoked Responses

	F	*p*
Time Domain		
N1-wave amplitude		
Main effect of modality change	12.268	.005**
Main effect of uncertainty	0.007	.93
Interaction	0.969	.35
N2-wave amplitude		
Main effect of modality change	56.456	<.001***
Main effect of uncertainty	6.230	.03*
Interaction	2.953	.11
P2-wave amplitude		
Main effect of modality change	16.964	.002**
Main effect of uncertainty	1.528	.24
Interaction	16.216	.002**
Time–Frequency Domain		
ROI LEP		
Main effect of modality change	22.357	<.001***
Main effect of uncertainty	2.043	.18
Interaction	0.249	.63
ROI ERS		
Main effect of modality change	21.805	<.001***
Main effect of uncertainty	0.058	.81
Interaction	1.42	.26
ROI ERD		
Main effect of modality change	4.832	.0502
Main effect of uncertainty	0.502	.49
Interaction	0.852	.38

*$p < .05$.

**$p < .01$.

***$p < .001$.

Laser-induced ERS and ERD

Grand-average time–frequency spectrograms of the laser-induced EEG responses obtained in the four experimental conditions are shown in Figure 6. Significances of ANOVA main effects and interactions are summarized in Table 1.

Effect of Modality Change

There was a significant main effect of the factor modality change on the ER% summary values of the LEP and ERS time–frequency responses elicited by S3. The magnitudes of the LEP and ERS responses were significantly larger when the S3 laser stimulus was preceded by auditory S1 and S2 stimuli (triplet different) than when it was preceded by laser S1 and S2 stimuli (triplet same) [LEP: $F(1, 11) = 22.357, p < .001$; ERS: $F(1, 11) = 21.805, p < .001$]. There was a suggestion of a main effect of modality change on the magnitude of the ERD response, in the same direction of the modulation of the LEP and ERS responses: The magnitude of the ERD tended to be larger when the S3 laser stimulus was preceded by auditory S1 and S2 stimuli (triplet different) [ERD: $F(1, 11) = 4.832$, $p = .0502$].

Effect of Uncertainty

There was no significant main effect of the factor uncertainty on the ER% summary values of all three time–frequency responses elicited by S3 [LEP: $F(1, 11) = 2.043$, $p = .18$; ERS: $F(1, 11) = 0.058, p = .81$; ERD: $F(1, 11) = 0.502, p = .49$].

Interaction between Modality Change and Uncertainty

There was no significant interaction between the factors modality change and uncertainty on the ER% summary values of all three time–frequency responses elicited by S3 [LEP: $F(1, 11) = 0.249, p = .63$; ERS: $F(1, 11) = 1.42$, $p = .26$; ERD: $F(1, 11) = 0.852, p = .38$].

Auditory-evoked Brain Potentials

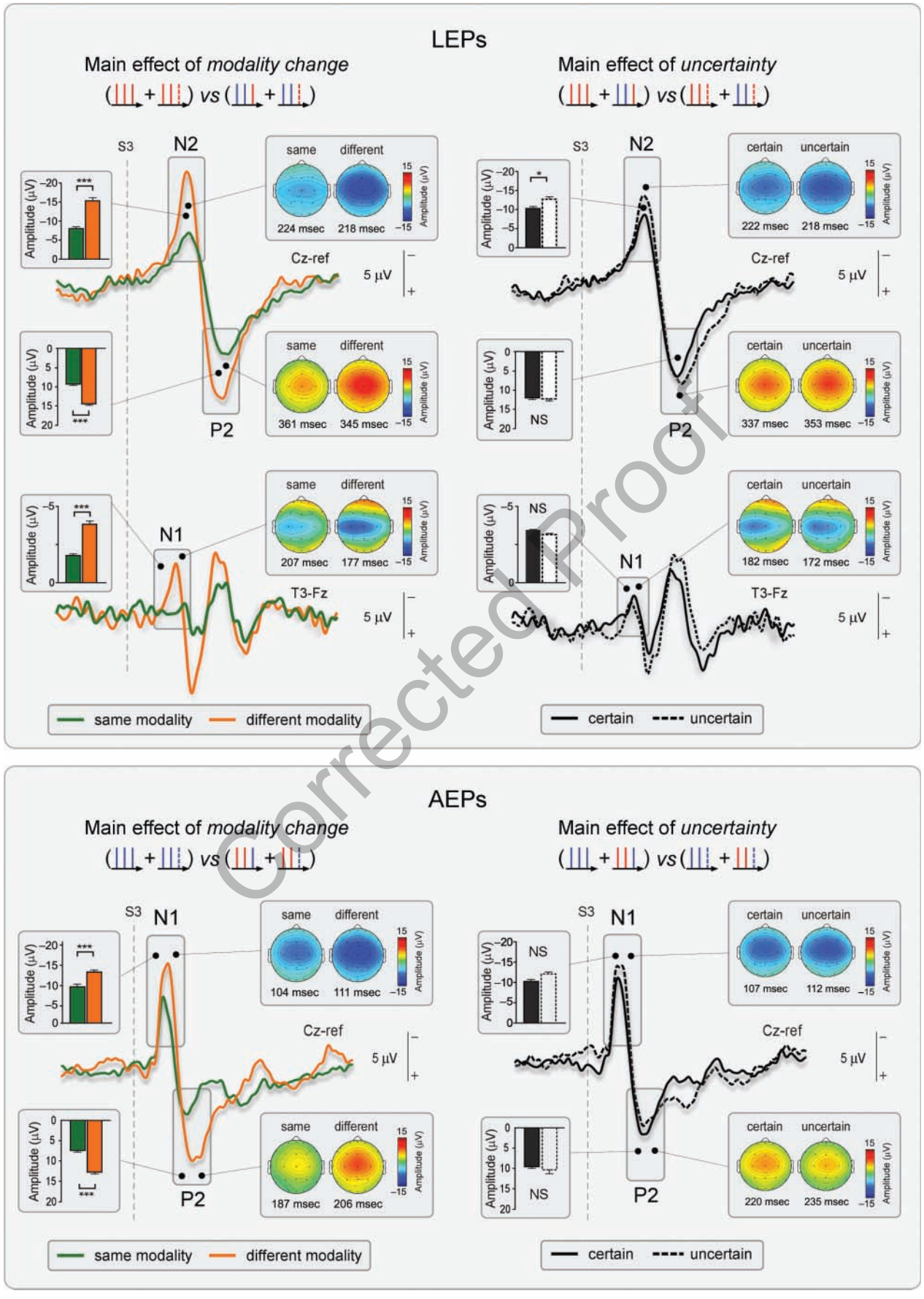
Grand-average waveforms of AEPs in the four different conditions are shown in Figure 3 (bottom). Significances of ANOVA main effects and interaction are summarized in Table 2.

Effect of Modality Change

There was a significant main effect of the factor modality change on the amplitude of the AEP elicited by S3 (Figure 4, bottom). The magnitude of the N1 and P2 waves was significantly larger when the S3 auditory stimulus was preceded by laser S1 and S2 stimuli (triplet different) than when it was preceded by an auditory S2 (triplet same) [N1: $F(1, 11) = 15.006, p = .003$; P2: $F(1, 11) = 38.834$, $p < .001$].

Effect of Uncertainty

Similarly to what observed in the LEP waveforms, there was a suggestion of significant main effect of uncertainty only on the amplitude of the N1 wave [$F(1, 11) = 4.763, p = .05$], but not on the amplitude of the P2 wave elicited by S3 [$F(1, 11) = 0.442, p = .52$]. Post hoc comparisons revealed



that the magnitude of the N1 wave tended to be significantly larger when the auditory stimulus was uncertain, independently of the change of its modality (Figure 4, bottom).

Interaction between Modality Change and Uncertainty

There was no significant interaction between the factors modality change and uncertainty on the amplitude of both the N1 [$F(1, 11) = 0.219, p = .65$] and the P2 waves of the AEP elicited by S3 [$F(1, 11) = 0.042, p = .84$].

Time Course of the Effect of Modality Change and Uncertainty

At electrode Cz, the factor modality change was a significant source of variance of the AEP waveform within two different time intervals: 75–120 msec (coinciding with the latency of the N1 wave) and 180–295 msec (coinciding with the latency of the P2 wave) (Figure 5, bottom). The factor uncertainty was a significant source of variance in the time interval (−)25–38 msec and 267–322 msec (coinciding with the latency of the second half of the P2 wave). The interaction between modality change and uncertainty across time was not significant ($p > .05$).

Auditory-induced ERS and ERD

Grand-average time–frequency spectrograms of the auditory-induced EEG responses obtained in the four experimental conditions are shown in Figure 6. Significances of ANOVA main effects and interactions are summarized in Table 2.

Effect of Modality Change

Similarly to what observed in the laser-induced time–frequency responses, there was a significant main effect of the factor modality change on the ER% summary values of AEP and ERS time–frequency responses elicited by S3. The magnitudes of the AEP and ERS responses were significantly larger when the S3 auditory stimulus was preceded by laser S1 and S2 stimuli (triplet different) than when it was preceded by auditory S1 and S2 stimuli (triplet same) [AEP: $F(1, 11) = 12.357, p = .005$; ERS: $F(1, 11) = 10.850, p = .007$]. There was no main effect of the factor modality change on the ERD response elicited by S3 auditory stimuli [$F(1, 11) = 0.093, p = .77$].

Effect of Uncertainty

There was no significant main effect of the factor uncertainty on the ER% summary values of all three time–frequency responses elicited by S3 [AEP: $F(1, 11) = 3.300, p = .10$; ERS: $F(1, 11) = 0.221, p = .65$; ERD: $F(1, 11) = 0.209, p = .66$].

Interaction between Modality Change and Uncertainty

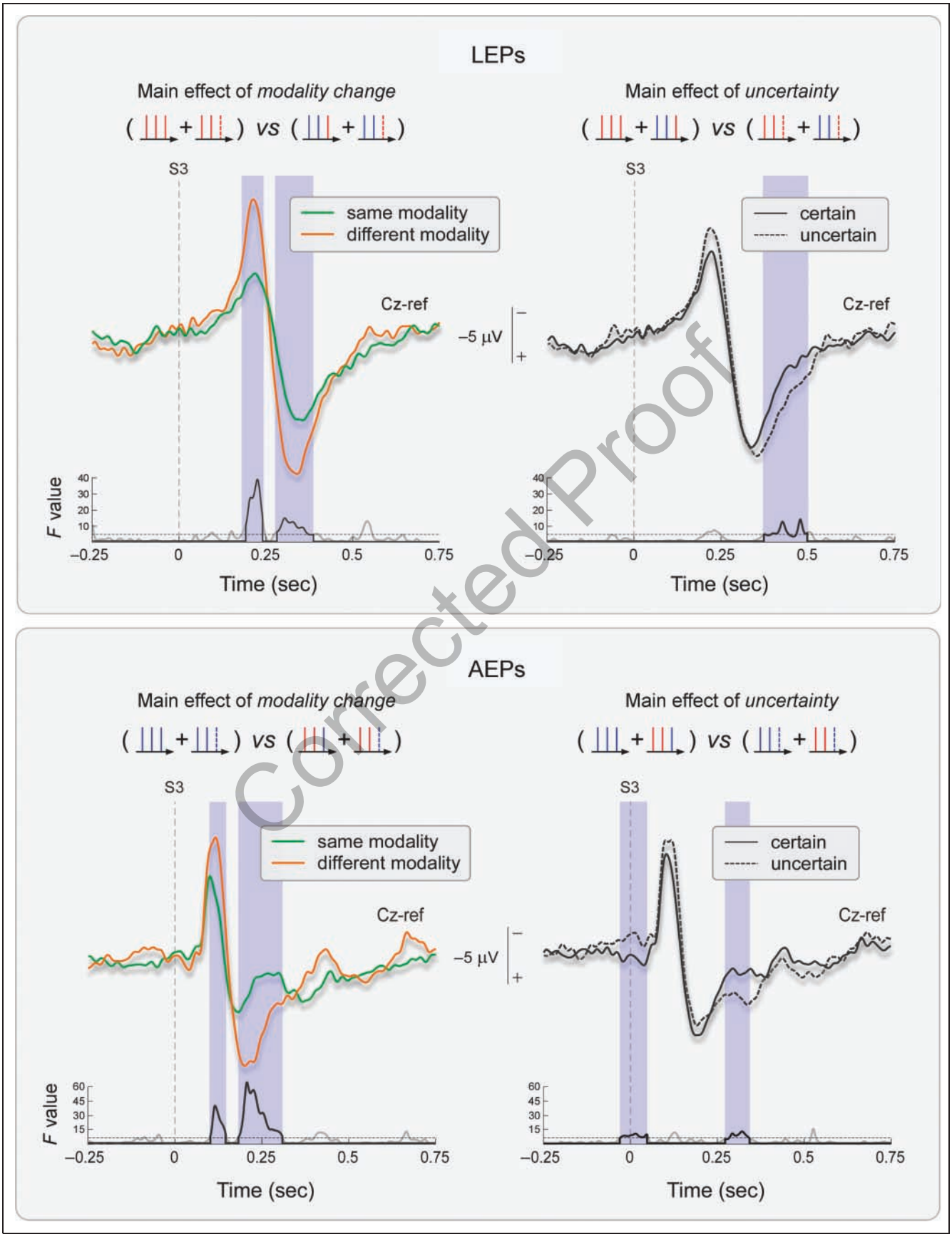
There was no significant interaction between the factors modality change and uncertainty on the ER% summary values of all three time–frequency responses elicited by S3 [AEP: $F(1, 11) = 0.007, p = .93$; ERS: $F(1, 11) < 0.001, p = .99$; ERD: $F(1, 11) = 0.371, p = .55$].

DISCUSSION

Here we aimed to tease out the selective contribution of (1) the occurrence of a change in stimulus modality and (2) the uncertainty of the occurrence of such a change in determining the magnitude of laser-evoked EEG responses. We observed two main findings. First, introducing a change in the sensory modality of the stimulus produced a significant dishabituation of the N1, N2, and P2 waves of LEPs and of the N1 and P2 waves of AEPs, as well as of the laser- and auditory-induced ERS and ERD. This finding is compatible with the view that all these responses reflect brain processes that are sensitive to bottom–up stimulus-driven changes in the pattern of a stream of sensory input, including changes in sensory modality. Second, the lack of knowledge of a possible change in stimulus modality (i.e., the uncertainty of such a change) did not increase the peak amplitude of the N1, N2, and P2 waves of LEPs and of the N1 and P2 waves of AEPs, as well as of the magnitude of laser- and auditory-induced ERS and ERD. The factor uncertainty only increased the ascending portion of the laser-evoked and auditory-evoked P2 wave. This finding indicates that, unlike bottom–up stimulus-driven changes of stimulus modality, top–down cognitive expectation of such a change is not a major determinant of the EEG response habituation induced by stimulus repetition.

Altogether, these results indicate that novelty resulting from the introduction of a change in the modality of a sensory stimulus, independently of explicit instruction-driven expectations, plays a major role in determining the magnitude of the laser-evoked EEG responses elicited

Figure 4. Main effect of modality change and uncertainty of modality change on LEPs (top) and AEPs (bottom). Superimposition of orange and green waveforms represents the main effect of modality change (left part of each panel). Superimposition of full and dashed black waveforms represents the main effect of the uncertainty of modality change (right part of each panel). The vertical dashed lines mark the onset of S3. Average peak amplitudes and scalp maps are shown in the insets. Note the significant main effect of modality change in determining the response magnitude of all ERP peaks.



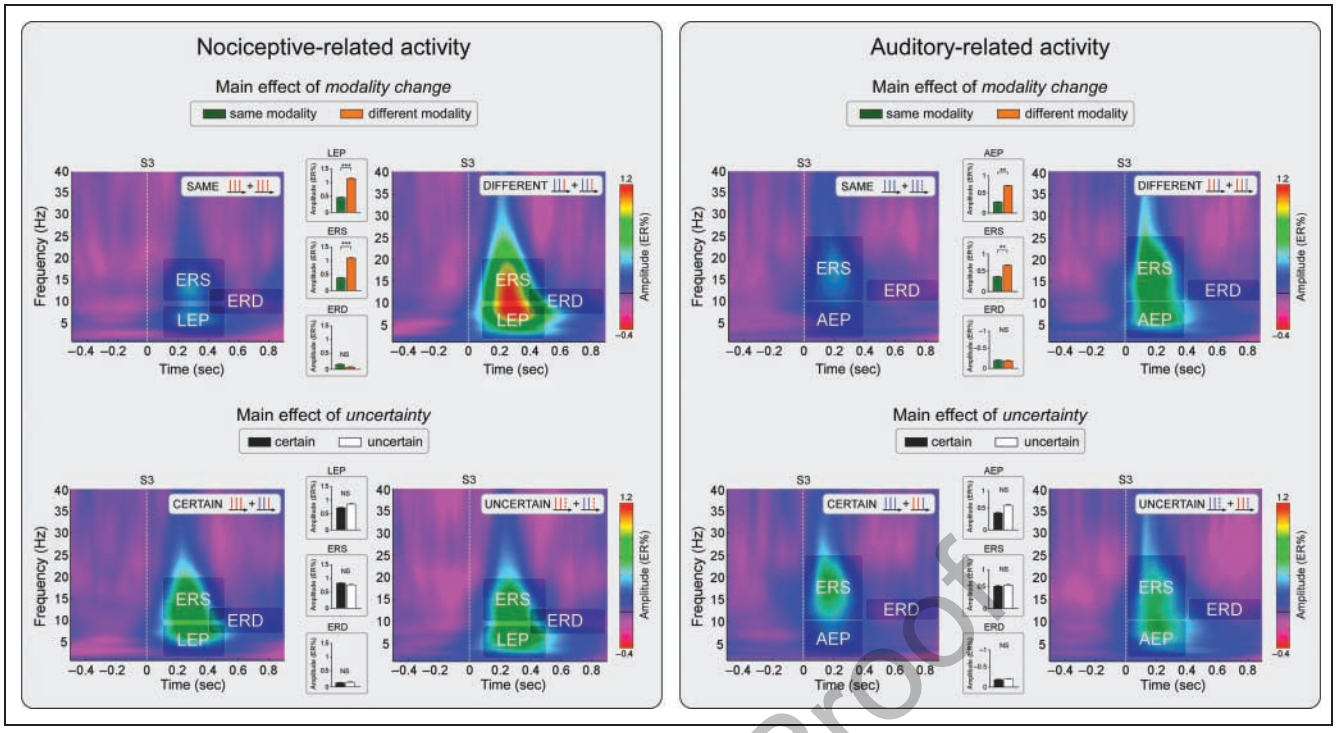


Figure 6. Main effect of modality change and uncertainty of modality change on laser-induced (left panel) and auditory-induced (right) ERS and ERD. Plots represent time–frequency representation of stimulus-induced modulation of EEG oscillation amplitude at electrode Cz (nose reference). *x*-axis, time (sec); *y*-axis, frequency (Hz). The vertical dashed lines mark the onset of the S3. The color scale represents the average increase (%ERS) or decrease (%ERD) of oscillation amplitude, relative to the prestimulus of S1 (−0.5 to −0.1 sec). Three time–frequency ROIs were defined. For each ROI, a summary measure was obtained by averaging the top 20% time–frequency points displaying the highest increase (ROIs "ERP" and "ERS") or decrease ("ERD") of signal. Note the significant effect of modality change in determining the response magnitude of both ERP and ERS.

by repeated stimulation, thus suggesting that these responses are mainly related to the bottom–up detection of salient sensory input.

Bottom–up Effect of Introducing a Change in Sensory Modality

When we described the habituation of the ERPs elicited by three repeated laser stimuli, each containing the same modality, location, intensity, and timing information (Iannetti et al., 2008), we were able to show that these responses habituate strongly to stimulus repetition, but we were unable to determine which type of sensory information is important to determine the habituation of these ERP responses. In the present experiment, we aimed to characterize the *selective* effect of stimulus modality in determining ERP habituation and dishabituation. Using a similar triplet paradigm, we observed that introducing a *selective* change of the modality of the repeated stimulus (i.e., without altering its intensity, location and timing) (Figure 1) significantly reverted the response reduction induced by stimulus repetition (Figure 2). Indeed, virtually all the EEG responses elicited by either nociceptive or auditory stimuli, both in the time domain (Figures 3, 4, and 5) and in the time–frequency domain (Figure 6), were significantly larger when the eliciting stimulus (S3) belonged to a sensory modality different from that of the two preceding stimuli (S1 and S2) (i.e., in the conditions AAL and LLA) than when the eliciting stimulus (S3) belonged to the same sensory modality (i.e., in the conditions LLL and AAA) (Figures 2 and 3).

The finding that introducing a change in sensory modality produces a strong dishabituation of the N1, N2, and P2 waves of LEPs as well as the N1 and P2 waves of AEPs is compatible with the notion that these waves reflect brain processes related to a multimodal network devoted to the detection and reaction to salient changes in the sensory environment (Mouraux & Iannetti, 2009; Downar, Crawley, Mikulis, & Davis, 2000, 2002). Indeed, the saliency of a sensory stimulus is often defined as a stimulus-driven bottom–up feature that is determined by the amount by which the

Figure 5. Whole-waveform ANOVA. To assess the time course of the effects of modality change and uncertainty on LEPs (top) and AEPs (bottom), we performed a repeated measures ANOVA using each time point of the averaged waveforms at Cz (nose reference). Below each waveform, the *F* values for each time point are represented. Time intervals in which the effect was significant (LEP threshold, $F = 4.8$; AEP threshold, $F = 4.4$) are highlighted in light blue (consecutivity threshold = 50 msec). Note that the factor modality change significantly modulated the ERP waveforms in time intervals coinciding with the main peaks, whereas the factor uncertainty significantly modulated both the LEP and AEP waveforms in the ascending part of the P2 wave.

Table 2. Summary of ANOVA Results: Auditory-evoked Responses

	F	*p*
Time Domain		
N1-wave amplitude		
Main effect of modality change	15.006	.003*
Main effect of uncertainty	4.763	.051
Interaction	0.219	.65
P2-wave amplitude		
Main effect of modality change	38.834	<.001**
Main effect of uncertainty	0.442	.52
Interaction	0.042	.84
Time–Frequency Domain		
ROI AEP		
Main effect of modality change	12.357	.005*
Main effect of uncertainty	3.300	.10
Interaction	0.007	.93
ROI ERS		
Main effect of modality change	10.850	.007*
Main effect of uncertainty	0.221	.65
Interaction	<.001	.99
ROI ERD		
Main effect of modality change	0.093	.77
Main effect of uncertainty	0.209	.66
Interaction	0.371	.55

*$p < .01$.

**$p < .001$.

sensory stimulus contrasts from its surroundings, and by the amount by which the stimulus contrasts from past experiences (Kayser, Petkov, Lippert, & Logothetis, 2005). In other words, in the present experiment, the saliency of the repeated stimulus was strongly determined by whether or not the physical features of the repeated stimulus differed from the physical features of the preceding stimuli. Both cingulate cortex, which is thought to be the main generator of the N2 and P2 waves of LEPs, and operculo-insular cortex, which is thought to be the main generator of the N1 wave and to contribute to the N2 wave of LEPs (Garcia-Larrea et al., 2003), have been identified as structures constituting the multimodal saliency-detection network identified by Downar et al. (2000) using fMRI.

An alternative possibility to the view that the N1, N2, and P2 waves of LEPs and the N1 and P2 waves of AEPs reflect mainly a *multimodal* network related to the detection of saliency independently of sensory modality is that they instead reflect *unimodal* brain processes related to the detection of saliency within the nociceptive and auditory modality, respectively. Indeed, Downar et al. (2000, 2002) showed that the introduction of a sudden change in the sensory environment elicits both multimodal responses, that is, responses related to the detection of saliency independently of sensory modality, and unimodal responses, that is, responses related to the detection of saliency within a specific sensory modality. Hence, the finding that the magnitude of the S3 response in a different triplet was stronger than the magnitude of the S3 response in a same triplet could be due to the fact that, in a different triplet, the S3 stimulus was the first stimulus belonging to the nociceptive (triplets AAL) or auditory (triplets LLA) modality, and not to the fact that the modality of S3 was different from the modality of the preceding stimuli.

Indeed, if one considers the possibility that LEPs and AEPs reflect, at least in part, neural activity originating from distinct cortical generators (e.g., nociceptive-specific activity possibly originating from operculo-insular cortices or auditory-specific activity possibly originating from auditory cortex; Yvert, Fischer, Bertrand, & Pernier, 2005; Frot & Mauguiere, 2003; Godey, Schwartz, de Graaf, Chauvel, & Liegeois-Chauvel, 2001; Frot, Rambaud, Guenot, & Mauguiere, 1999; Giard et al., 1994), then the observed results could be explained by refractoriness of these cortical generators.

However, this interpretation cannot account fully for the observed results, for the following reasons. First, several studies have shown that the magnitude of both LEPs and AEPs is strongly conditioned by the *context* within which the repetition of the nociceptive or auditory stimuli occurs: Stimulus repetition reduces the magnitude of LEPs and AEPs when the stimuli are presented using an ISI that is constant from trial to trial, but not when the ISI is variable from trial to trial (Wang, Mouraux, Liang, & Iannetti, 2010; Mouraux & Iannetti, 2008a; Mouraux, Guerit, & Plaghki, 2004; Budd & Michie, 1994; Loveless, Hari, Hamalainen, & Tiihonen, 1989). Hence, these studies demonstrate that, at the ISIs used in the present study, most of the neural generators underlying LEPs and AEPs are *not* in a refractory state. Second, if the greater reduction of S3 response magnitude observed in same than in different triplets was due to refractoriness of unimodal generators, one would have expected the scalp topography of the S3-ERP to be noticeably different between the two conditions, because of the change in the relative contribution of unimodal versus multimodal generators.

Although the functional significance of the N1, N2, and P2 waves of LEPs is progressively being clarified (e.g., Legrain et al., 2009; Mouraux & Iannetti, 2009; Iannetti et al., 2008; Legrain, Bruyer, Guerit, & Plaghki, 2005), the functional significance of laser-induced ERS and ERD (Mouraux et al., 2003) has not been investigated to the same extent. Here we show that the laser-induced ERS at ~15 Hz and 270 msec (Figure 6) undergoes a modulation similar to that observed in the N1, N2, and P2 waves of LEPs. Although there is no

reliable information about the cortical origin of this laser-induced ERS, the observation that its magnitude is strongly reduced by stimulus repetition (Iannetti et al., 2008) and is increased by the introduction of a change in the modality of the repeated stimulus (Figure 6) suggests that, similar to the N1, N2, and P2 waves of LEPs, the laser-induced ERS also reflects the activity of a multimodal network related to the detection of salient sensory events. It is worth noting that a similar lack of dissociation between N1, N2, and P2 waves of LEPs and laser-induced ERS was already observed in response to a different experimental manipulation (Iannetti et al., 2008). Altogether, these findings suggest that all these responses reflect functionally similar neural activities.

Top–down Effect of Uncertainty of Change of Modality

In striking contrast with the robust effect of introducing a change in the sensory modality of the repeated stimulus, lack of explicit knowledge (uncertainty) of the occurrence of such a change (i.e., top–down cognitive expectations) contributed only marginally to the observed response dishabituation. Indeed, the only robust effect consisted in a difference in the amplitude of the ascending portion of the P2 wave, which was greater when subjects had no explicit knowledge of the occurrence of a change in the sensory modality of S3.

The finding that the uncertainty of the occurrence of a change in stimulus modality has only a limited effect on the amplitude of the elicited ERPs is important. Indeed, it indicates that the magnitude of LEPs is largely dependent on the novelty of the stimulus, independently of cognitive expectations. It has been recently shown that when two consecutive laser stimuli are applied using the same short (e.g., 1 sec or less) interstimulus interval across all trials of a block, the magnitude of the LEP elicited by the second stimulus is reduced compared to the magnitude of the LEP elicited by the first stimulus (e.g., Truini et al., 2004). In contrast, when two consecutive laser stimuli are applied using the same short interstimulus interval, but randomly varied from trial to trial, the LEP elicited by the second stimulus is entirely unaffected by stimulus repetition, even at ISIs as short as 280 msec (Mouraux et al., 2004). The results of the current study indicate that these two opposite findings could be largely due to differences in bottom–up stimulus-driven novelty, independently of top–down cognitive expectations.

The finding that cognitive expectation modulates the magnitude of the ascending portion of the P2 wave, which was enhanced when the modality of S3 was uncertain (Figure 4), agrees with previous literature on both behavioral (Clark, Brown, Jones, & El-Deredy, 2008) and neural effects of expected versus unexpected stimuli (Legrain, Bruyer, et al., 2003; Legrain, Guerit, et al., 2003; Legrain et al., 2002). Indeed, it was shown that when the occurrence of a laser stimulus is uncertain and behaviorally relevant, part of the response in the latency range of the laser-evoked P2 can be explained by the enhancement of overlapping neural activities, such as the P3a wave (sometimes referred to as P400; Legrain, Guerit, et al., 2003), interpreted as reflecting neural processes related to the triggering of an involuntary shift of attention toward unexpected sensory events (see also Legrain et al., 2009), and the P3b wave, interpreted as reflecting neural processes related to the detection of novel attended events (Polich, 2007; Legrain et al., 2002).

Laser-induced and Auditory-induced ERD

The only EEG responses that were not modulated either by the occurrence of a change in stimulus modality or by the uncertainty of such a change were the laser-induced and auditory-induced event-related desynchronization centered at ~13 Hz (Figure 6). The fact that the magnitude of this ERD does not correlate with the intensity of perceived pain (Iannetti et al., 2008; Mouraux et al., 2003), and is not modulated by either stimulus repetition (Iannetti et al., 2008) or changes in the modality of the repeated stimulus (Figure 6) seems to agree with the view that the laser-induced ERD reflects aspecific brain processes, which are possibly related to sustained attentional and mnemonic processes. Indeed, the magnitude of alpha-band oscillations has been shown to vary with sensory, motor, and cognitive operations (reviewed in Pfurtscheller & Lopes da Silva, 1999), and alpha-band ERD has also been shown to occur in a wide range of cognitive tasks that engage specific attentional and mnesic processes. Also, stimuli of any sensory modality induce a transient suppression of alpha-band power, hypothesized to indicate activation (or disinhibition) of the cortical areas related to the processing of the incoming sensory stimulus (Yordanova, Kolev, & Polich, 2001; Sergeant, Geuze, & van Winsum, 1987).

Conclusion

Our results provide additional evidence that the magnitude of laser-evoked EEG responses is strongly dependent on the context within which the nociceptive stimuli are delivered, and thus, are far from being a faithful index of the incoming sensory information. Furthermore, our results indicate that these responses reflect neural processes strongly determined by the occurrence of a bottom–up change in the modality of the eliciting stimulus, and less strongly determined by explicit, top–down cognitive expectations.

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