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 (I) 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 activates 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 mechanohot 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 pathophysiological 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 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-
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 > 10 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 noxious 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; Raji 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.

M E T H O D S

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 (Electronics 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...
and its diameter was set at approximately 7 mm (∼38 mm²) 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 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.

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 right and left hand dorsum, in two separate sessions on the same day. The order of sessions was balanced across subjects. 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.
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 down-sampled 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 EEG 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 2.5σ0 (σ0 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%).

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 at 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 ERIt,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.
The correlation between response magnitude and intensity of perception (Fig. 5). Post hoc analyses revealed a significant linear higher response magnitudes for stimuli perceived as more intense (N2: F9.74, 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 (coincident with the latency of N2), 275–372 ms (coincident 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 (coincident with the latency of N2), 275–404 ms (coincident with the latency of P2), and 542–673 ms. A significant 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 = 2.53, P < 0.05; P2: F = 9.45, P < 0.005).
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 and S3 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. 8B). 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. 8B).

**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. 8C). 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. 8C).

**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”: \(P = \)
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
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).
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...
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...
ocurrence 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 mnemonic 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 mnemonic 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.

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\(^3\) Salience 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).
Laser-evoked brain responses reflect stimulus saliency


