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Determinants of Laser-Evoked EEG Responses: Pain Perception or Stimulus Saliency?

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Iannetti GD, Hughes NP, Lee MC, Mouraux A. Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? *J Neurophysiol* 100: 815–828, 2008. First published June 4, 2008; doi:10.1152/jn.00097.2008. Although laser-evoked electroencephalographic (EEG) responses are increasingly used to investigate nociceptive pathways, their functional significance remains unclear. The reproducible observation of a robust correlation between the intensity of pain perception and the magnitude of the laser-evoked N1, N2, and P2 responses has led some investigators to consider these responses a direct correlate of the neural activity responsible for pain intensity coding in the human cortex. Here, we provide compelling evidence to the contrary. By delivering trains of three identical laser pulses at four different energies, we explored the modulation exerted by the temporal expectancy of the stimulus on the relationship between intensity of pain perception and magnitude of the following laser-evoked brain responses: the phase-locked N1, N2, and P2 waves, and the non-phase-locked laser-induced synchronization (ERS) and desynchronization (ERD). We showed that increasing the temporal expectancy of the stimulus through stimulus repetition at a constant interstimulus interval *1*) significantly reduces the magnitudes of the laser-evoked N1, N2, P2, and ERS; and *2*) disrupts the relationship between the intensity of pain perception and the magnitude of these responses. Taken together, our results indicate that laser-evoked EEG responses are not determined by the perception of pain per se, but are mainly determined by the saliency of the eliciting nociceptive stimulus (i.e., its ability to capture attention). Therefore laser-evoked EEG responses represent an indirect readout of the function of the nociceptive system.

INTRODUCTION

Brief radiant heat pulses, generated by infrared laser stimulators, are used to excite selectively Aδ- and C-fiber free nerve endings located in the superficial layers of the skin (Bromm et al. 1984). Such stimuli elicit a number of electrical brain responses, some of which can be detected in the human electroencephalogram (EEG) (Carmon et al. 1976; Mouraux et al. 2003). Although the laser stimulus coactivates several distinct ascending somatosensory pathways (e.g., Iannetti et al. 2003), the detected responses have been shown to be exclusively related to the activation of type II Aδ mechano-heat nociceptors (Treede et al. 1995) and spinothalamic neurons located in the anterolateral quadrant of the spinal cord (Treede 2003). Several studies have shown that C-fiber input can also elicit detectable responses in the human EEG, but only if the concomitant activation of Aδ nociceptors is avoided (reviewed in Plaghki and Mouraux 2002). Aδ-related laser-evoked potentials (LEPs) have been used extensively to investigate the peripheral and central processing of nociceptive sensory input, both in physiological (e.g., Iannetti et al. 2003) and in physiopathological studies (reviewed in Treede et al. 2003), and are currently considered the best available diagnostic tool to assess the function of Aδ nociceptive pathways in patients (Cruccu et al. 2004).

LEPs consist of a number of deflections, time locked to the onset of the laser stimulus and embedded in the ongoing EEG signal. The largest deflections form a negative–positive complex (N2–P2; 160–390 ms when stimulating the hand dorsum; Bromm and Treede 1984), maximal at the scalp vertex. This complex is preceded by a smaller negative deflection (N1; ~160 ms; Garcia-Larrea et al. 1997) maximal over the temporal region contralateral to the stimulated side. 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 et al. 2003). They seem to result from sources in bilateral operculoinsular cortices, the anterior cingulate cortex, and, possibly, the contralateral primary sensory cortex. LEPs are known to be significantly modulated by attentional factors (reviewed in Lorenz and Garcia-Larrea 2003). In particular, Legrain et al. (2002, 2003) showed that the laser-evoked N1 and N2 waves are enhanced by spatial attention, suggesting that their sources are sensitive to "top-down" attentional mechanisms, whereas the laser-evoked P2 wave is enhanced by the probability of stimulus occurrence, suggesting that its sources are sensitive to "bottom-up" stimulus-driven mechanisms of arousal or attentional orientation.

Sensory stimuli do not only elicit time-locked deflections in the EEG (i.e., event-related potentials [ERPs]); they may also induce transient modulations of the ongoing oscillatory EEG activity. Because this oscillatory activity is not phase locked to the onset of the stimulus, it is cancelled out by the across-trial averaging procedures commonly used to reveal ERPs. Therefore alternative signal-processing techniques, based on the joint time–frequency decomposition of signals, must be used to reveal these stimulus-related modulations of ongoing EEG activity (Mouraux and Iannetti 2008a). These modulations may appear either as a transient increase (event-related synchronization [ERS]) or as a transient decrease (event-related desyn-

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chronization [ERD]) of EEG power, usually confined within a specific frequency band. The functional significance of ERS and ERD is thought to differ according to the frequency at 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 Lopes da Silva and Pfurtscheller 1999). In contrast, ERS in the gamma band (frequencies >40 Hz) has been hypothesized to reflect the formation of transient cortical assemblies and thus to play a role in cortical integration (Rodriguez et al. 1999; Tallon-Baudry et al. 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 (Mouraux et al. 2003; Ohara et al. 2004a; Ploner et al. 2006): a short-lasting ERS, starting about 160 ms after stimulus onset, followed by a long-lasting ERD, starting about 500 ms 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.

Numerous studies have shown that the magnitude of perceived pain is strongly correlated with the magnitude of the laser-evoked N2–P2 response (Arendt-Nielsen 1994; Beydoun et al. 1993; Bromm and Treede 1991; Iannetti et al. 2005a; Ohara et al. 2004b). In contrast, far less studies have demonstrated a comparable positive correlation between the magnitude of perceived pain and the magnitude of the earlier N1 response (Iannetti et al. 2005a) or the magnitude of the laser-induced ERS and ERD (Mouraux et al. 2003). Similarly, functional magnetic resonance imaging (fMRI) studies have shown a significant correlation between the magnitude of perceived pain and the magnitude of the hemodynamic response in an array of brain regions, including the primary and secondary somatosensory cortices, the insular cortex, and the anterior cingulate cortex (e.g., Coghill et al. 1999; Derbyshire et al. 1997). This reproducible finding has led to the often-accepted notion that these responses reflect "neural mechanisms for pain intensity coding in the human cortex" (Porro 2003) and arise from brain structures specifically involved in the conscious perception of pain (Coghill et al. 1999; Schnitzler and Ploner 2000; Timmermann et al. 2001; Tracey and Mantyh 2007). It is for these reasons that LEPs are sometimes called "pain-evoked potentials" (e.g., Edwards et al. 2007; Kakigi et al. 2000; Schmidt et al. 2007).

Keeping in mind the clear evidence for a significant correlation between the magnitude of perceived pain and the magnitude of laser-evoked brain responses, it is important to highlight that a number of studies have shown that when identical laser stimuli are presented at a short and constant interstimulus interval (ISI), the magnitude of the N2–P2 response is strongly reduced (Bromm and Treede 1987; Raij et al. 2003; Truini et al. 2004). However, most of these experiments aimed primarily at characterizing the reduction in N2–P2 magnitude as a function of the ISI, but did not examine the effect of stimulus repetition on the intensity of perceived pain. Thus a crucial question remains open: is the positive correlation between magnitude of the N2–P2 response and magnitude of perceived pain preserved when the magnitude of the N2–P2 response is reduced by stimulus repetition? A single anecdotal report suggests that this is not the case and that when a laser stimulus is shortly preceded by another identical laser stimulus, the intensity of the perceived pain elicited by both stimuli is the same, whereas the magnitude of the N2–P2 response elicited by the second stimulus is significantly reduced compared with the magnitude of the N2–P2 response elicited by the first stimulus (Treede et al. 2003). In other words, stimulus repetition at a constant ISI could lead to a strong reduction of LEP magnitude, without concomitantly reducing the intensity of pain perception.

Furthermore, as all these studies focused on the effect of stimulus repetition on the magnitude of the N2–P2 response, another important question remains unaddressed: are the other features of the laser-evoked EEG response (i.e., the earlier N1 response, the laser-induced ERS, and the laser-induced ERD) similarly reduced when identical laser stimuli are presented at short and constant ISIs?

Addressing these two questions would represent a significant step toward understanding of the functional significance of laser-evoked EEG responses. If the magnitude of these brain responses and the magnitude of perceived pain are equally reduced by stimulus repetition, this would suggest that laser-evoked EEG responses are closely related to neural mechanisms for pain intensity coding (Arendt-Nielsen 1990; Iannetti et al. 2005a; Kakigi et al. 2000; Ohara et al. 2004b; Price 2000) and that the observed reduction in response magnitude could be related, as suggested by some investigators (Truini et al. 2004, 2007), to refractoriness of the nociceptive afferent pathway. On the contrary, if stimulus repetition produces a clear dissociation between the magnitude of these brain responses and the magnitude of perceived pain (Treede et al. 2003), an alternative explanation would have to be put forward.

Here we addressed these questions by recording EEG responses elicited by laser pulses of different energies, delivered in trains of three identical stimuli with constant ISI of 1 s (see Fig. 1, *top*). This experimental design allowed us to characterize the respective effect of stimulus energy and stimulus repetition on both the intensity of perceived pain and the magnitude of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS and ERD.

METHODS

Subjects

Seven healthy subjects (five men and two women) aged 24–42 yr (mean 29 ± 6) participated in the study. The participants were recruited among research staff and PhD students of the University of Oxford (UK). All participants gave their written informed consent. The study conformed to the standards set by the Declaration of Helsinki and was approved by the local ethics committee.

Radiant-heat stimulation

Noxious radiant-heat stimuli were generated by an infrared neodymium yttrium aluminum perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Florence, Italy). At this short wavelength, the skin is very transparent to the laser radiation and, consequently, the laser pulses activate directly Aδ- and C-fiber nociceptive terminals located in the superficial layers of the skin (Iannetti et al. 2006). Laser pulses were directed at the dorsum of both the right and the left hands and a He–Ne laser pointed to the area to be stimulated. The laser beam was transmitted through an optic fiber

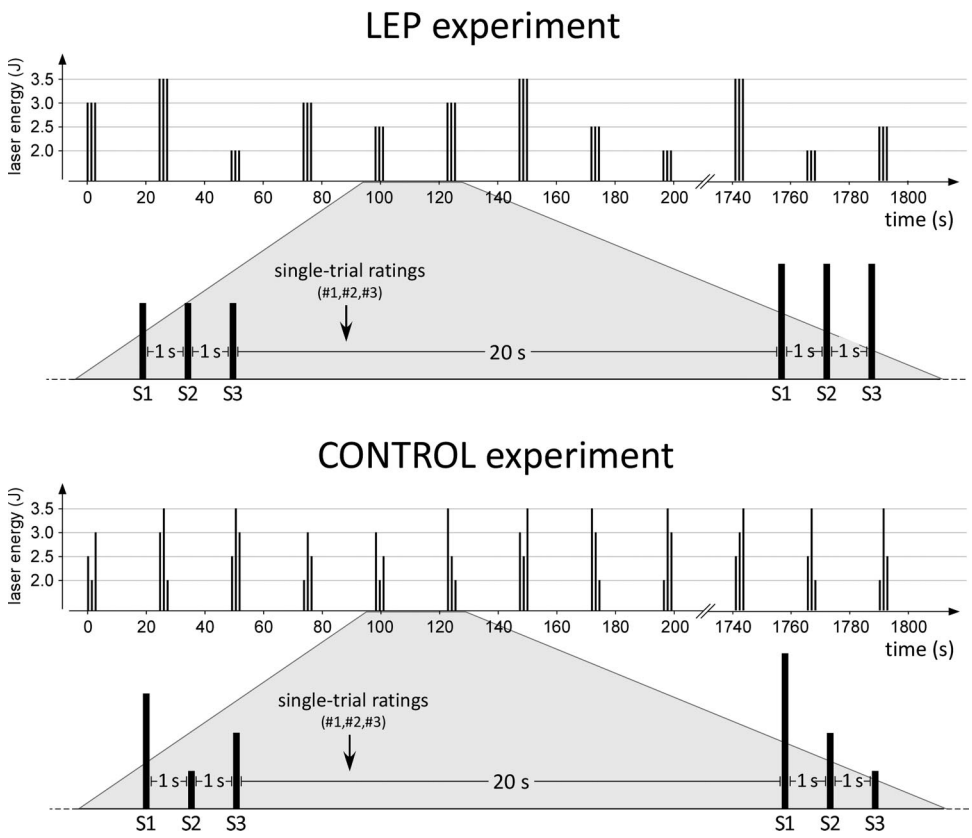

FIG. 1. Experimental design. *Top*: laser-evoked electroencephalographic (EEG) responses were recorded following the stimulation of the right and left hand dorsum, in two separate sessions on the same day. The order of sessions was balanced across subjects. In each session, stimuli were delivered in trains. Each train consisted of three laser stimuli of identical energy (S1–S2–S3: a triplet), delivered at constant interstimulus interval (ISI) of 1 s. The time interval between each triplet was 20 s. Four different stimulation energies were used (2, 2.5, 3, and 3.5 J). In each session, 20 triplets of each of the 4 stimulus energies were delivered in random order, for a total of 80 triplets per session. Between 3 and 6 s after the end of each triplet, subjects were asked to rate verbally the intensity of perceived pain using a numerical rating scale ranging from 0 to 100. *Bottom*: a control experiment was conducted to ensure that subjects were able to rate independently and reliably the intensity of perception of three laser pulses delivered at constant ISI of 1 s. As in the laser-evoked potential (LEP) experiment, 4 different laser energies were used and each train consisted of three stimuli (S1–S2–S3: a triplet) delivered at constant ISI of 1 s. However, the energy of each of the three stimuli was pseudorandomly varied within each triplet. Each of the four stimulus energies was presented 20 times in each stimulus of the triplet. The timing of both stimulus presentation and psychophysical rating was identical to that used in the LEP experiment.

and its diameter was set at approximately 7 mm (~38 mm^2) by focusing lenses. The duration of the laser pulses was 4 ms. Four different energies of stimulation were used (E1: 2 J; E2: 2.5 J; E3: 3 J; E4: 3.5 J). In a preliminary experiment we had found that stimuli with these characteristics produce a clear pinprick sensation, and result in subjective reports of a range of perceived intensities.

Experimental design

Before starting the recording, we delivered a small number of laser pulses to the dorsum of the right and the left hands in a pseudorandomized order, with the aim of familiarizing the subjects with the stimuli.

A schematic illustration of the experimental design is shown in Fig. 1. Laser-evoked EEG responses were recorded following the stimulation of the dorsum of the right and left hands, in two separate sessions on the same day. The order of the two sessions was balanced across subjects. In each recording session we delivered 20 trains at each of the four stimulus energies (E1–E4), in random order, for a total of 80 trains. Each train consisted of three stimuli (a triplet: S1–S3) of identical energy, delivered at a constant ISI of 1 s. The time interval between each triplet was 20 s. Between each laser pulse of a given triplet the target of the laser beam was manually displaced by about 1 cm along a proximal–distal line on the hand dorsum. The direction of this displacement was balanced in each session (40 stimuli in proximal and 40 stimuli in the distal direction). A proximal–distal spatial displacement was preferred to a medial–lateral displacement because the former minimizes the variations in thickness and innervation of the irradiated skin and, consequently, in the intensity of the nociceptive input (Schlereth et al. 2001). An auditory tone simultaneous to the onset of each laser pulse prompted the experimenter to displace the handheld optical device connected to the end of the optic fiber. Between 3 and 6 s after the end of each triplet, subjects were asked to rate verbally the intensity of the Aδ-related pricking sensation elicited by each of the three laser stimuli constituting the triplet, using a numerical rating scale ranging from 0 to 100, where 0 was defined as "no pain" and 100 was defined as "pain as bad as it could be" (Jensen et al. 1989). This procedure provided ratings for each individual percept elicited by each individual laser pulse of the triplet. Subjects were not asked to rate the burning, C-related second pain, because no LEP response can be elicited by a C-fiber afferent volley when it is preceded by an Aδ afferent volley (Plaghki and Mouraux 2003). Because variations in baseline skin temperature could bias results (Tjolsen et al. 1988), an infrared thermometer was used to ensure that baseline skin temperatures were similar at the beginning and at the end of each recording session.

To ensure that subjects were able to independently and reliably rate the intensity of three consecutive laser stimuli presented at 1-s ISI, we performed a control psychophysical experiment (Fig. 1, *bottom*). In this additional experiment 80 triplets of laser pulses were delivered to

the dorsum of the left hand of five subjects. As in the LEP experiment, four different laser energies were used. However, instead of using the same stimulus energy for S1, S2, and S3 (Fig. 1, *top*), the stimulus energy was pseudorandomly varied *within* each triplet (Fig. 1, *bottom*). For each stimulus of the triplet, each of the four stimulus energies was presented 20 times. The timing of stimulus presentation and psychophysical rating was identical to that used in the LEP experiment.

EEG recording

Participants were seated in a comfortable chair and wore protective goggles. They were asked to focus on the stimulus, relax their muscles, keep their eyes open, and gaze slightly downward. Acoustic isolation was ensured using earplugs and headphones. Brain electrical activity was recorded from seven silver disc electrodes placed on the scalp, according to the international 10–20 system: Fz, Cz, Pz, C3, C4, T3, and T4. The nose was used as a common extracephalic reference. Signals were digitized at a sampling rate of 4,096 Hz and a precision of 12 bits, giving a resolution of 0.195 μV (System Plus; Micromed, Treviso, Italy). To monitor ocular movements and eye blinks, electro-oculographic (EOG) signals were simultaneously recorded from two surface electrodes, one placed over the right lower eyelid, the other placed 1 cm lateral to the outer canthus of the right eye.

EEG analysis

Preprocessing and statistical analysis of EEG data were carried out using Letswave (http://amouraux.webnode.com; see also Mouraux and Iannetti 2008b), a free signal-processing tool developed in Delphi 6.0 (Borland Software, Austin, TX). Additional statistical analyses were carried out using Prism 5.0 (GraphPad Software, San Diego, CA).

PREPROCESSING OF EEG DATA. Continuous EEG data were downsampled to 512 Hz and band-pass filtered from 0.5 to 30 Hz (for analysis in the time domain) and from 0.5 to 100 Hz (for analysis in the time–frequency domain) using a fast Fourier transform filter. EEG data were then segmented into epochs using a time window ranging from 2 s before the first stimulus (S1) to 2 s after the third stimulus (S3) of each triplet (total epoch duration: 6 s). Each EEG epoch was baseline corrected, using the time interval ranging from −0.5 to 0 s as reference. EEG epochs were then visually inspected and trials contaminated by artifacts due to gross movements were removed. Finally, artifacts due to eye blinks or eye movements were subtracted using a method based on an independent-component analysis (FastICA algorithm; Hyvarinen and Oja 2000). In a study examining EEG responses evoked by visual stimuli, this method was shown to be more efficient than more conventional regression-based methods (Jung et al. 2000). In all data sets, individual eye movements, showing a large EOG channel contribution and a frontal scalp distribution, could be clearly seen in the removed independent components.

EEG ANALYSIS IN THE TIME DOMAIN. For each subject, the EEG epochs were averaged time locked to the onset of the first stimulus (S1) of each triplet. Furthermore, EEG epochs were classified in four categories according to the intensity of pain perception (I1–I4). This was achieved after rescaling the ratings of each subject between 0 and 100, defining 0 as the smallest pain rating and 100 as the largest pain rating of that subject. This procedure yielded four average waveforms for each subject (I1: 0–25; I2: 26–50; I3: 51–75; I4: 76–100). The number of trials contributing to each category was not significantly different.

The amplitude and the latency of the laser-evoked N2 and P2 peaks were measured at all channels. All amplitudes were measured from baseline to peak. The N2 wave was defined as the most negative deflection following the onset of each stimulus of the triplet. The P2 wave was defined as the most positive deflection following the onset of each stimulus of the triplet. The latency and amplitude of the laser-evoked N1 peak were estimated by averaging the signals recorded at the temporal electrode contralateral to the stimulated side (electrode T3 when stimulating the right hand dorsum; electrode T4 when stimulating the left hand dorsum). Within this average waveform, the N1 wave was defined as the most negative deflection preceding N2.

To assess the effect of the factor "stimulus repetition" (S1–S3, which refers to the repetition of three identical laser pulses at constant 1-s ISI) and the factor "intensity of perception" (I1–I4), as well as the interaction between these two factors, we performed a two-way repeated-measures ANOVA using the measured amplitude and latency of the laser-evoked N1, N2, and P2 peaks. When the effect of the factor "stimulus repetition" was significant, we performed a post hoc analysis using a paired-sample *t*-test to compare the responses elicited by S1, S2, and S3. When the effect of the factor "intensity of perception" was significant, we performed a post hoc analysis using a linear regression between intensity of perception and response magnitude to examine their correlation. When the interaction between the factors "stimulus repetition" and "intensity of perception" was significant, we performed a post hoc analysis comparing the slopes of the linear regression between intensity of perception and response magnitude for S1, S2, and S3, to assess how the correlation between intensity of perception and response magnitude was affected by stimulus repetition.

Furthermore, to disclose the time course of the effects of "stimulus repetition" and "intensity of perception," we performed the same repeated-measures ANOVA, but using each time point of the averaged ERP waveforms. This yielded two waveforms expressing the significance of the effect of each of the two experimental factors across time.

EEG ANALYSIS IN THE TIME—FREQUENCY DOMAIN. *Continuous wavelet transform.* A time–frequency representation of each single EEG epoch was obtained using the continuous wavelet transform. As compared with the windowed Fourier transform, which decomposes the signal using a fixed window of analysis, the wavelet transform adapts the width of its window of analysis as a function of frequency, and thereby offers an optimal compromise for time–frequency resolution (Mouraux and Iannetti 2008a; Mouraux et al. 2003). At low frequencies, temporal resolution is less important than frequency resolution because low-frequency changes (e.g., a slow drift in the signal) cannot be precisely located in time, but can be precisely defined in frequency. Therefore when estimating low frequencies, the wavelet transform uses a wide window, resulting in a low temporal resolution but a high-frequency resolution. In contrast, high-frequency changes (e.g., a brief discontinuity in the signal) can be precisely located in time, but not in frequency. Therefore when estimating high frequencies, the wavelet transform uses a narrow window, resulting in a high temporal resolution but a low-frequency resolution. For this reason, the wavelet transform is particularly well suited to explore the wide frequency spectrum of the EEG. A Morlet wavelet, used as a basis function, consists of a complex exponential function that is localized in time by a Gaussian envelope. The initial spread of the Morlet function was set to $2.5/\pi\omega_0$ (ω_0 being the central frequency of the wavelet). This "mother" wavelet was then contracted (resulting in an increase of its central frequency and a decrease of its window width) or dilated (resulting in a decrease of its central frequency and an increase of its window width) to obtain a set of "daughter" wavelets used to explore frequencies ranging from 1 to 101 Hz in 1-Hz steps (for details of the analysis see Mouraux and Iannetti 2008a; Mouraux et al. 2003). The modulus of the transform expressed the oscillation amplitude as a function of time and frequency. 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 to identify non-phase-locked, laser-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 (−900 to −100 ms before the onset of S1; ER%).[1]

Quantitative analysis of time–frequency spectrograms. To summarize the differences between the brain responses observed in the different experimental conditions (S1–S3; I1–I4), three time–frequency windows of interest were defined, centered around the locations of the three main foci of activity. Time and frequency limits of each window of interest were as follows: LEP: 100–500 ms and 2–8 Hz; ERS: 100–500 ms and 10–20 Hz; and ERD: 400–900 ms and 7–13 Hz. Within each window of interest, ER% values were extracted to compute the mean of the 20% of points displaying the highest increase (LEP 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 we have successfully used to analyze blood oxygen level–dependent fMRI data (Iannetti et al. 2005b; Mitsis et al. 2008), shows several advantages for disclosing condition-specific effects (Mouraux and Iannetti 2008a): *1*) it takes into account the functional variability between subjects; *2*) it avoids the problem of selecting just outlier values; *3*) it allows for comparisons between the same number of points in each window of interest across different periods; and *4*) it avoids the "regression to the mean" problem that would have been introduced if the same points had been compared across experimental conditions. Resulting summary values were then compared using a two-way repeated-measures ANOVA, with "stimulus repetition" (S1–S3) and "intensity of perception" (I1–I4) as factors. When the effect of "stimulus repetition" was significant, we performed a post hoc analysis using a paired-sample *t*-test to compare the responses elicited by S1, S2, and S3. When the effect of "intensity of perception" was significant, we performed a post hoc analysis using a linear regression between intensity of perception and response magnitude. When the interaction between the factors "stimulus repetition" and "intensity of perception" was significant, we performed a post hoc analysis comparing the slopes of the linear regression between intensity of perception and response magnitude for S1, S2, and S3, to assess how the correlation between intensity of perception and response magnitude was affected by stimulus repetition.

CORRELATION WITH PERCEPTION AT SINGLE-TRIAL LEVEL. The linear correlation between intensity of pain perception and magnitude of the laser-evoked brain responses elicited by S1, S2, and S3 was computed at the single-trial level, both in the time domain and in the time–frequency domain.

Correlation in the time domain. In the time domain this was achieved by computing, for each time point, the linear correlation (Pearson's *r*) between the EEG signal amplitude of that time point and the corresponding intensity of pain perception. Time points from −0.25 to 1 s were correlated with the magnitude of pain elicited by S1, time points from 1 to 2 s were correlated with the magnitude of pain elicited by S2, and time points from 2 to 3 s were correlated with the magnitude of pain elicited by S3.[2] For each subject, this procedure yielded a waveform expressing Pearson's *r* against time.

Correlation in the time–frequency domain. In the time–frequency domain this was achieved by computing, for each time–frequency point, the linear correlation (Pearson's *r*) between the signal amplitude of that time–frequency point (ER%) and the corresponding intensity of pain perception. For each subject, this yielded a time–frequency map expressing Pearson's *r* against time and frequency.

[1] $ER(t,f)\% = [A(t,f) - R(f)]/R(f)$. For each estimated frequency f, $A(t,f)$ is the signal amplitude at a given time t, and $R(f)$ is the signal amplitude averaged within the reference interval (Pfurtscheller and Lopes da Silva 1999).

[2] The correlation between intensity of perception and signal magnitude in the 0.25-s time interval before the onset of S1 was computed to show that time points that do not contain stimulus-related activity do not correlate with intensity of perception.

RESULTS

Quality and intensity of perception

For each of the four energies used (E1–E4), laser stimuli elicited a clear pinprick sensation in all subjects, related to the activation of Aδ fibers (Bromm and Treede 1984). As expected, the intensity of pain perception was significantly and positively correlated with the energy of the laser stimulus ($P < 0.0001$; Fig. 2, *left*). In contrast, stimulus repetition (S1–S3) did not affect the intensity of pain perception ($P > 0.5$; Fig. 2, *left*). Last, there was no interaction between the experimental factors "stimulus energy" and "stimulus repetition" ($P > 0.5$).

The control experiment showed that the stimulus–response functions obtained in S2 and S3 were remarkably similar to the stimulus–response function obtained in S1 (Fig. 2, *right*). This finding demonstrates that subjects were able to independently and reliably rate the intensity of three consecutive laser stimuli presented at 1-s ISI.

Laser-evoked N1, N2, and P2 waves

In contrast to the intensity of pain perception, the magnitude of the laser-evoked N1 and of the following laser-evoked N2 and P2 was strongly modulated by the factor "stimulus repetition" (S1–S3; Figs. 3 and 4). Furthermore, although the magnitude of the responses elicited by S1 was strongly correlated with the intensity of perception (I1–I4; Figs. 5, 6, and 7), the magnitude of the responses elicited by S2 and S3 was significantly less correlated with the intensity of perception. The latency of N1, N2, and P2 was not modulated by either "stimulus repetition" or "intensity of perception."

EFFECT OF "STIMULUS REPETITION". The magnitudes of the laser-evoked N1, N2, and P2 elicited by S1, S2, and S3 were significantly different (N1: $F = 25.06$, $P < 0.0001$; N2: $F = 38.95$, $P < 0.0001$; P2: $F = 65.86$, $P < 0.0001$; see also Fig. 5). Post hoc comparisons revealed that the magnitudes of the responses elicited by S2 and S3 were significantly reduced compared with the magnitude of the responses elicited by S1

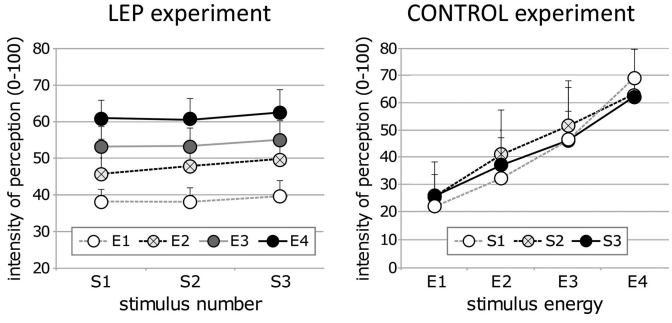

FIG. 2. Relationship between stimulus energy, stimulus repetition, and intensity of pain perception. Radiant heat (Nd:YAP laser) stimuli were delivered in triplets (S1–S3), using 4 stimulus energies (E1–E4), and constant ISI of 1 s. *Left*: the stimulus energy was identical across the 3 stimuli constituting the triplet ("LEP experiment"; see *top panel* of Fig. 1). *x*-axis, stimulus number; *y*-axis, rescaled intensity of pain perception. *Right*: the energy was pseudorandomly varied across the 3 stimuli constituting the triplet ("CONTROL experiment"; see *bottom panel* of Fig. 1). *x*-axis, stimulus energy; *y*-axis, rescaled intensity of pain perception. Error bars represent the SE. In both experiments, the intensity of perception was significantly and positively correlated with the energy of the laser stimulus (E1–E4), stronger stimuli leading to higher intensities of perception. Note how stimulus repetition (S1–S3) did not affect the intensity of pain perception.

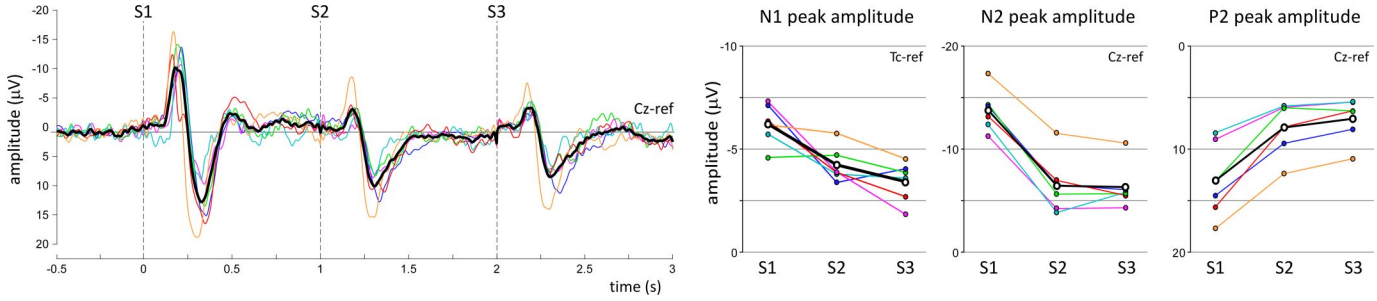

FIG. 3. Effect of stimulus repetition on laser-evoked brain potentials. *Left*: single-subject and group-level average LEPs elicited by S1, S2, and S3, and recorded at electrode Cz (nose reference). *x*-axis, time (s); *y*-axis, amplitude (μV). The vertical dashed lines mark the onset of the 3 laser stimuli (S1–S3). The colored waveforms represent single subjects, whereas the black waveform is the grand average across subjects. *Right*: single-subject and group-level average peak amplitudes of the N1, N2, and P2 waves elicited by S1, S2, and S3. *x*-axis, stimulus number (S1–S3); *y*-axis, amplitude (μV). Colored lines represent single subjects, whereas the black line is the group-level average. Note the significant decrease in N1, N2, and P2 amplitude between S1 and S2, with no further reduction between S2 and S3.

(N1: $P < 0.05$; N2: $P < 0.001$; P2: $P < 0.001$). However, the magnitude of the responses elicited by S2 was not significantly different from that elicited by S3 ($P > 0.1$). In other words, the laser-evoked N1, N2, and P2 all showed a similar modulation profile with a significant decrease in amplitude between S1 and S2 (N1: $-32 \pm 22\%$; N2: $-55 \pm 13\%$; P2: $-39 \pm 10\%$), but no further decrease between S2 and S3 (N1: $-17 \pm 25\%$; N2: $+3 \pm 25\%$; P2: $-9 \pm 8\%$). As shown in Fig. 4, this effect was significant across all scalp electrodes.

EFFECT OF "INTENSITY OF PERCEPTION". The magnitudes of the laser-evoked N1, N2, and P2 were significantly modulated by the factor "intensity of perception" (N1: $F = 7.44$, $P < 0.005$; N2: $F = 10.27$, $P < 0.0005$; P2: $F = 17.58$, $P < 0.0001$), with higher response magnitudes for stimuli perceived as more intense (Fig. 5). Post hoc analyses revealed a significant linear correlation between response magnitude and intensity of perception (N1: $r^2 = 0.56$, $P < 0.0001$; N2: $r^2 = 0.59$, $P < 0.0001$; P2: $r^2 = 0.70$, $P < 0.0001$; Fig. 7).

INTERACTION BETWEEN "STIMULUS REPETITION" AND "INTENSITY OF PERCEPTION". For all three laser-evoked responses (N1, N2, and P2), there was a significant interaction between the factors "stimulus repetition" and "intensity of perception" (N1: $F = 4.11$, $P < 0.005$; N2: $F = 2.33$, $P < 0.05$; P2: $F = 9.45$, $P < 0.0001$). Post hoc analysis revealed that the slopes of the linear correlation between response magnitude and intensity of perception for S1 were significantly different from those for S2 and S3 (N1: $F = 7.05$, $P < 0.005$; N2: $F = 4.23$, $P < 0.05$; P2: $F = 9.74$, $P < 0.0005$; see also Fig. 7); whereas the slopes of the linear regression between response magnitude and intensity of perception for S2 and S3 were remarkably similar (N1: $P = 0.69$; N2: $P = 0.77$; P2: $P = 0.36$), that for S1 was significantly steeper than those for S2 and S3 (N1: $P < 0.05$; N2: $P < 0.05$; P2: $P < 0.005$).

TIME COURSE OF THE EFFECT OF "STIMULUS REPETITION" AND "INTENSITY OF PERCEPTION". To follow the effect of these two experimental factors across time, we computed a two-way repeated-measures ANOVA for each time point of the averaged ERP waveforms. Results of this analysis are shown in Fig. 6. At electrode Cz, the factor "stimulus repetition" was a significant source of variance within three distinct time intervals: 152–230 ms (coinciding with the latency of N2), 275–372 ms (coinciding with the latency of P2), and 425–534 ms. The factor "intensity of perception" was also a significant source of variance and this within three similar time intervals: 156–214 ms (coinciding with the latency of N2), 275–404 ms (coinciding with the latency of P2), and 542–673 ms. A significant

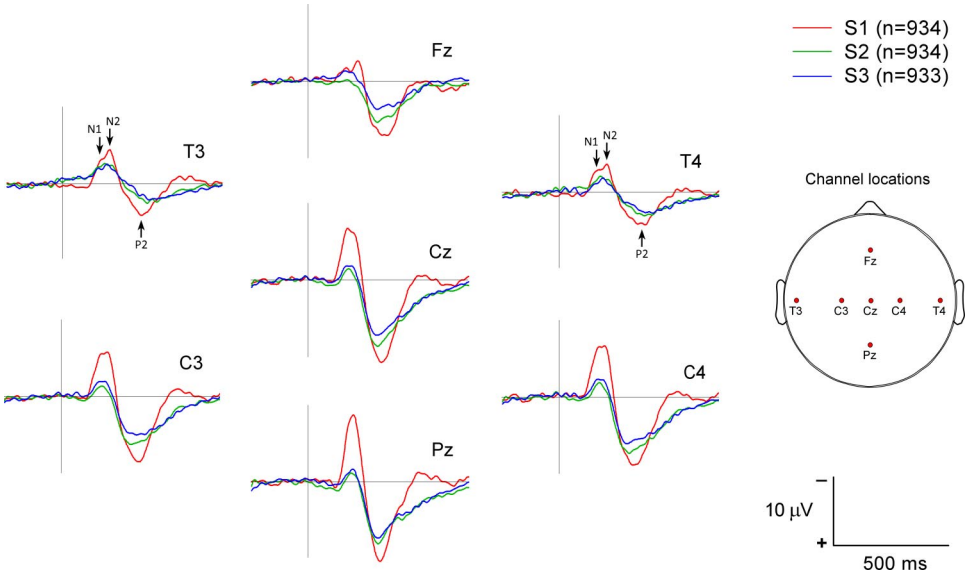

FIG. 4. Scalp distribution of the effect of stimulus repetition on LEPs. Group-level average waveforms recorded at different scalp electrodes (nose reference) whose positions are shown in the sketch on the *right*. LEPs elicited by the 1st (S1: red), the 2nd (S2: green), and the 3rd (S3: blue) stimuli are color coded and superimposed. *x*-axis, time (ms); *y*-axis, amplitude (μV). At all recorded electrodes there was a significant decrease in response amplitude between S1 and S2, with no further reduction between S2 and S3. Arrows indicate N1, N2, and P2 LEP peaks.

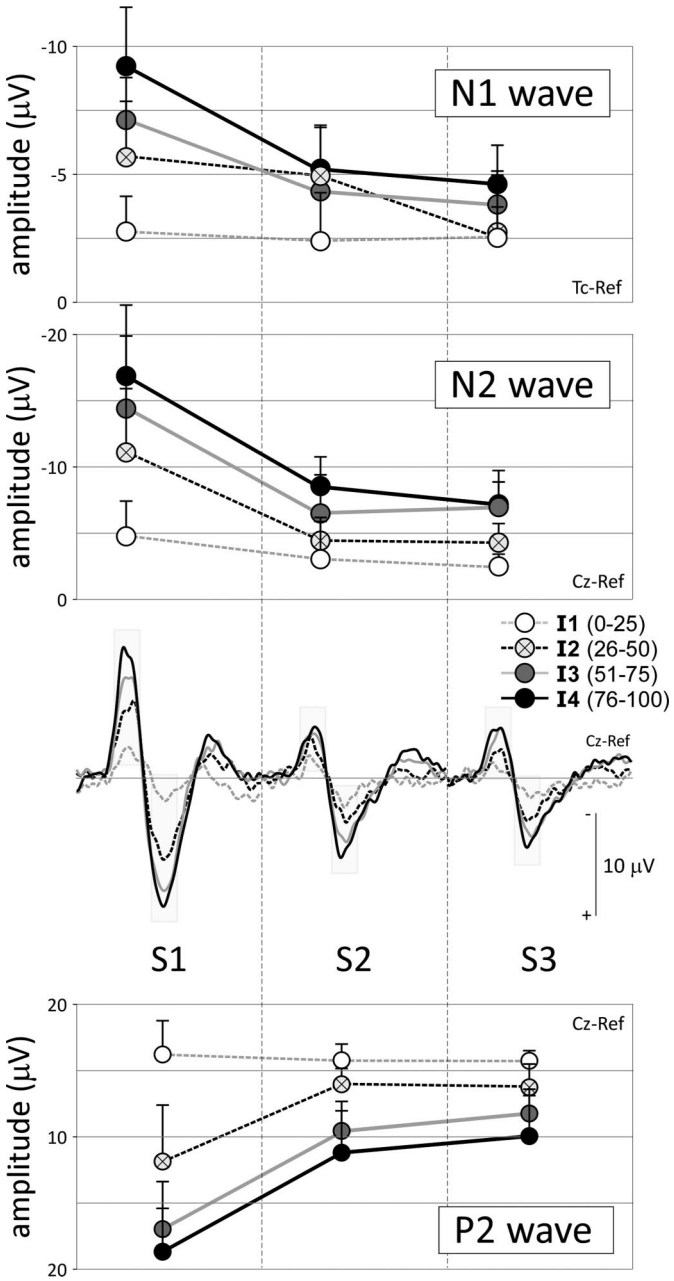

FIG. 5. Effect of stimulus repetition (S1–S3) and intensity of pain perception (I1–I4) on the peak amplitude of laser-evoked N1, N2, and P2 waves. EEG epochs were classified in 4 categories according to the rescaled intensity of pain perception (I1: 0–25; I2: 26–50; I3: 51–75; and I4: 76–100). Group-level average waveforms recorded at electrode Cz (nose reference) are displayed in the central plot. *x*-axis, time (s); *y*-axis, amplitude (μV). Group-level N1, N2, and P2 peak amplitude values are plotted in the *top* and *bottom graphs*. *x*-axis, stimulus number (S1–S3); *y*-axis, amplitude (μV). Note the strong effect of stimulus repetition on all 3 peaks, with a significant reduction in magnitude between S1 and S2, with no further reduction between S2 and S3. Note also the strong relationship between intensity of pain perception and amplitude of the N1, N2, and P2 peaks elicited by S1, and how this relationship is reduced when the same responses are elicited by S2 and S3.

interaction between the two factors was found in the first two time intervals: 153–196 ms (coinciding with the latency of N2) and 260–371 ms (coinciding with the latency of P2), showing that stimulus repetition reduced the strength of the relationship between intensity of perception and response magnitude.

Laser-induced ERS and ERD

The time–frequency analysis of EEG signals (Fig. 8) revealed that, in addition to the phase-locked N1, N2, and P2 waves ("LEP": window of interest, maximal at 285 ms, 3.7 Hz), the first laser stimulus (S1) elicited two distinct foci of non-phase-locked activity: an ERS ("ERS": maximal at 199 ms, 15.4 Hz), followed by an ERD ("ERD": maximal at 865 ms, 9.4 Hz). Despite the large size of the defined windows of interest, the peak latency and frequency of both responses were remarkably similar across subjects (Fig. 8, *bottom*).

The magnitude of the responses in windows "LEP" and "ERS" was strongly modulated by the factor "stimulus repetition" (S1–S3; Fig. 8). Furthermore, their magnitude was significantly and positively correlated with the factor "intensity of perception" (I1–I4; Figs. 7 and 8). In contrast, the magnitude of the response in window "ERD" was not modulated by either "stimulus repetition" or "intensity of perception."

EFFECT OF "STIMULUS REPETITION". The magnitudes of the responses in the windows of interest "LEP" and "ERS" elicited by S1, S2, and S3 were significantly different ("LEP": $F = 127.2$, $P < 0.0001$; "ERS": $F = 30.87$, $P < 0.0001$). Post hoc comparison revealed that the magnitude of the responses elicited by S2 and S3 were significantly reduced compared with the magnitudes of the responses elicited by S1 ("LEP": $P < 0.001$; "ERS": $P < 0.001$; see also Fig. 8). However, the magnitude of the responses elicited by S2 were not significantly different from that elicited by S3 ("LEP": $P = 0.63$; "ERS": $P = 0.11$). In other words, windows of interest "LEP" and "ERS" showed a similar modulation profile with a significant decrease in amplitude between S1 and S2 ("LEP": $-73 \pm 15\%$; "ERS": $-89 \pm 10\%$), but no further decrease between S2 and S3 ("LEP": $+17 \pm 5\%$; "ERS": $+5 \pm 29\%$). This nonlinear pattern of modulation was remarkably consistent across subjects (Fig. 8*B*). In contrast, stimulus repetition had no effect on the magnitude of the response in window of interest "ERD" ($F = 2.25$; $P = 0.319$, Fig. 8*B*).

EFFECT OF "INTENSITY OF PERCEPTION". The magnitudes of the responses in windows of interest "LEP" and "ERS" were significantly modulated by the factor "intensity of perception" ("LEP": $F = 8.34$, $P < 0.001$; "ERS": $F = 2.99$, $P < 0.05$), with higher response magnitudes for stimuli perceived as more intense (Fig. 8*C*). Post hoc analyses revealed a significant linear correlation between response magnitude and intensity of perception ("LEP": $r^2 = 0.55$, $P < 0.0001$; "ERS": $r^2 = 0.21$, $P < 0.05$). In contrast, intensity of perception had no effect on the magnitude of the response in window of interest "ERD" ($F = 0.48$; $P = 0.71$; Fig. 8*C*).

INTERACTION BETWEEN "STIMULUS REPETITION" AND "INTENSITY OF PERCEPTION". For both windows of interest "LEP" and "ERS" there was a significant interaction between the factors "stimulus repetition" and "intensity of perception" ("LEP": $F = 6.91$, $P < 0.0001$; "ERS": $F = 2.36$, $P < 0.05$), whereas there was no significant interaction for window of interest "ERD" ($F = 1.21$; $P = 0.32$). Post hoc analysis revealed that the slopes of the linear correlation between response magnitude and intensity of perception for S1 were significantly different from those for S2 and S3 ("LEP": $F = 8.68$, $P < 0.0005$; "ERS": $F = 4.66$, $P < 0.05$; see also Fig. 7); whereas the slopes for S2 and S3 were remarkably similar ("LEP": $P = 0.76$; "ERS": $P =$

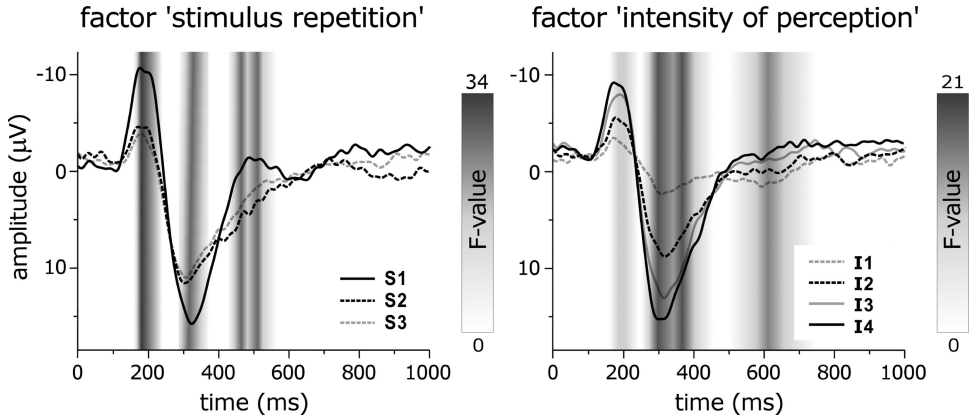

FIG. 6. Whole-waveform ANOVA. To assess the time course of the effect of "stimulus repetition" and "intensity of pain perception" on LEPs, we performed a repeated-measures ANOVA using each time point of the averaged waveforms (electrode Cz, nose reference). *x*-axis, time (ms); *y*-axis, amplitude (μV). *F*-values obtained for each time point are coded using a gray scale. *Left graph*: group-level LEP waveforms elicited by the 1st (S1), the 2nd (S2), and the 3rd (S3) stimulus of the triplet. The factor "stimulus repetition" significantly modulated the waveform in 3 distinct time intervals: 152–230 ms (coinciding with the latency of the N2 wave), 295–372 ms (coinciding with the latency of the P2 wave), and 425–534 ms. *Right graph*: group-level LEP waveforms categorized according to the intensity of pain perception (I1–I4; see also Fig. 5). The factor "intensity of perception" significantly modulated the waveform in 3 distinct time intervals: 156–214 ms (coinciding with the latency of the N2 wave), 275–404 ms (coinciding with the latency of the P2 wave), and 542–673 ms. In addition, a significant interaction between the 2 factors was found in 2 time intervals (153–196 and 260–371 ms), showing that stimulus repetition reduced the strength of the relationship between intensity of perception and response magnitude.

0.28), that for S1 was much steeper than those for S2 ("LEP": $P < 0.005$; "ERS": $P = 0.061$) and S3 ("LEP": $P < 0.005$; "ERS": $P < 0.05$).

Correlation with perception at the single-trial level

CORRELATION IN THE TIME DOMAIN. For each subject, the linear correlation between intensity of perception and waveform amplitude of each single EEG epoch was examined. Results of this analysis are shown in Fig. 9*A*. Following S1, the amplitude of the EEG signal at Cz was significantly ($P < 0.05$) correlated with the intensity of perception within two distinct time intervals. The first interval (144–220 ms) coincided with the latency of the N2 wave and the second interval (267–378 ms) coincided with the latency of the P2 wave. Following S2 and S3, the correlation between signal amplitude and intensity of perception was notably reduced.

CORRELATION IN THE TIME–FREQUENCY DOMAIN. For each subject, the linear correlation between intensity of perception and signal amplitude of the time–frequency decomposition of each single EEG epoch was examined. Results of this analysis are shown in Fig. 9*B*. Following S1, the signal amplitude showed a significant ($P < 0.05$) correlation with the intensity of perception in two distinct foci, located at 230 ms/3.7 Hz and 187 ms/14.8 Hz. The location of these foci matched the center of windows of interest "LEP" and "ERS." Notably, no significant correlation was found in the time–frequency window of interest "ERD" (500–800 ms, 8–12 Hz). Following S2 and S3, the correlation between signal amplitude and intensity of perception was notably reduced.

DISCUSSION

Our results show that the repetition of three identical laser pulses (S1–S3) at constant 1-s ISI does not affect the intensity of the elicited pain sensation (Fig. 2, *left*): the intensity of perceived pain elicited by the second (S2) and third (S3) stimuli of the triplet was not significantly different from the intensity of perceived pain elicited by the first (S1) stimulus of the triplet. Furthermore, the intensity of the pain elicited by each of the three stimuli of the triplet was strongly and positively correlated with the energy of the laser stimulus.

In contrast, the repetition of three identical laser pulses at constant 1-s ISI greatly reduces the magnitude of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS elicited by S2 and S3 (Figs. 3, 6, and 8). This reduction occurred entirely between S1 and S2, with no further reduction between S2 and S3. Furthermore, the magnitudes of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS elicited by S1 were strongly correlated with the intensity of perceived pain, whereas this correlation was markedly reduced for the responses elicited by S2 and S3 (Fig. 7).

Last, our results show that neither stimulus repetition nor intensity of pain perception affects the magnitude of the laser-induced ERD responses (Figs. 7 and 8).

The "refractoriness" hypothesis

We observed that stimulus repetition at short and constant ISI led to a significant reduction of the magnitude of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS (Figs. 3, 6, and 8), and that this reduction in magnitude occurred entirely between S1 and S2, with no further reduction between S2 and S3. This observation could be interpreted as a consequence of "neuronal refractoriness" of the polysynaptic nociceptive afferent pathway, an explanation put forward by some investigators (Truini et al. 2004) and currently debated (Mouraux and Iannetti 2008b). According to the "neuronal refractoriness" interpretation, the observed response decrement would be the consequence of basic neurophysiological mechanisms, related to the changes in the kinetics of potassium current that follow an action potential, leading to a transiently reduced state of neuronal excitability (Hille 1992). However, this interpretation is unlikely, since the duration of "neuronal refractoriness" is in the order of a few milliseconds (Hodgkin and Huxley 1952). Another mechanism

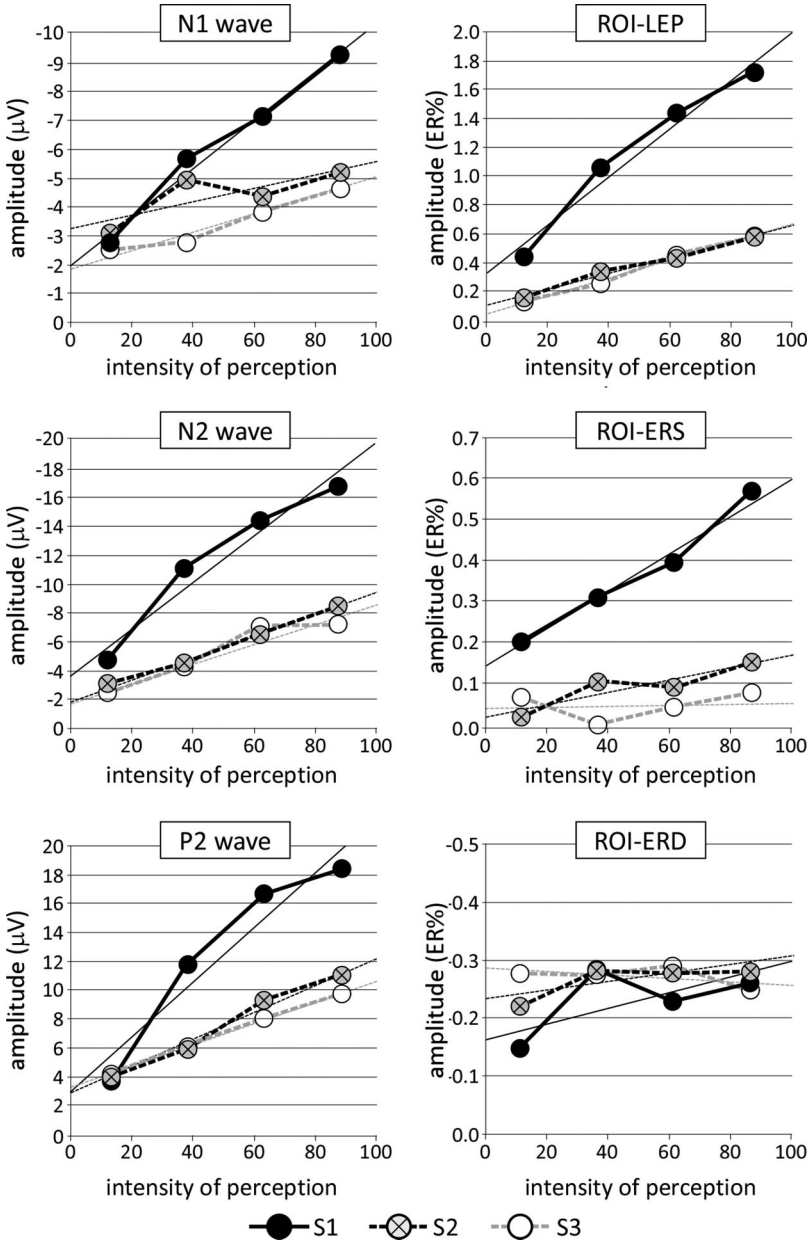

FIG. 7. Effect of stimulus repetition on the relationship between intensity of pain perception and magnitude of laser-evoked EEG responses. For each subject, trials were classified into 4 categories according to the rescaled ratings of perceived pain (I1: 0–25; I2: 26–50; I3: 51–75; I4: 76–100; see also Fig. 5). *x*-axis, intensity of pain perception; *y*-axis, response amplitude (μV or ER%). Lines connect the average peak amplitude of the laser-evoked N1, N2, and P2 waves (*left graphs*) and of the response contained in windows of interest "LEP," event-related synchronization ("ERS"), and event-related desynchronization ("ERD") (*right graphs*) obtained at each intensity of perception. Responses elicited by the 1st stimulus of the triplet (S1) are represented using a filled black line. Responses elicited by the 2nd (S2) and the 3rd (S3) stimuli of the triplet are represented using black and gray dashed lines. Note how the slopes of the linear regression between response magnitude and intensity of perception for S1 are, for N1, N2, and P2 waves, as well as for the windows of interest "LEP" and "ERS," significantly steeper than the slopes of the corresponding linear regressions obtained for S2 and S3. Also note how the slopes obtained for S2 and S3 are remarkably similar. In contrast, note how the magnitude of the response in the window of interest "ERD" does not correlate with intensity of perception and how it is unaffected by stimulus repetition.

of refractoriness that could explain the decrement of LEP amplitude is "psychological refractoriness" whose duration is in the order of hundreds of milliseconds. Psychological refractoriness is thought to reflect the fact that cortical processing resources of limited capacity are consumed by the first stimulus of a pair, leaving fewer resources to process the second stimulus of the pair (Pashler 1984).

Nevertheless, neither "neuronal refractoriness" of the nociceptive afferent pathway nor "psychological refractoriness" can explain the observed reduction in LEP magnitude, for the following two reasons. First, stimulus repetition did not affect the magnitude of perceived pain (Fig. 2, *left*). If the magnitude reduction of the brain responses elicited by S2 and S3 was related to refractoriness of the afferent pathway, the magnitude of perceived pain would have been expected to be similarly reduced. Second, when laser stimuli are delivered in pairs at unpredictable ISIs, thus ensuring that the occurrence of the second stimulus is as unexpected as the occurrence of a single stimulus, the amplitude of the laser-evoked N2–P2 is totally unaffected by the preceding stimulus, even at ISIs as short as 280 ms (Mouraux et al. 2004).

Taken together, these findings rule out refractoriness as a possible explanation for the observed modulation of the laser-evoked N1, N2–P2, and ERS responses.

Pain-related potentials?

The finding that the magnitude of the N2–P2 correlates better with the intensity of perceived pain than with the actual intensity of the laser stimulus (Carmon et al. 1978) has supported the notion that the laser-evoked N2–P2 constitutes a direct correlate of neural mechanisms underlying pain intensity coding in the human cortex (Frot et al. 2008; Iannetti et al. 2005a; Kakigi et al. 2000; Schmidt et al. 2007; Schnitzler and Ploner 2000; Timmermann et al. 2001).

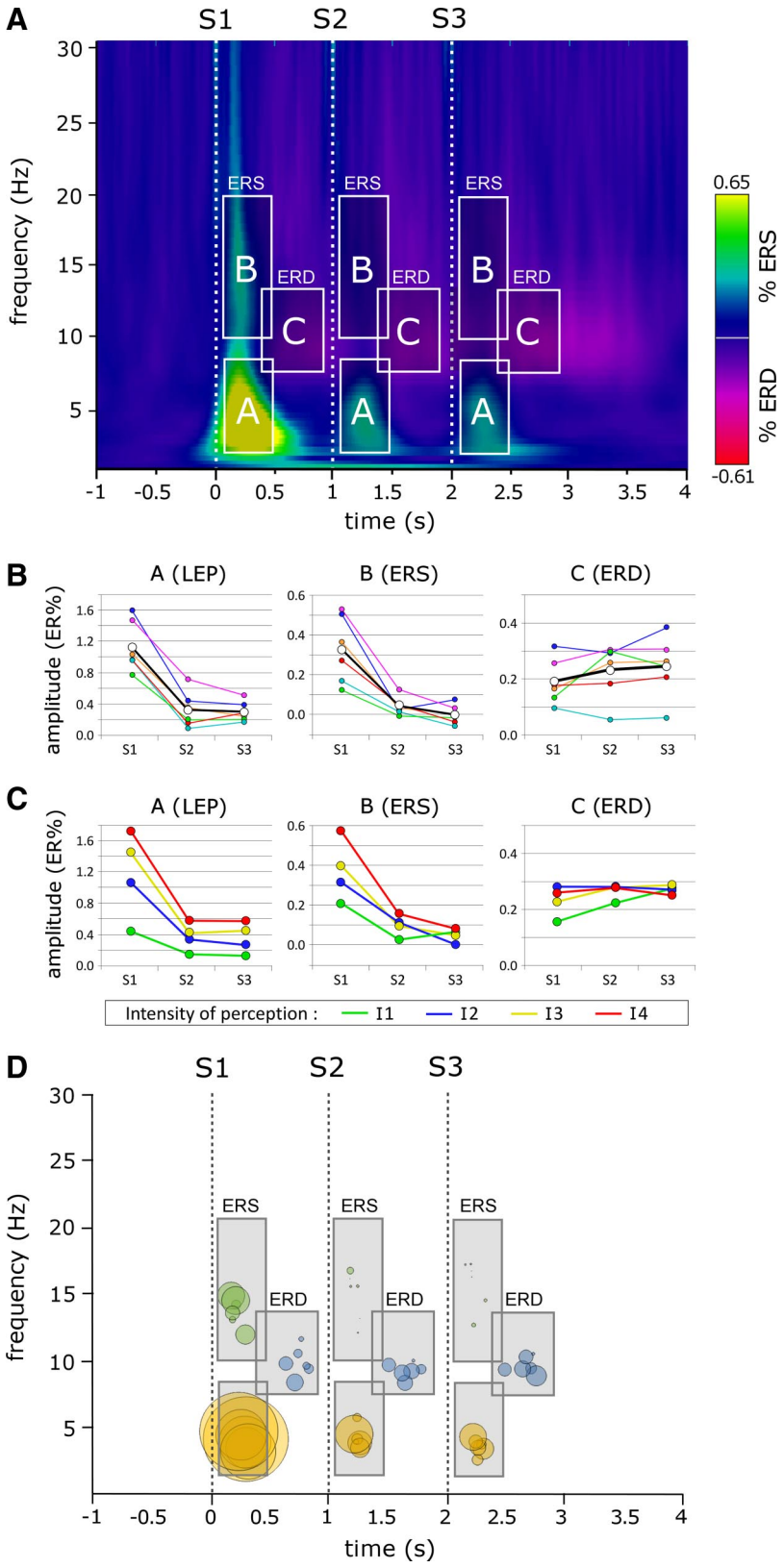

FIG. 8. Effect of stimulus repetition (S1–S3) and intensity of pain perception (I1–I4) on laser-induced ERS and ERD. *A*: time–frequency representation of laser-induced modulation of EEG oscillation amplitude at electrode Cz (nose reference). *x*-axis, time (s); *y*-axis, frequency (Hz). The vertical dashed lines mark the onset of the 3 laser stimuli (S1–S3). The color scale represents the average increase (%ERS) or decrease (%ERD) of oscillation amplitude, relative to a prestimulus reference interval (−0.9 to −0.1 s before the onset of S1). Following each stimulus, 3 windows of interest were defined. For each window of interest, a summary measure was obtained by averaging the top 20% time–frequency points displaying the highest increase (windows of interest "LEP" and "ERS") or decrease ("ERD") of signal amplitude (see METHODS for details). *B*: effect of stimulus repetition (S1–S3) on the activity within each window of interest. The colored waveforms represent single subjects, whereas the black waveform is the average across subjects. *C*: effect of stimulus repetition (S1–S3) and intensity of perception (I1–I4) on the activity within each window of interest. *x*-axis, stimulus number; *y*-axis: percentage of change relative to the reference interval (ER%). The activity within windows of interest "LEP" and "ERS" was significantly modulated by both stimulus repetition (the activity following S1 was significantly greater than the activity following S2 and S3) and intensity of perception (with higher response magnitudes for stimuli perceived as more intense). In contrast, the amplitude of the signal within window of interest "ERD" was not modulated by either stimulus repetition or intensity of perception. *D*: landscape representation of window-of-interest peaks across subjects. *x*-axis, time (s); *y*-axis, frequency (Hz). The responses of each individual subject are represented as circles (window of interest: "LEP": yellow; "ERS": green; "ERD": blue). The center of each circle corresponds to the location of the peak in time and frequency. The radius of each circle represents the magnitude of the response relative to the reference interval (ER%). Note that, despite the large size of the windows of interest, the peak location of the identified responses was remarkably similar across subjects.

Furthermore, the repeated observation that N2–P2 amplitude is negatively correlated with the histological assessment of fiber loss and altered pain sensitivity in small-fiber peripheral neuropathies (Kakigi et al. 1991b), and with altered pain sensitivity in lesions of the spinothalamic tract (e.g., syringomyelia; Kakigi et al. 1991a; Treede et al. 1991), has further corroborated this notion.

However, that evidence is not sufficient to conclude that LEPs constitute a direct readout of the function of the nociceptive system. When graded nociceptive sensory stimuli are

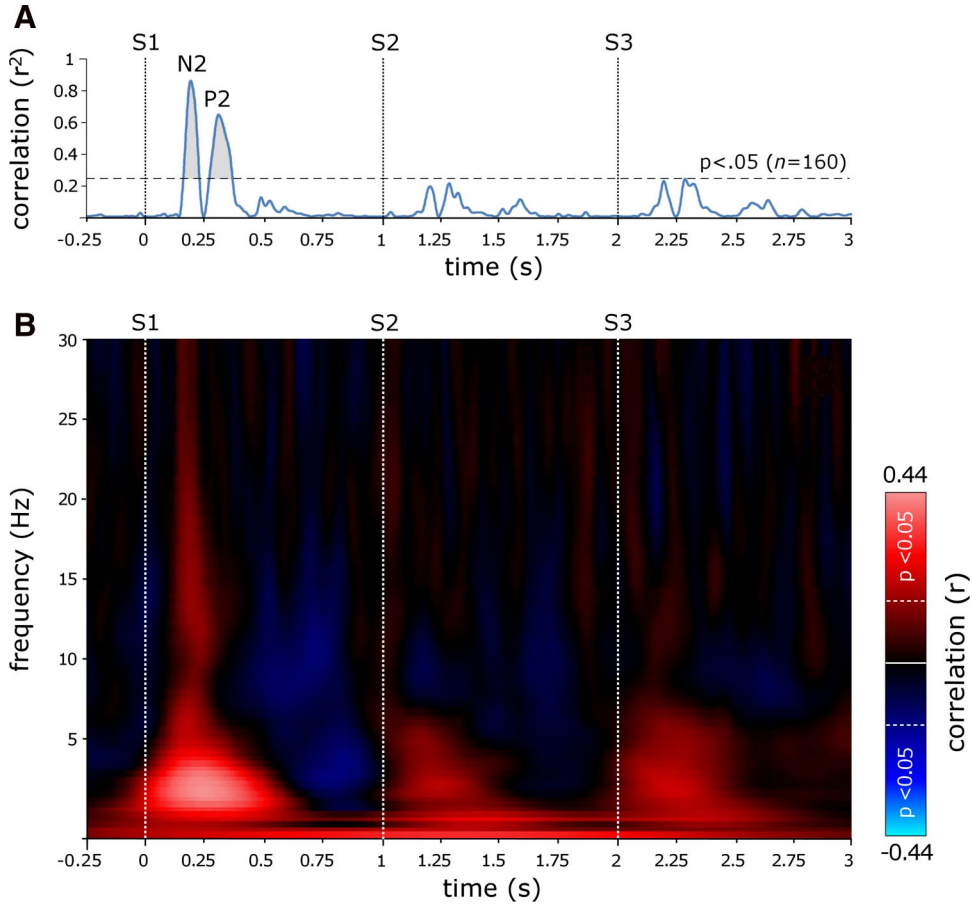

FIG. 9. Time course of the correlation with perception at single-trial level. *A*: correlation in the time domain. Group-level correlation waveform between signal amplitude at electrode Cz and intensity of perception. *x*-axis, time (s); *y*-axis, correlation (Pearson's r^2). The horizontal dashed line ($r^2 = 0.024$) indicates the threshold for $n = 160$ ($P < 0.05$, 2-tailed). The vertical dashed lines indicate the onsets of the 3 laser stimuli (S1–S3). The waveform was obtained by computing, for each time point, the linear correlation (Pearson's r^2) between the EEG signal amplitude of that time point and the corresponding intensity of pain perception. Note that following S1, the correlation was significant in 2 time intervals (highlighted in gray: 144–220 and 267–378 ms) corresponding to the latencies of the laser-evoked N2 and P2 waves. In contrast, following S2 and S3, the correlation between signal amplitude and intensity of perception was notably reduced. *B*: correlation in the time–frequency domain. Group-level time–frequency plot of the correlation between intensity of pain perception and signal amplitude at electrode Cz (ER%). *x*-axis, time (s); *y*-axis, frequency (Hz). Correlation coefficient (Pearson's *r*) is color coded. The horizontal dashed lines on the color scale ($|r| = 0.154$) indicate the threshold for $n = 160$ ($P < 0.05$, 2-tailed). The vertical dashed lines indicate the onset of the 3 laser stimuli (S1–S3). This plot was obtained by computing, for each time–frequency point, the linear correlation (Pearson's *r*) between the signal amplitude of that point (ER%) and the corresponding intensity of pain perception. Note that following S1, the correlation was significant in 2 time–frequency regions, centered around 230 ms/3.7 Hz and 187 ms/14.8 Hz, and corresponding to the activities contained in windows of interest "LEP" and "ERS." In contrast, following S2 and S3, the correlation between signal amplitude and intensity of perception was notably reduced.

applied (e.g., by delivering laser stimuli of varying energies), first-order sensory neurons and projection neurons are also activated in a graded manner (Gybels et al. 1979; Kenshalo et al. 2000; Kenshalo et al. 1979), and the resulting intensity of perception may be expected to vary accordingly. The observation that, when laser stimuli are repeated at short and constant ISI, the relationship between intensity of the stimulus and intensity of pain perception is preserved (Fig. 2, *left*)—whereas the relationship between intensity of pain perception and magnitude of the laser-evoked N1, N2–P2, and ERS (Fig. 8) is not—constitutes a clear indication that all these responses, although elicited by a stimulus that is selectively nociceptive, reflect cortical activities that are not related to the neural coding of pain intensity.

Saliency-related potentials?

We observed that the magnitudes of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS were strongly conditioned by both stimulus repetition and intensity of perceived pain. Could the effect of these two experimental factors be explained by a single, common determinant?

The first stimulus of each triplet (S1) was preceded by the last stimulus of the previous triplet (S3) by a 20-s-long interval. In contrast, a constant interval of only 1 s separated the onset of the second and third stimuli of each triplet (S2 and S3) from the onset of the preceding stimulus. Therefore the temporal expectancy of S2 and S3 was far greater than that of S1 (i.e., the onset of S2 and S3 was much more predictable than the onset of S1). Furthermore, because stimulus energy was constant across all three stimuli of each triplet, but randomly varied from triplet to triplet, the stimulus energy of S1 was a predictor of the stimulus energy of S2 and S3, whereas the stimulus energy of S3 was not a predictor of the stimulus energy of the first stimulus (S1) of the following triplet. Therefore because both the time of occurrence and the stimulus energy of S1 were much more unexpected than the time of

occurrence and the stimulus energy of S2 and S3, S1 was much more *salient*[3] than S2 and S3. Could these differences in stimulus saliency fully explain the observed effect of stimulus repetition on response magnitude? Because the saliency of S2 and S3 was similar, this would explain why the reduction in response magnitude occurred entirely between S1 and S2, with no further reduction between S2 and S3 (Figs. 3 and 8). It would also explain why stimulus repetition at constant ISI affected the response magnitude without affecting the intensity of pain perception. Finally, it would explain why, when pairs of identical laser stimuli are delivered at unpredictable ISIs (i.e., when the temporal expectancy of the two stimuli, and thus their saliency, are identical), stimulus repetition does not affect response magnitude (Mouraux et al. 2004).

In addition to being strongly modulated by stimulus repetition, the magnitudes of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS elicited by S1 were strongly correlated to the intensity of pain perception (Fig. 7). Because a laser stimulus that is perceived as intense is by definition more salient than a laser stimulus that is perceived as weak (Downar et al. 2000), it could well be that the correlation between response magnitude and the intensity of pain perception is, in fact, an indirect reflection of the modulation of response magnitude by stimulus saliency.

If stimulus saliency is the main determinant of the magnitude of the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS, possibly through the modulation of a specific subset of their neural generators, what could be the functional significance of these responses? One possibility is that they reflect neural activities that are involved in stimulus-triggered mechanisms of arousal or attentional capture (Bromm et al. 1984; Garcia-Larrea 2004; Mouraux and Plaghki 2006). In accordance with this hypothesis are the following observations. First, innocuous stimuli belonging to the somatosensory, the auditory, and the visual sensory modality can elicit brain responses whose shape and scalp topography closely resemble the shape and scalp topography of the laser-evoked N2–P2 (Kunde and Treede 1993; Naatanen and Picton 1987; Vogel and Luck 2000). Second, the magnitude of all these responses, similarly to the magnitude of the laser-evoked N2–P2, is strongly conditioned by stimulus saliency.

Because we collected data from seven scalp electrodes, it was impossible to define which of the distinct neural generators known to contribute to scalp LEPs (Garcia-Larrea et al. 2003) were modulated by stimulus saliency. However, by showing that all main LEP peaks (i.e., the N1, N2, and P2 waves) were modulated by stimulus saliency, our results suggest that both the cingulate cortex, which is thought to be the main generator of the N2 and P2 waves, and the operculoinsular cortex, which is thought to be the main generator of the N1 wave and to contribute to the N2 wave, were affected. In agreement with this suggestion, Downar et al. (2000, 2002) recently identified, using fMRI, a number of cortical areas sensitive to stimulus saliency. These areas would constitute a "multimodal network for involuntary attention to events in the sensory environment." Interestingly, this network included all brain regions (e.g., anterior cingulate cortex, bilateral operculoinsular cortices) that are commonly considered to contribute to scalp LEPs.

Laser-induced ERD

In striking contrast with the behavior of all other laser-evoked brain responses, the laser-induced ERD, starting about 500 ms after the onset of the stimulus, and centered in the alpha band (8–12 Hz), was neither correlated with the intensity of perception nor affected by stimulus repetition.

The magnitude of alpha-band oscillations has been shown to vary with sensory, motor, and cognitive operations (reviewed in Lopes da Silva and Pfurtscheller 1999). In particular, it is well known that auditory, visual, and somatosensory stimuli induce a transient suppression of alpha-band power, which has been hypothesized to reflect activation (or disinhibition) of the cortical areas related to the processing of the incoming sensory stimulus. Alpha-band ERD has also been shown to occur during cognitive tasks that engage specific attentional and mnesic processes (Sergeant et al. 1987; Van Winsum et al. 1984; Yordanova et al. 2001).

In the current experiment subjects were asked to recall and report, at the end of each trial, the intensity of the perception elicited by each of the three consecutive stimuli. Therefore although a contribution of C-fiber unmyelinated input to the observed laser-induced ERD cannot be excluded on the basis of its onset and offset latencies, it could well be that the observed laser-induced ERD reflects brain activities mainly related to the attentional and mnemonic processes that this task required. This hypothesis would explain *1*) why the magnitude of the laser-induced ERD was unrelated to the intensity of pain perception, *2*) why it was unaffected by stimulus repetition, and *3*) why its duration appeared to outlast well after the onset of S3 (Fig. 8), as one would expect that such an activity would end only at task closure.

Conclusion

What are the practical implications of our results? Here we show that laser-evoked brain responses represent an indirect readout of central nociceptive processing. Whereas the laser-evoked N1, the laser-evoked N2–P2, and the laser-induced ERS are mainly related to stimulus saliency, the laser-induced ERD is probably related to cognitive or mnesic task-related processes. The fact that none of these responses appears to be a direct correlate of the neural activity responsible for pain intensity coding in the human cortex certainly does not mean that their recording is not useful to explore the function of the nociceptive system. However, it questions the appropriateness of relying on these brain responses to pinpoint activity arising in specific brain structures and, assuming that these structures are specifically involved in the processing of nociceptive input, thereby build models of the cortical processes underlying the perception of pain. Indeed, scientists and clinicians should be well aware that although the eliciting laser stimulus activates the nociceptive system in a fully selective manner (Bromm and Treede 1984), these responses mostly reflect neural processes that are not unique to the nociceptive system, but are instead triggered by any salient stimulus occurring in the sensory environment, regardless of its sensory modality.

[3] Saliency refers here to the "ability of the stimulus to disrupt the current cognitive focus and elicit an attentional or behavioural switch" (Downar et al. 2000).

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