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OPERCULOINSULAR CORTEX ENCODES PAIN INTENSITY AT THE EARLIEST STAGES OF CORTICAL PROCESSING AS INDICATED BY AMPLITUDE OF LASER-EVOKED POTENTIALS IN HUMANS

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Abstract—Converging evidence from different functional imaging studies indicates that the intensity of activation of different nociceptive areas (including the operculoinsular cortex, the primary somatosensory cortex, and the anterior cingulate gyrus) correlates with perceived pain intensity in the human brain. Brief radiant laser pulses excite selectively Aδ and C nociceptors in the superficial skin layers, provide a purely nociceptive input, and evoke brain potentials (laser-evoked potentials, LEPs) that are commonly used to assess nociceptive pathways in physiological and clinical studies. Aδ-related LEPs are constituted of different components. The earliest is a lateralised, small negative component (N1) which could be generated by the operculoinsular cortex. The major negative component (N2) seems to be mainly the result of activation in the bilateral operculoinsular cortices and contralateral primary somatosensory cortex, and it is followed by a positive component (P2) probably generated by the cingulate gyrus.

Currently, early and late LEP components are considered to be differentially sensitive to the subjective variability of pain perception: the late N2–P2 complex strongly correlates with perceived pain, whereas the early N1 component is thought to be a pre-perceptual sensory response. To obtain physiological information on the roles of the pain-related brain areas in healthy humans, we examined the relationship between perceived pain intensity and latency and amplitude of the early (N1) and late (N2, P2) LEP components. We found that the amplitude of the N1 component correlated significantly with the subjective pain ratings, both within and between subjects. Furthermore, we showed that the N2 and P2 late LEP components are differentially sensitive to the perceived sensation, and demonstrated that the N2 component mainly explains the previously described correlation between perceived pain and the amplitude of the N2–P2 vertex complex of LEPs. Our findings confirm the notion that pain intensity processing is distributed over several brain areas, and suggest that the intensity coding of a noxious stimulus occurs already at the earliest stage of perception processing, in the operculoinsular region and, possibly, the primary somatosensory area. © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: nociceptive system, Aδ fibres, perception, electrophysiology, single-trial analysis, parasylvian region.

The current concept of pain intensity coding does not comply with the classical distinction between brain structures dedicated to processing the sensory and emotional components of pain perception (Melzack and Casey, 1968): pain intensity-dependent activations have been found in cortical regions pertaining both to the "lateral" (primary and secondary somatosensory areas: SI and SII, insular cortex) and "medial" (cingulate cortex, prefrontal cortex) pain systems (for a review see Porro, 2003).

Functional neuroimaging techniques like positron emission tomography (PET), functional magnetic resonance (fMRI), electroencephalography (EEG), and magnetoencephalography (MEG) provide consistent information on the spatial–temporal characteristics of pain processing. Despite their higher spatial resolution, PET and fMRI measure haemodynamic brain responses with a minimal temporal window of some seconds, and thus cannot investigate with high temporal resolution (e.g. less than 1 s) the sequence of pain-related events; this information can be achieved on a millisecond scale by recording the EEG or MEG responses to noxious stimuli.

Brief radiant heat pulses, generated by laser stimulators, selectively excite the superficial thermal-pain receptors and evoke EEG responses (laser-evoked potentials, LEPs) related to the activation of type II mechano-heat nociceptors (AMH II units), small-myelinated primary afferents (Aδ), and spinothalamic tract neurons (Bromm and Treede, 1991; Treede et al., 1995). The largest signal is a negative-positive complex (N2–P2), with maximal amplitude at the vertex: several studies have clearly demonstrated a role for the bilateral operculoinsular areas in generating the N2 component (Tarkka and Treede, 1993; Valeriani et al., 1996; Frot et al., 1999) and for the cingulate gyrus in generating the P2 component (Tarkka and Treede, 1993; Bromm and Chen, 1995; Iannetti et al., 2003; for review see Garcia-Larrea et al., 2003). More recently, converging evidence from intracranial and source localisation LEP studies has also suggested a role of the contralateral SI in generating the N2 component (Ohara et al., 2004; Schlereth et al., 2003), in agreement with previ-

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Abbreviations: AMH II, type-II Aδ mechano-heat; EEG, electroencephalography; EMG, electromyography; fMRI, functional magnetic resonance imaging; LEPs, laser-evoked potentials; MEG, magnetoencephalography; Nd:YAP, neodymium:yttrium–aluminium–perovskite; PET, positron emission tomography; SI, primary somatosensory cortex; SII, secondary somatosensory cortex.

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ous MEG reports showing a contralateral SI source partly overlapping with the N2 time window of LEPs (Ploner et al., 1999, 2000).

In addition, a negative component (N1), earlier than the large N2–P2 vertex complex, has been consistently described: this is a dipolar, lateralized response, originating from the operculoinsular cortex (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani et al., 1996; Frot and Mauguiere, 2003; Vogel et al., 2003).

These Aδ-related LEPs, investigated in physiological and clinical studies in patients with peripheral or central lesions (Bromm and Treede, 1991; Iannetti et al., 2001; Spiegel et al., 2003), are now considered the best tool for assessing function of nociceptive pathways (Cruccu et al., 2004).

The current view on LEPs states that early and late components are differentially sensitive to subjective perception, whereas the amplitude of the early N1 LEP wave does not appear to correlate with pain intensity perception, and consequently this component has been suggested to represent a pre-perceptual sensory response (Garcia-Larrea et al., 1997); a positive relationship between the intensity of laser stimuli, magnitude of perceived pain, and amplitude of the main N2–P2 scalp response has been confirmed by different groups (Kakigi et al., 1989; Bromm and Treede, 1991; Beydoun et al., 1993; Arendt-Nielsen, 1994; Garcia-Larrea et al., 1997). In contrast, PET and fMRI studies indicate that both the cingulate and operculoinsular cortices encode the intensity of perceived pain (Porro et al., 1998; Coghill et al., 1999; Peyron et al., 1999; Craig et al., 2000; Buchel et al., 2002). Their poor temporal resolution, however, makes it impossible to ascertain the stage at which different brain areas deal with the intensity coding.

As the intensity of a painful stimulus largely dictates any ensuing behaviour, it is most likely that the intensity of a noxious stimulus is processed first. Our aim was therefore to verify if the intensity coding of a noxious stimulus occurs in the parasylvian region at the earliest stages of pain perception processing. We recorded the scalp potentials and pain ratings to noxious laser stimuli, and analysed the relationships between subjective ratings and latency and amplitude of the early (N1) and late (N2, P2) LEP components.

EXPERIMENTAL PROCEDURES

Subjects

Eight healthy volunteers (five men and three women) aged 25–30 years (mean: 27±2.1) participated in the study. The subjects were recruited from research staff and PhD Students of the University of Oxford (Oxford, UK). All participants gave their informed consent, and the local ethics committee approved the procedures.

Laser stimulation

Painful heat stimuli were generated by an infrared neodymium: yttrium–aluminium–perovskite (Nd:YAP) laser (Electronical Engineering, Florence, Italy; www.elengroup.com; energy 0.5–15 J; duration 2–20 ms) with a wavelength of 1.34 μm. A He-Ne pilot laser pointed to the area to be stimulated. The laser beam was transmitted through a fibre optic and its diameter was set at approximately 5 mm (~20 mm^2).

In previous experiments we found that Nd:YAP laser pulses of high intensity (up to a 2-J energy directed to a skin area of about 20 mm^2) were optimal to elicit painful pinprick sensation (A-delta input) and readily evoked LEPs after stimulation of different body districts, without inducing damage to the skin (Cruccu et al., 2003; Iannetti et al., 2004).

In the present study laser pulses were directed to the skin of the right hand dorsum. To avoid nociceptor fatigue or sensitisation, the laser beam was moved slightly after each stimulus, and stimuli were delivered arrhythmically with 8–15 s intervals to minimise central habituation. We set the stimulus at a fixed energy (1 J) of radiation, eliciting a pinprick, moderately painful sensation that the subject could tolerate across 60 stimuli.

Scalp recording

Participants were seated in a comfortable chair, wore protective goggles, and were asked to stay awake and relax their muscles. They were instructed to keep their eyes open and gaze slightly downwards. Complete acoustic isolation was ensured using earplugs and headphones. Brain electrical activity was recorded with silver disc electrodes from Fz, Cz, Pz (versus linked earlobes), T3 and T4 (versus Fz) according to the international 10–20 system, with a bandwidth of 0.3–50 Hz, a sampling rate of 1024 Hz, and a conversion of 12 bit giving a resolution of 0.195 μV/digit (SystemPlus; Micromed, Treviso, Italy). The electrode impedance was always kept below 5 kΩ). In order to monitor ocular movements or eye-blinks and discard contaminated trials, electrooculographic signals were simultaneously recorded with surface electrodes, with the active electrode over the mid lower eyelid and the reference 1 cm lateral to the lateral corner of the orbit. In two subjects we recorded the electromyographic (EMG) activity from the orbicularis oculi, masseter, and cervical muscles.

For each subject, two series of 30 trials, separated by a 15-min interval, were collected and analysed off-line. The window analysis time was 2 s (500 ms pre-stimulus+1500 ms post-stimulus). Between 4 and 8 s after each stimulus the subjects were visually invited to rate verbally the evoked sensation on a numerical rating scale ranging from 0 to 10, where 0 was “no pain” and 10 “pain as bad as it could be” (Jensen and Karoly, 2001). This produced 60 (two series of 30) single-trial pain ratings for each subject. Subjects were instructed to signal any perception different from a clear pinprick sensation, and were unaware of any detail of the stimulation paradigm (in particular they did not know that the energy was kept constant across the whole recording session).

Data analysis and statistics

Preliminary analysis of the EEG recordings included visual inspection and removal of trials contaminated by ocular movements. LEP components were identified with three different procedures: (i) standard averaging, (ii) single-trial and (iii) few-trial analysis.

Standard averaging (N1 and N2–P2). The standard, time-locked averaging approach was first used to identify LEP components, and, for each subject, the two series were averaged separately. We measured the peak latencies of the contralateral early response (N1) and the negative and positive components (N2, P2) of the late vertex response, the baseline-to-peak amplitude of N1, N2 and P2 responses, and the peak-to-peak amplitude of the vertex complex (N2–P2). This analysis produced three values of latencies (N1, N2, P2) and four values of amplitude (N1, N2, P2, N2–P2) for each series of each subject (14 LEP measures for each subject).

Single-trial analysis (N2 and P2 components). Each EEG trial recorded from Cz was checked by visual inspection for the presence of a waveform similar to the averaged N2–P2 complex, within the expected time range (the subject's average latency±60 ms; Purves and Boyd, 1993). Using this approach, although the N2 and P2 vertex components were easily identified, the latency measurement was occasionally more difficult. Therefore we decided to follow, in all trials, this procedure: signals were digitally smoothed with a 30-Hz low-pass filter in order to eliminate small deflections indenting the main negative and positive waves, latencies were then taken at the peak of the smoothed N2 and P2 waves, and the amplitude was calculated at these two points (peak-to-peak and in respect to the baseline line) in the non-smoothed signal.

This analysis produced between 39 and 52 values of latencies and amplitudes for each subject (60 trials minus trials with EMG artefact minus trials without an identifiable response).

Few-trial analysis (N1). Because of its lower signal-to-noise ratio, the early N1 component cannot be seen in single trials, and in preliminary experiments we found that the waveform, latency, and amplitude of the N1 component became stable after a low number of averaged trials (approximately 10). The pain ratings given by each subject were intra-individually divided into three groups of equal number: one third with the highest scores, one third with the intermediate scores, and one third with the lowest scores. This provided three averages of an equal number of trials for three levels of pain rating (low, medium, and high) in each series of each subject. In total, this approach produced 48 averages (three levels×two series×eight subjects). For each average, the N1 peak latency and baseline-to-peak amplitude were measured.

We assessed the correlations between the following variables:

(i) mean N1, N2, P2, and N2–P2 values yielded by standard averaging and corresponding mean pain rating of each series;

(ii) single-trial N2, P2 and N2–P2 values and corresponding single-trial pain rating for each subject, and the same analysis with the data pooled from all subjects;

(iii) mean N1 values yielded by few-trial analysis and corresponding pain intensity group (low, medium, high) for each subject, and the same analysis with the data pooled from all subjects.

All pain ratings and LEP data had a Gaussian distribution. Correlations between pain ratings and LEP data from "standard averaging" and "single-trial analysis" were evaluated with the coefficient of correlation for parametric data (Pearson *r*). When two parametric variables had a significant correlation, we also calculated the linear regression. In the case of "few-trial analysis," because the pain ratings were divided in three categories (low, medium, high), we used the coefficient of correlation for nonparametric data (Spearman *R*).

Lastly, we tested for a possible effect of the order of stimulus presentation on the following data: single-trial pain ratings; N2, P2 and N2–P2 amplitudes of single-trial LEP responses. The Mann-Whitney test was used to analyse the difference in pain ratings and LEP amplitudes *between* the first and the second series, for each subject. The Kruskall-Wallis test was used to analyse the difference in pain ratings and LEP amplitudes *within* each series of each subject.

For all statistics and graphs, we used Prism 4.0 (GraphPad, Sorrento Valley, CA, USA). Results are given as mean±S.D. In order to facilitate comparisons, the absolute *P* values are given for the group analysis results.

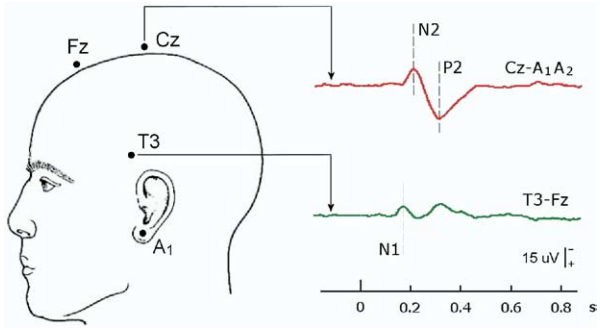

Fig. 1. Grand mean of LEPs after stimulation of the right hand dorsum (n=16). The largest scalp signal is a late, negative–positive complex (N2–P2) peaking at about 230–320 ms, with maximal amplitude recorded at the vertex (Cz) against the linked earlobes (A_1A_2); its major negative component (N2) seems to be mainly the result of activation in the bilateral operculoinsular cortices and contralateral SI, and it is followed by a positive component (P2) probably generated by the cingulate gyrus. An earlier negative component (N1, peaking at about 180 ms) has maximal scalp amplitude over the Sylvian fissure contralateral to the stimulated hand (T3), and arises from the parasylvian cortex, namely the operculoinsular areas (lower trace, green). LEPs allow investigating the sequential processing of noxious inputs by distinct brain structures.

RESULTS

Quality and intensity of sensation

Laser stimuli elicited a clear, pinprick sensation in all subjects. The average pain rating across all subjects was 3.9±1.6. The range of the average pain ratings was 2.3–5.9 (n=8), whilst that of pain ratings in individual trials was 0–7 (n=480). The within-subject variability of the pain ratings (S.D.s ranged from 0.64–1.43) was far lower than the between-subject variability of the individual mean pain ratings (S.D.=1.63).

Components of laser evoked potentials (LEPs)

Standard averaging. In all subjects, standard averaging analysis easily disclosed clear and reproducible LEPs, time-locked to laser stimulation. EMG recordings showed that no reflex response was elicited in the orbicularis oculi, masseter, or cervical muscles. The earliest identifiable scalp component was the early-latency negative wave (N1) visible in EEG data recorded from the right and left temporal leads, with a latency of approximately 180 ms and an amplitude of approximately 7 μV (Fig. 1). One run of one subject did not yield a clear N1 component.

The N1 component was always followed by the late negative–positive complex (N2–P2) in the midline (Fz, Cz and Pz) leads, with a mean N2 latency of approximately 230 ms and a mean P2 latency of approximately 320 ms; the mean N2, P2 and peak-to-peak (N2–P2) amplitudes (all maximal at Cz) were 7, 21 and 28 μV.

Single-trial analysis of N2–P2 complex. A clear N2–P2 complex was identifiable at Cz in almost 80% of single trials entering the analysis (352/447).

Fig. 2 shows 15 consecutive single-trial LEPs recorded from Cz in one representative subject: the N2–P2 complex is clearly visible in most sweeps.

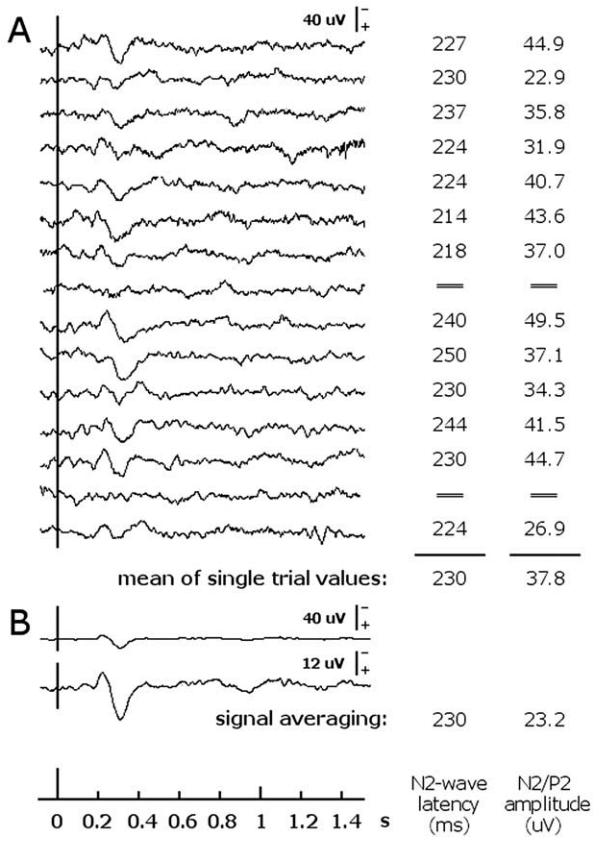

Fig. 2. Single-trial analysis of the N2–P2 complex. LEP responses from one representative subject. Scalp potentials are recorded from the vertex, referred to linked-earlobes. (A) Fifteen consecutive single-trial responses, with their latency and amplitude; note that the late N2–P2 complex can be detected easily in most of the trials and that the latency jitters widely between trials. (B) Time-locked, standard averaging of the single-trials shown in A; the same averaged signal is displayed with the same amplification of single-trials (upper trace) and with a higher amplification (lower trace); note that the amplitude of the signal yielded by standard averaging (B) is lower than the sum of amplitudes of single-trial responses, mostly because the latency jitter between trials causes the averaging of signals in opposing phase.

The means of single-trial latencies of the N2 and P2 components were 226 ms and 330 ms respectively, i.e. similar to those measured in the standard averaging. Because of the latency jitter, the means of single-trial amplitudes of N2 and P2 components, and that of N2–P2 complex (16, 30 and 46 μV, respectively), were far higher than the corresponding ones measured in the standard averaging (7, 21 and 28 μV).

Few-trial analysis of N1 component. In the few-trial analysis, the EEG sweeps of each series were divided and averaged according to three pain levels (high, medium, low). Of the 48 averages, eight did not yield a clear N1 component. The mean N1 latency of the remaining 40 averages was 173 ms (similar to the one measured in the standard averaging) and amplitude was approximately 10 μV (while that measured in the standard averaging was 7 μV). Fig. 3 shows the averaged EEG sweeps using few-trials analysis, in one representative subject.

Correlation between pain ratings and LEP components

The amplitude of LEP responses (whether early or late) showed a better correlation with perceived pain than the latency. Correlations between pain ratings and LEP latencies and amplitudes are summarised in Figs. 4 and 5.

Standard-averaging values. Whereas the mean latency of N1, N2 and P2 components did not correlate with the mean pain ratings (N1: $P=0.27$, $n=15$; N2: $P=0.35$, $n=16$; P2: $P=0.6$, $n=16$), the mean amplitude of N1, N2 and N2–P2 components did correlate significantly with the mean pain rating (N1: $r=0.5540$, $P=0.032$, $n=15$; N2: $r=0.591$, $P=0.016$, $n=16$; N2–P2: $r=0.5576$, $P=0.025$, $n=16$), whilst the mean amplitude of P2, despite it showed a clear trend ($P=0.051$, $n=16$), did not.

Single-trial analysis of N2–P2. The absolute single-trial N2 and P2 latencies did not correlate with the corresponding pain ratings (N2: $P=0.24$, $n=352$; P2: $P=0.14$, $n=352$).

The absolute values of single-trial N2–P2 amplitudes showed a significant within-subject correlation with the corresponding pain ratings, in all subjects (in the eight subjects the r correlation coefficient ranged from 0.4113 to 0.8096, P values from <0.01 to <0.0001, and n values from 39 to 52). When amplitudes and pain ratings from all subjects were pooled together, the level of significance was even higher ($r=0.2509$, $P=0.0002$, $n=352$). The correlation with pain ratings was even more significant when the amplitude of N2 alone was analysed ($r=0.3024$, $P<0.0001$, $n=352$). In contrast, the amplitude of P2 alone failed to reach statistical significance ($P=0.074$, $n=352$).

Few-trial analysis of N1. The correlation between N1 latency and the corresponding pain levels showed a clear negative trend, with shorter latencies for higher psychophysical ratings. However, possibly because the division of N1 values and pain ratings in three groups (low, medium, high) strongly reduced the degrees of freedom, the correlation between N1 latency and the corresponding pain levels did not reach statistical significance, either in single subjects, or when the data from all subjects were pooled together ($P=0.057$, $n=40$).

The absolute values of N1 amplitude significantly correlated with pain level in two subjects only (subject 2: $r=0.6308$, $P<0.001$, $n=6$; subject 6: $r=0.8145$, $P<0.05$, $n=6$). But when the data from all subjects were pooled together the correlation reached a strong statistical significance ($r=0.5828$, $P=0.0003$, $n=40$).

Effect of the order of stimulus presentation

Since LEP amplitudes and perceived pain intensity can habituate across the experimental session (Lorenz and Garcia-Larrea, 2003), we tested for the effect of stimulus presentation order by analysing these responses between and within the two recording series. The between-series comparison demonstrated that, in all subjects, all responses (pain ratings, N2, P2 and N2–P2 amplitudes) did not differ between first and second series (Mann-Whitney,

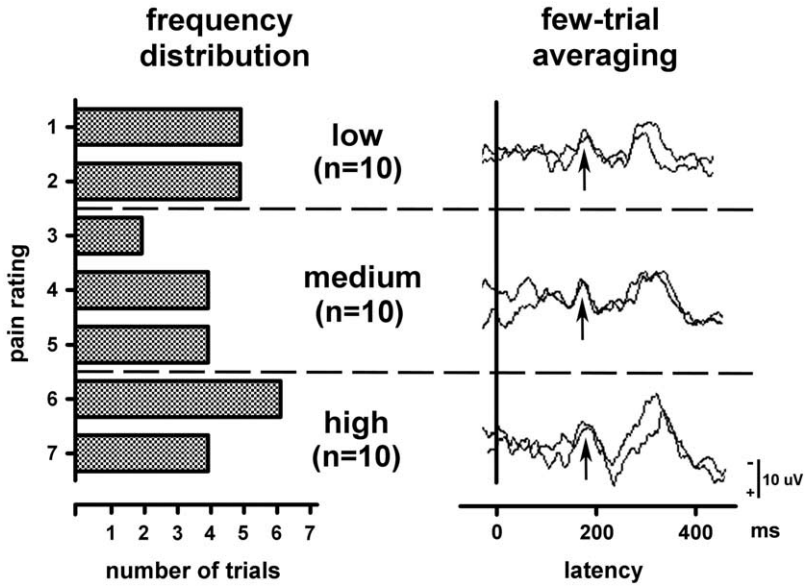

Fig. 3. Few-trial analysis of the N1 component. Pain ratings and LEP responses from one representative subject. Left panel: frequency distribution of pain ratings in one series (total number of stimuli=30; bin width=1). The pain ratings given by each subject were intra-individually divided into three groups: one third with the highest scores, one third with the intermediate scores, and one third with the lowest scores. The corresponding signals were averaged, thus providing one averaged signal for each of the three levels of pain rating (low, medium, high). Right panel: N1 components obtained by averaging of 10 trials; waveforms from the two collected series are superimposed. Scalp potentials are recorded from the contralateral temporal electrode (T3), referenced to Fz. Arrows indicate N1.

$P>0.05$). Also the within-series comparison did not disclose significant differences in each response (Kruskall-Wallis, $P>0.05$).

DISCUSSION

Using a laser stimulator, we investigated the correlations between subjective pain ratings (as a psychophysiological measure of perceived pain) and the EEG brain responses (as an electrophysiological measure of nociceptive processing) to noxious stimuli. EEG responses are advantageous because they disclose the temporal sequence of brain evoked events on a millisecond timescale. We found a strong correlation between the intensity of perceived pain and the amplitude of N1 and N2 components of LEPs, which are known to be generated in the operculoinsular cortex and SI. Besides confirming the notion that pain intensity processing is distributed over several brain areas, here we show that the intensity coding of a noxious stimulus might occur in the operculoinsular cortex and SI already at the earliest stage of cortical pain processing.

Afferent input and brain signals

Although used less widely than CO_2 lasers, solid-state (YAG/YAP) lasers provide reliable pain-related brain responses (LEPs), selectively related to the activation of AMH II nociceptors. Compared with CO_2 lasers, solid-state lasers emit a radiation with a much shorter wavelength, and consequently their radiation penetrates deeper and energy is dispersed in a bigger skin volume (the CO_2 radiation is almost completely absorbed at the epidermal surface; Bromm and Treede, 1983). This implies that: 1. with solid-state lasers the output energy required to produce a similar perceptual correlate (e.g. a moderately painful pinprick) is much higher (about 10 times); 2. the heat is directly absorbed at nociceptor depth (approximately 200 μm), and nociceptive afferents are activated more rapidly and mostly directly. In addition, solid-state lasers have shorter stimulus duration (in the range of single milliseconds) producing a faster increase of skin temperature; thereby they yield a highly synchronised afferent volley which provides a stronger nociceptive input to the brain (Spiegel et al., 2000; Iannetti et al., 2004).

In this study we used a solid-state Nd:YAP laser stimulator because, by improving the signal-to-noise ratio, it allowed us to identify and measure small signals without having to average many trials, and to correlate these signals with pain perception in single trials.

Because the late N2–P2 complex exhibits a wide variability of latency and morphology (Arendt-Nielsen, 1990; Purves and Boyd, 1993), standard time-locked averaging techniques do not provide a reliable estimate of its amplitude. We avoided this problem by adopting visual identification of single-trial components (Purves and Boyd, 1993), and found that the N2–P2 complex was easily recognizable in almost 80% of trials and that the mean of single-trial amplitudes was 64% higher than the amplitude of the signal yielded by standard averaging (Fig. 2). The lower amplitude of the signal yielded by standard time-locked averaging is explained by the wide latency jitter between trials, which causes the averaging of signals in opposing phase. Hence, the single-trial approach yields a mean amplitude value that approximates better the real N2–P2 amplitude. Because less influenced by latency jitter, the

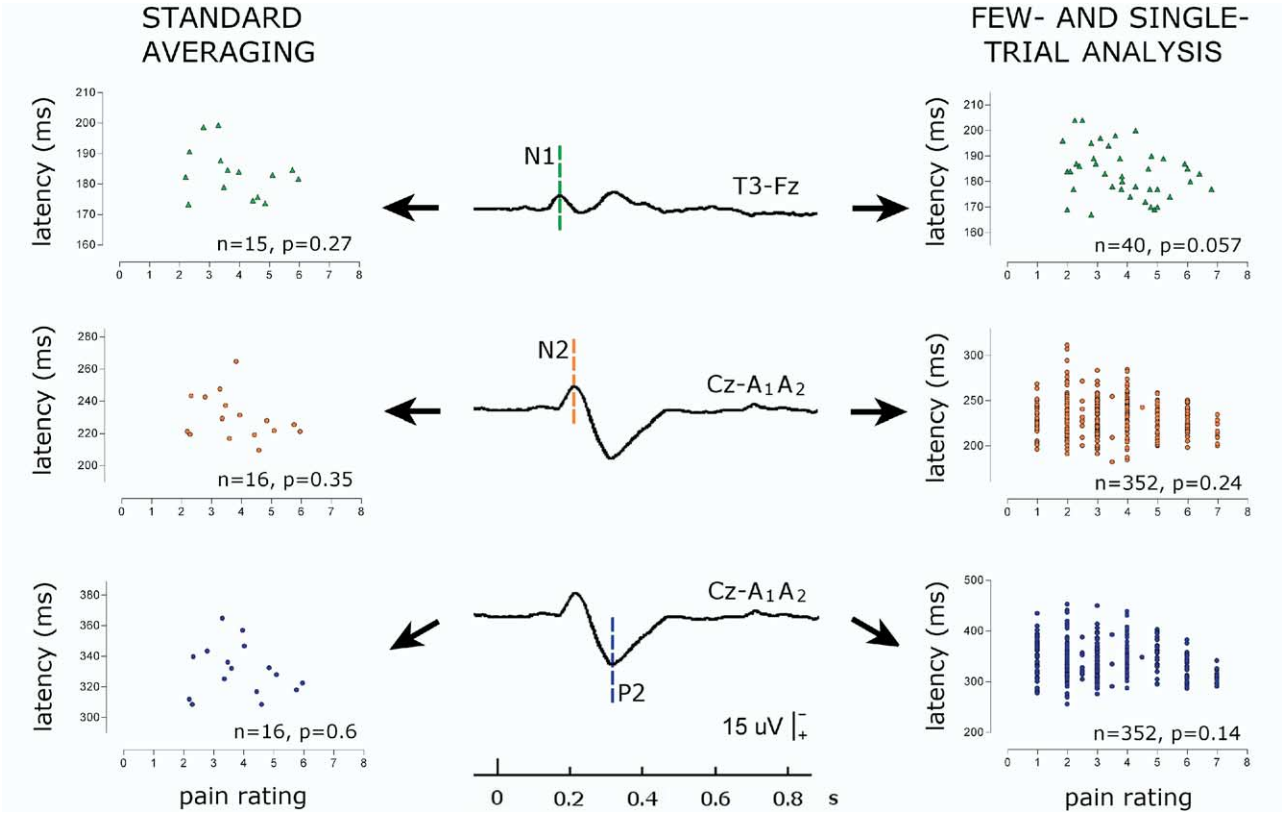

Fig. 4. Pain rating-LEP correlations. Latency. The correlations between pain ratings and the latency of N1, N2 and P2 components were not significant. The latency of N1 was measured at the contralateral temporal electrode (T3 versus Fz) and those of N2 and P2 at the vertex (Cz versus linked earlobes, A_1A_2). Left column: latency values obtained with standard averaging are plotted against the average pain ratings; *y* axis: peak latency; *x* axis: pain rating. Each symbol indicates the mean peak latency obtained from one series of each subject (N1 component: $n=15$; N2 and P2 components: $n=16$). Middle column: LEP waveforms with latency peaks of the three components highlighted (N1, green; N2, orange; P2, blue). Right column: latency values obtained with few-trial (N1) or single-trial (N2, P2) averaging are plotted against corresponding pain ratings; *y* axis: peak latency; *x* axis: pain rating. Each symbol indicates the peak latency obtained with few-trial (N1 component: $n=40$) or single-trial averaging (N2 and P2 components: $n=352$).

mean of single-trial latencies and the latency measured from the standard averaging were similar.

Regarding the N1 component, we could not measure it in single trials, because of its lower signal-to-noise ratio, but we were able to measure it by averaging only 10 trials or less ("few-trial" analysis). Therefore we had to divide the pain ratings into three groups (high, medium, low) instead of exploiting the single-trial analysis used for the later LEP components (N2 and P2). This provided us with N1 measures that could be correlated with perceived pain, if not on a single-trial basis, at least with a three-category pain level, thus allowing within-subject analyses (Fig. 3). Similarly to N2–P2, the mean N1 amplitude yielded by few-trial analysis was higher than that yielded by standard averaging.

Correlation with perceived pain

Pain is a complex sensory experience. Because of its inherent subjectivity, the search for techniques that objectively measure pain in humans has been a crucial challenge. A positive relationship between intensity of laser stimulation, magnitude of the perceived pain and amplitude of the main late N2–P2 LEP response has been repeatedly demonstrated (Arendt-Nielsen, 1994). It is now generally agreed that this response represents a reliable biomarker of perceived pain in normal subjects (Bromm and Lorenz, 1998).

In this study we kept a constant output energy for all laser stimuli, and exploited the intrinsic variability of subjective rating and electrophysiological responses, in order to characterise at best the relationship between these two kinds of response, minimising as much as possible the chance of finding spurious correlations induced by the introduction of more variables (e.g. the different levels of sensory input). The observed variance of within subject pain ratings (S.D.s ranging from 0.64 to 1.43) may be due to both central (variations of subject cognitive state) and peripheral reasons (variations in skin thickness and spatial distribution of nociceptive terminals).

Various cognitive factors, such as attention paid to the stimulus and vigilance, are known to influence both pain perception and LEP components (Legrain et al., 2002; Lorenz and Garcia-Larrea, 2003). LEP amplitude decreases across the recording session (Arendt-Nielsen, 1990). However, we did not find any significant effect of stimulus presentation order on psychophysical responses or LEP amplitude, probably because the single-trial rating

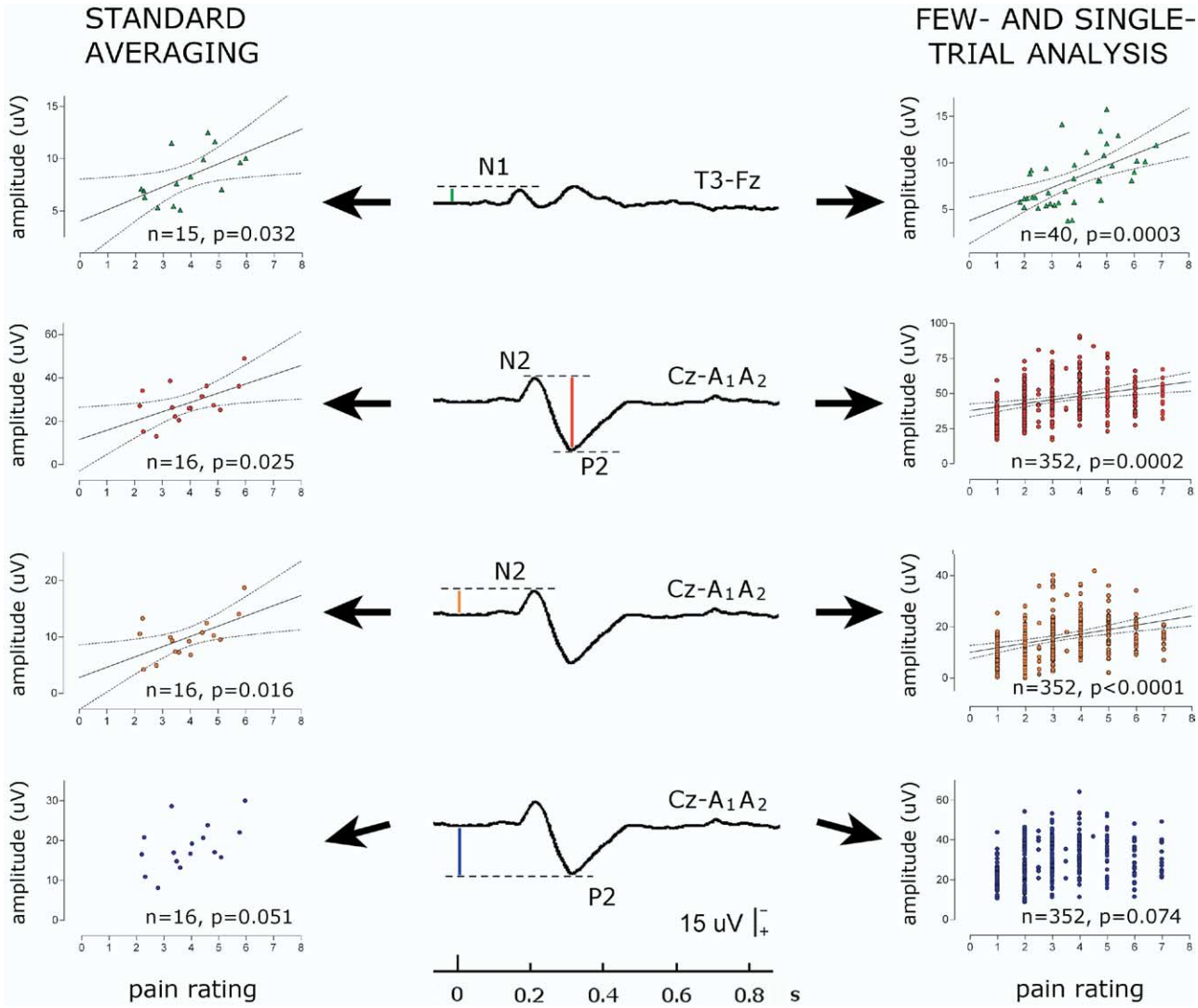

Fig. 5. Pain rating-LEP correlations. Amplitude. The amplitudes of all the explored LEP components showed a clear positive trend, with higher amplitudes for higher pain ratings, but this correlation was statistically significant for N1, N2 and N2–P2 only. The amplitude of N1 was measured baseline-to-peak at the contralateral temporal electrode (T3 versus Fz), that of N2 and P2 components was measured baseline-to-peak at the vertex (Cz versus linked earlobes, A_1A_2) and that of N2–P2 complex was measured peak-to-peak at the vertex. Left column: amplitude values obtained with standard averaging are plotted against the average pain ratings; *y* axis: amplitude; *x* axis: pain rating. Each symbol indicates the mean amplitude obtained from one series of each subject (N1 component: $n=15$; N2, P2 components and N2–P2 complex: $n=16$). Middle column: LEP waveforms with amplitude of the four components highlighted (N1, green; N2–P2, red; N2, orange; P2, blue). Right column: amplitudes obtained with few-trial (N1) or single-trial (N2, P2, N2–P2) averaging are plotted against corresponding pain ratings; *y* axis: amplitude; *x* axis: pain rating. Each symbol indicates the amplitude obtained with few-trial (N1 component: $n=40$) or single-trial averaging (N2, P2 components and N2–P2 complex: $n=352$). The continuous line on each graph is the mean regression calculated on all amplitude values; dashed lines indicate the 95% confidence limits.

task demanded a constant attentional level across the experimental session. The correlations between perceived pain and LEPs were by no means due to time-dependent variations of the cognitive state of the subject.

Naturally, the number of nociceptors activated by each laser pulse might vary, because, in order to avoid skin damage and nociceptor fatigue or sensitisation, the irradiated skin spot must be changed after each stimulus. Hence the sensory input varied slightly because of the laser–skin interaction.

As described above, the standard averaging techniques hide important physiological information. Single-trial analysis permits assessing the between-trial variations of psychophysiological and electrophysiological responses and their relationship, and increases the power of statistical analysis. The inherent variability of single pain-related neural responses further suggests the use of a single-trial analysis approach.

We found that the amplitude of N1 and N2 LEP components strongly correlated with the intensity of perceived pain (Fig. 5). In contrast, latency correlations did not reach statistical significance, although the latency of N1 component yielded by the few-trial approach showed a clear negative trend, with shorter latencies for higher psycho-

physical ratings (Fig. 4). The higher significance of amplitude correlations may be due to the fact that the total number of activated neurons is critical for encoding noxious events and discriminating their intensity (Price, 1988), and stronger inputs activate more cortical neurons synchronously, which necessarily induces an amplitude increase of the scalp response. Alternatively, the latency is affected similarly, but the latency change is very small and sometimes smaller than the precision of the latency measurement of these long-latency responses.

Taking advantage of the single-trial analyses we were able to demonstrate that the correlation between psychophysiological and electrophysiological responses, when present, was higher within- than between-subjects. This finding, although expected, is novel in LEP studies, and it is explained by the importance in pain perception of inter-individual cognitive differences, from education to affective-motivational aspects (Turk and Melzack, 2001).

Role of operculoinsular cortex and SI in coding pain intensity

The correlation between pain ratings and amplitude of LEP components was as strong for the N1 component as for the later N2–P2 complex. Whereas many studies consistently found a positive correlation between pain ratings and the N2–P2 amplitude (Arendt-Nielsen, 1994), far fewer studies have dealt with the role of N1 and operculoinsular areas in coding pain intensity.

A key function of operculoinsular cortex in pain processing has been established with different techniques (for review, see Peyron et al., 2000), and the location of operculoinsular activities is concordant when results from different techniques (PET, fMRI, intracerebral and scalp LEPs) are compared within a common reference space (Peyron et al., 2002).

Some experimental results indirectly suggest an early role of the operculoinsular cortex in coding the intensity of noxious inputs: Schlereth et al. (2003), in particular, found that the N1 (generated by operculoinsular areas) was the most enhanced LEP component during a discrimination task between two levels of stimulus intensity. One EEG study (Garcia-Larrea et al., 1997) and one MEG study (Timmermann et al., 2001) partly contrast with our conclusion that operculoinsular areas encode pain intensity. Garcia-Larrea et al. (1997) did not find any significant correlation; but they used a CO_2 laser (and thus were provided with a less synchronous afferent volley and a smaller cortical signal), adopted a different montage of scalp electrodes (which may make the measurement of N1 amplitude more difficult), and, most of all, did not perform a within-subject analysis. In the MEG study, Timmermann et al. (2001) found that the activation pattern of SII activity in response to a solid-state laser stimulation pointed against a significant contribution of SII to the sensory-discriminative aspects of pain perception. Although they did not directly correlate the activity of MEG sources with pain ratings, the SII activity clearly increased with increasing pain ratings, supporting our findings (Fig. 2B in Timmermann et al., 2001).

In addition, we found a dissociation between the two components constituting the N2–P2 complex: the correlation between N2 amplitude and pain ratings was, both between and within subjects, more significant than that of the whole N2–P2 complex, and far more significant than that of P2, which alone failed to reach statistical significance (Fig. 5). This indicates a different contribution of N2 and P2 to the previously described correlation between the N2–P2 complex and perceived pain. This is particularly interesting in relation to recent evidence showing that the N2 and P2 components are differentially modulated by cognitive tasks (Legrain et al., 2002; Bentley et al., 2004) and have different neural generators (Garcia-Larrea et al., 2003). A bilateral dipolar source in operculoinsular areas has been proposed as generator of the N2 component (Tarkka and Treede, 1993; Valeriani et al., 1996; Frot et al., 1999), and a contribution of the contralateral SI to N2 has been recently demonstrated in an intracranial LEP study (Ohara et al., 2004). Schlereth et al. (2003) have also detected an electrical source in the contralateral postcentral gyrus (SI) within the N2 time-window (with a peak activity occurring 20 ms after the operculoinsular source); interestingly, the activity of this SI source (as well as that of the preceding operculoinsular source) was significantly enhanced during an intensity discrimination task. Furthermore, in their MEG study, Timmermann et al. (2001) found that the pattern of SI activity closely matched the intensity of the perceived pain.

Taken together, these results seem to indicate that SI contributes to the intensity coding of pain as it does for other somatosensory modalities.

To interpret these findings with caution, it is important to consider that EEG scalp signals often cannot resolve neural sources overlapping in time; according to the time course of LEP dipolar sources (e.g. Valeriani et al., 1996; Schlereth et al., 2003), the scalp N2 possibly lumps together the neural activity of different cerebral structures (SI, operculoinsular and cingulate). Nevertheless, the clear dissociation that we found between N1 and N2 amplitudes on one side, and P2 amplitude on the other side, suggests a minor role of the cingulate cortex in coding pain intensity, if compared with the operculoinsular cortex and SI.

In this study we demonstrated that the amplitude of the early N1 and N2 LEP components correlates significantly with subjective pain ratings, indicating that the operculoinsular cortex and perhaps SI, which have been demonstrated to contribute to their generation, encode pain intensity at the earliest stage of nociceptive processing. In all sensory modalities, the first coding of elementary attributes of afferent inputs reaching the brain is essential for the ensuing sensory processing (Kandel et al., 2000). Our data suggest that the nociceptive system, notwithstanding its different characteristics from other, less ancient somatosensory modalities, probably follows this rule, and an accurate, early coding of the noxious input constitutes the necessary basis to which the affective-motivational aspects are subsequently added to produce the full pain experience. This hypothesis is consistent with recent reports indicating an involvement of the operculoinsular cor-

tex in the discriminative representation of thermal-pain perception. Peyron et al. (1999) have shown a significant encoding activity of stimulus intensity in the SII, and Craig et al. (2000) have observed that the activation of the dorsal margin of the middle/posterior insula is linearly correlated with both the intensity of cold stimuli and the subjective rating of perceived temperature. Indeed, patients with lesions in the parietal operculum and posterior insula display an increase of pain and thermal threshold on the contralateral body (Greenspan and Winfield, 1992; Greenspan et al., 1999).

In conclusion, our main finding, made possible by the high temporal resolution of EEG responses and by a laser input that allowed within subject correlations, is that the N1 and N2 components of LEPs significantly correlate with the perceived pain intensity, thus indicating that the intensity-coding of noxious stimuli occurs in the operculoinsular region and, possibly, the SI at the earliest stages of pain processing.

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