Human Brain Oscillatory Activity Phase-Locked to Painful Electrical Stimulations: A Multi-Channel EEG Study

Claudio Babiloni,^{1,2*} Fabio Babiloni,^{1,2} Filippo Carducci,^{1,2} Febo Cincotti,¹
Fabrizio Rosciarelli,¹ Lars Arendt-Nielsen,⁴ Andrew C.N. Chen,⁴
and Paolo Maria Rossini³

¹Dipartimento di Fisiologia Umana e Farmacologia, Università di Roma "La Sapienza," Rome, Italy

²A.Fa.R. CRCCS, Dipartimento di Neuroscienze, Osp. FBF Isola Tiberina, Rome, Italy

³IRCCS "S. Giovanni di Dio." Via Pilastroni, Brescia, Italy

⁴Human Brain Mapping and Cortical Imaging Laboratory, Center for Sensory-Motor Interaction, Aalborg, Denmark

Abstract: The main aims of this study were 1) a fine spatial analysis of electroencephalographic (EEG) oscillations after galvanic painful stimulation (nonpainful stimulation as a reference) and 2) a comparative evaluation of phase- and nonphase-locked component of these EEG oscillations. Preliminary surface Laplacian transformation of EEG data (31 channels) reduced head volume conductor effects. EEG phase values were computed by FFT analysis and the statistical evaluation of these values was performed by Rayleigh test ($P < 0.05$). About 50% of the EEG single trials presented statistically the same FFT phase value of the evoked EEG oscillations (phase-locked single trials), indicating a preponderant phase-locked compared to nonphase-locked component. The remaining single trials showed random FFT phase values (nonphase-locked single trials), indicating a preponderant nonphase-locked compared to phase-locked component. Compared to nonpainful stimulation, painful stimulation increased phase-locked theta to gamma band responses in the contralateral hemisphere and decreased the phase-locked beta band response in the ipsilateral hemisphere. Furthermore, nonphase-locked alpha band response decreased in the ipsilateral fronto-central area. In conclusion, both decreased and increased EEG oscillatory responses to galvanic painful stimulation would occur in parallel in different cortical regions and in the phase- and nonphase-locked EEG data sets. This enriches the actual debate on the mapping of event-related oscillatory activity of human brain. *Hum. Brain Mapping* 15:112–123, 2002.

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INTRODUCTION

Previous electroencephalographic (EEG) studies on somatosensory evoked brain potentials (SEPs) after galvanic painful stimulations have been mainly based on the averaging of EEG data with respect to the stimulus delivery. These studies have shown a typical negative-positive peak complex 100–300 msec post-stimulus, which is largest in amplitude at the scalp vertex [Bromm and Lorenz, 1998; Chen, 1993; Chen et al., 1998a; Chudler and Dong, 1983]. The specificity of this complex was suggested by the correlation among the vertex potential amplitude, pain magnitude, and

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*Correspondence to: Dr. Claudio Babiloni, Dipartimento di Fisiologia Umana e Farmacologia, Sezione di EEG ad Alta Risoluzione, Università degli Studi di Roma "La Sapienza," P.le Aldo Moro 5, 00185 Rome, Italy.

E-mail: claudio.babiloni@uniroma1.it

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analgesics administration. The negative-positive peak complex would roughly model the event-related response of cingulate cortex, deeply involved in the attentional and affective aspects of the cortical information processing [Bromm and Lorenz, 1998; Chen et al., 1998b].

Another way to investigate human brain responses to painful stimulations is the analysis of event-related EEG rhythms or oscillations. The most striking findings for tonic painful stimulations concerned with an increment of delta power [Chen et al., 1989; Chen and Rappelsberger, 1994; Veerasarn and Stohler, 1992] as well as with a decrement of alpha power (8–12 Hz) and an increment of beta power (13–30 Hz) [Backonja et al., 1991; Chen et al., 1989; Chen and Rappelsberger, 1994; Veerasarn and Stohler, 1992]. On the other hand, enlarged EEG oscillations after a brief galvanic painful stimulation were observed in delta (0.5–3.5 Hz), theta (4–7 Hz) and low alpha (8–10 Hz) bands [Bromm et al., 1989]. The preliminary administration of opioid/antidepressant reduced the power of all EEG frequency bands [Bromm et al., 1989].

The EEG oscillations are generated by the reciprocal coupling between excitatory and inhibitory neurons, whereby the feedback via cortico-cortical and reciprocal thalamo-cortical connections plays an important role [Pfurtscheller and Lopes da Silva, 1999]. Neural generators producing EEG oscillations are usually randomly active, but change their functional state in response to a sensory stimulation. Synchronization and enhancement of post-stimulus EEG activity would mainly indicate a phase locking of EEG oscillations [Basar, 1980]. The phase-locked EEG activity is roughly depicted in the averaged evoked potentials [Basar, 1980; Sayers et al., 1974]. The phase-locked EEG activity can model the post-stimulus reorganization of oscillatory neural networks [Brandt and Jansen, 1991; Jansen and Brandt, 1991]. On the other hand, the nonphase-locked EEG activity contains brain oscillations not rigidly time-locked to the stimulus, which may provide additional information on sensory information processing [Eckhorn et al., 1988; Makeig 1993; Pfurtscheller, 1988; Pfurtscheller and Lopes da Silva, 1999; Pfurtscheller and Neuper, 1994]. According to the additive model of event-related potentials, the nonphase-locked brain oscillations produce the so-called background rhythmic EEG activity [Basar, 1998].

Previous EEG studies have also shown that phase-locked and nonphase-locked components of visual and auditory evoked potentials might co-exist at different latencies in each single trial and might be relatively independent of each other [Brandt and Jansen,

1991; Jansen et al., 1993; Jansen and Brandt, 1991]. In the present EEG study, it was assumed that the phase-locking of evoked EEG oscillations is represented by a polarization of the FFT phase values of EEG single trials around a certain value. According to this assumption, we sorted in two classes the single trials of potentials evoked by painful and nonpainful galvanic stimulations, which were previously analyzed in time domain [Babiloni et al., 2001]. The first class included single trials having such a polarized phase value in the post-stimulus EEG oscillations (phase-locked single trials). The second class included single trials having random phase values (nonphase-locked single trials).

In the present study, we defined the concept of phase-locked component as the signal component that affects the (FFT) phase value of the post-stimulus EEG oscillations. When this component is preponderant, the phase of the post-stimulus EEG oscillations would be fixed at a certain value. This would be the case of the phase-locked single trials. In contrast, a slight or negligible phase-locked component would be incapable of fixing the phase value of the post-stimulus EEG oscillations. As a result, the nonphase-locked component of the EEG oscillations would be disclosed. This would be the case of the nonphase-locked single trials.

The use of such a sorting procedure does not imply that phase-locked component is present only in the phase-locked single trials and nonphase-locked component is present only in the nonphase-locked single trials. Indeed, a minor nonphase-locked component (i.e., incapable of randomizing the phase of EEG oscillations) might be present in the phase-locked single trials. Furthermore, a minor phase-locked component (i.e., incapable of fixing the phase of EEG oscillations) might be present in the nonphase-locked single trials. Therefore, the sorting of the single trials in two mutually exclusive classes does not allow the investigation of the pure EEG phase-locked component vs. the pure EEG nonphase-locked component, co-existing at each single trial [Jansen and Brandt, 1991].

A main working hypothesis of the present study is that our sorting procedure allows a rough but insightful mapping of the phase- vs. nonphase-locked components of the evoked EEG oscillations. Of course, the underlying assumption is that the emerging features of the phase-locked component can be extracted by the phase-locked single trials, even if they have a minor nonphase-locked component. Analogously, it is assumed that the emerging features of the nonphase-locked component can be extracted by the nonphase-locked single trials, even if they have a minor phase-locked component. On the whole, the proposed sorting procedure would have intrinsic limits (i.e.,

classification of EEG single trials in two mutually exclusive classes) but would enrich the actual debate on the mapping of phase- vs. nonphase-locked oscillatory activity of human brain. In particular, the present study addressed the following novel issues: 1) a finer spatial analysis of painful stimulus-related EEG oscillations (i.e., theta, alpha, beta, and gamma bands) by the use of surface Laplacian transformation acting as a mathematical spatial high-pass filter that reduces head volume conductor effects and annuls the influence of electrode reference [Nunez, 1995]; 2) the comparative analysis of phase- and nonphase-locked EEG oscillations after painful stimulations; and 3) the evaluation of the encoding of painful intensity from the point of view of EEG oscillations. Of note, the aims of the present study do not include the evaluation of the phase-reordering phenomenon (i.e., the time evolution of EEG phase) and the analysis of power increase/decrease of the evoked EEG oscillations compared to pre-stimulus baseline.

MATERIALS AND METHODS

Generalities

The following description of subjects, stimulation procedure, EEG recordings, and preliminary data analysis was sketched here. Further details can be found in the companion study [Babiloni et al., 2001]. Twelve healthy, informed volunteers participated to the present study. During the experimental sessions, the median nerve was stimulated (3 Hz) at the left wrist. The stimulation levels were determined by a series of increasing and decreasing stimulus intensities at the beginning of each recording block. The painfulness of the stimuli was rated verbally by the subjects on a numerical scale ranging from 0 (no sensation) to 10 (pain tolerance threshold). In this scale, values of 2 (no pain, thumb muscle twitch) and 4 (slight pain) indicated the motor and pain thresholds, respectively. Furthermore, value of 6 (moderate pain) corresponded to a pain level proportionally distributed between values of 4 and 10.

SEPs were recorded (linked-earlobe reference, bandpass of 0.05–500 Hz, sampling rate of 2,000 Hz) from 31 electrodes, according to an augmented 10-20 system. An electro-oculographic channel was used to monitor eye movements and blinking. Subjects performed no involuntary hand motor response after the galvanic stimulations (apart stimulus-locked thumb muscle twitch), during the preliminary experimental sessions. Therefore, no electromyographic channel was used during the EEG recording. The experiment-

ers controlled the occurrence of post-stimulus movements during the EEG recordings by visual inspection. About 600 EEG segments (single trials) from 50 msec before to 250 msec post-stimulus were collected for each of the three stimulus conditions or blocks. The recording blocks were pseudo-randomized across the subjects to balance the order effects

EEG single trials contaminated by blinking, eye movements, or other artifacts were carefully rejected off-line. EEG data after painful and non-painful stimulations were not diversely affected by these artifacts. To enhance spatially the EEG data, surface Laplacian estimate over a spherical model was performed by a 3-D spline function [Babiloni et al., 1996]. In some cases, the Laplacian values on the border electrodes were zeroed because of unreliability of the spline Laplacian estimate for these electrodes [Babiloni et al., 1998].

Spectral data analysis

Selection of the (EEG) single trials phase- and non-phase-locked to the galvanic stimulus delivery was performed as described below.

Power density spectrum analysis of the single trials was computed by FFT (Bartlett set) for a period ranging from +10 msec (i.e., after stimulus artifact) to +250 msec after the stimulus delivery (zerotime). Zero-padding procedure with about 50% zeros was used to obtain frequency bins of 2 Hz, after that a simulation study demonstrated that the implemented procedure caused no phase shift. The individual spectral reactivity was computed for the three conditions (moderate painful, slight painful, and nonpainful stimulations) within each band of interest, i.e., theta (4–6 Hz), alpha (8–12 Hz), beta 1 (14–22 Hz), beta 2 (24–34 Hz), and gamma (36–50 Hz). Owing to the brief period of 250 msec in the post-stimulation period, no EEG delta band was investigated in this study. Whereas, such a brief period may allow the computation of theta EEG oscillations, taking into account recent evidence that maximum theta response to a stimulation is observed within 250–300 msec post-stimulus, i.e., about a cycle of the theta rhythm [Basar, 1999].

For each condition, the phase (FFT) of bandpassed data was extracted from each single trial (individual frequency reactivity). Results from individual subjects were represented by raster and histogram graphs (Fig. 1), which emphasized possible polarization or direction of the phase around certain values within 50 bins of 7.2° (i.e., obtained dividing the $0-360^\circ$ range of the phase by 50). Such a direction would indicate the

Figure 1.

Raster and histogram graphs illustrating the phase (FFT) of band-passed (theta, alpha, beta 1, beta 2, and gamma) EEG oscillations after galvanic nonpainful and (moderate and slight) painful stimulations at left wrist (i.e., overlying median nerve stimulation). The EEG oscillations refer to an electrode position (FC2), in which the

phase-locked EEG oscillations were maximum in a representative subject. The FFT phase values (degrees) are illustrated for each single trial in the raster. The histograms (last three rows) plot the number of single trials within 50 bins of 7.2°, obtained dividing the 0–360° phase range by 50.

phase locking of the EEG oscillations in the post-stimulus period. For each subject/condition/frequency of interest, phase-locking resulted in a peak (“polarization”) on the phase histograms (Fig. 1).

The statistical significance of this “polarization” for each subject/condition/electrode was computed by Rayleigh test, which determined if the phase (angle) of the single trials belonged to a uniform circular distribution. Rayleigh’s R was calculated by

$$R = nr,$$

where n is the single trials number and r is the length of the mean phase (angle) vector. Useful information on the r-values was given by

$$r = \sqrt{X^2 + Y^2},$$

where X and Y are the rectangular coordinates of the mean phase or angle ($\bar{a}$) calculated by

$$X = \frac{\sum_{i=1}^n \cos a_i}{n} \quad \text{and} \quad Y = \frac{\sum_{i=1}^n \sin a_i}{n}.$$

An excellent approximation of the probability (P) of Rayleigh’s R [Zar, 1996] was provided by

$$P = \exp[\sqrt{1 + 4n + 4(n^2 - R^2)} - (1 + 2n)].$$

For each non-border electrode, Rayleigh test indicated the FFT phase values associated with a statistical significance ($P < 0.05$). The reference electrode was defined as that electrode showing the most statistically significant phase-locked FFT phase values for a given subject/condition/frequency of interest. The phase-locked single trials were those presenting the polarized or phase-locked values at the reference electrode. The nonphase-locked single trials were the remaining ones. On the whole, the classification of phase-locked

TABLE I. Topography of maximum phase-locked EEG oscillations

Subject	Theta	Alpha	Beta1	Beta2	Gamma
AN	FC2	Fz	FC2	Fz	Fz
AP	FC2	FC2	PC6	PC6	FC2
BH	FC2	Fz	FC2	Fz	FC2
BK	FC2	Fz	FC2	PC6	FC2
CN	FC2	FC2	FC2	FC2	PC6
DN	FC2	PC6	PC6	FC2	FC2
JO	P4	P4	P4	P4	FC2
JR	FC2	FC2	FC2	FC2	FC2
MN	FC2	FC2	FC2	PC6	FC2
MV	FC2	FC2	FC2	FC2	FC2
RL	FC2	FC2	FC2	PC6	PC6
ST	Fz	Fz	FC2	FC2	PC6

Scalp topography of maximum phase-locked EEG oscillations following left galvanic nonpainful and (moderate and slight) painful stimulations in all participating subjects. The scalp topography is indicated by the electrode site (augmented 10-20 system) in which this phase-locked EEG activity was maximum and statistically significant (Rayleigh test, $p < 0.05$).

and nonphase-locked single trials for each subject/condition/frequency band was based only on the statistically most “responsive” (“reference”) electrode in terms of phase “polarization” (see Table I).

To substantiate our sorting procedure, two-way ANOVA for repeated measures was used for the statistical comparison of amplitudes of SEPs generated by averaging separately phase- and nonphase-locked single trials, which were classified on the basis of the (FFT) phase of alpha band oscillations. The two SEP data sets were formed averaging the same number of single trials, to avoid differences in amplitude due to the disparity of the single trials averaged. Tukey’s test was used for post-hoc comparisons.

The power spectral reactivity after the painful stimulation was defined for each band as the maximum of the spectral ratio between EEG oscillations to moderate painful vs. nonpainful stimulations. In addition, the spectral ratio was computed between EEG oscillations to moderate vs. slight painful stimulations, to evaluate brain processes for the encoding of painful stimulation intensity. In total, we computed the following four spectral ratios: 1) phase-locked moderate painful vs. nonpainful stimulation data; 2) nonphase-locked moderate painful vs. nonpainful stimulation data; 3) phase-locked moderate painful vs. slight painful stimulation data; and 4) nonphase-locked moderate painful vs. slight painful stimulation data.

Descriptive statistical analysis included the computation of across-subjects mean and standard error of the aforementioned spectral ratios. The grand averaging of individual power density distributions allowed the removal of the effects of inter-individual frequency band variations from across-subjects mean.

This was particularly important, given that EEG data related to painful stimuli might present a high inter-subject variability. On the other hand, inferential statistical comparisons were performed with paired t -test. Maximum differences of each spectral ratio (i.e., moderate painful vs. nonpainful stimulations) were selected based on the color mapping of statistical t -values. A Bonferroni-corrected ($P < 0.05$) paired t -testing was then performed only at most responsive electrodes, i.e. electrodes nearest to the maximum mapped t -values. Noteworthy, a Bonferroni or Holm correction for all electrodes/comparisons would have been inadequate. In fact, due to residual head volume conduction effects and distributed EEG oscillatory activity, the spatial information conveyed by each electrode is not independent of that of close electrodes.

Across-subjects mean of the spectral ratio, standard error, t -statistics, and correlation between phase- and nonphase-locked data were mapped on a 3-D quasi-realistic head model by a spline interpolating function [Babiloni et al., 1995]. This model was constructed based on the magnetic resonance data of 152 subjects digitized at Brain Imaging Center of the Montreal Neurological Institute (SPM96).

RESULTS

Averaged seps to painful stimulation

The quality and reliability of averaged SEPs after painful and non-painful stimulation can be ascertained in the companion study [Babiloni et al., 2001]. To summarize, the subtraction waveform of centromedian SEPs to the moderate painful minus nonpainful

Figure 2.

Most representative waveforms of spatially-enhanced somatosensory evoked potentials (SEPs) to the nonpainful stimulations in a representative subject (Subject 2). Electrode sites are labeled according to 10-20 system.

stimulation disclosed the typical SEP negative-positive complex observed after a painful stimulation [Bromm and Lorenz, 1998]. As an example, typical individual waveforms of spatially enhanced SEPs to the nonpainful stimulations are depicted in Figure 2 (Subject 2). Based on their topography, polarity, and latency, the SEPs to nonpainful stimulations were labeled frontal P20-N30-N60-N120-P170, central P22-P45, and parietal N20-P30-P60-P120 (N = negativity; P = positivity; number = latency in msec). On the other hand, SEPs to the painful stimulations were labeled parietal P80 and central N125-P170-P200.

Classification of phase- and nonphase-locked single trials

The phase locking of the EEG oscillations was observed in all subjects/conditions/frequency bands. The most significant phase-locked EEG oscillations were generally computed in the contralateral fronto-central scalp region (Table I). The percentage of the

phase-locked EEG oscillations increased significantly with the intensity of the galvanic stimulation, particularly in the alpha and beta bands ($P < 0.05$ to $P < 0.001$). The percentage range of these oscillations (Table II) was from 49–54% (nonpainful stimulation) to 52–59% (slight painful stimulation) and 56–59% (moderate painful stimulation). The mean phase of the phase-locked EEG oscillations ranged from 123° to 220° and did not differ significantly across the conditions and bands (Table III). Remarkably, RUN test indicated that the phase- and nonphase-locked EEG oscillations were randomly inter-mingled across each condition, in that they did not tend to occur in clusters ($P > 0.05$) [Zar, 1996].

Figure 3 shows across-subjects grand average waveforms of spatially enhanced SEPs, which were located at right frontal (F4 electrode site of 10-20 system), central (C4), and parietal (PC6) leads contralateral to the nonpainful median nerve stimulations. These waveforms refer to the SEPs obtained averaging separately phase-locked (thick black traces) and nonphase-locked (thin gray traces) single trials. It is noted that, within 100 msec post-stimulus, the potential amplitude was greater in the SEPs generated averaging phase- rather than nonphase-locked single trials, especially at the contralateral frontal and central leads.

Individual data analysis of SEPs from phase- vs. nonphase-locked single trials allowed the detection of N60 and P170 potentials at right (contralateral) frontal leads of all but one subject. These potentials were further considered because they showed the main negative (N60) and positive (P170) SEP peaks in two post-stimulus periods of interest, i.e., from +20 to +100 msec and from +100 to +250 msec, respectively. The N60 peaked at about +69 msec (± 5 SE, phase-locked data) and +71 msec (± 6 SE, nonphase-locked data) post-stimulus. Its amplitude ranged from $-19 \mu\text{V}/\text{cm}^2$ (± 3.6 SE, phase-locked data) to $-7 \mu\text{V}/\text{cm}^2$ (± 3.5 SE, nonphase-locked data). On the other hand, the P170 peaked at about +143 msec (± 6 SE, phase-locked data) and +146 msec (± 7 SE, nonphase-locked data) post-stimulus. The amplitude of the P170 was of

TABLE II. Percentage of phase-locked single trials

Condition	Theta	Alpha	Beta1	Beta2	Gamma
No pain	53 ± 1	55 ± 1	49 ± 1	49 ± 1	54 ± 1
Slight pain	59 ± 1	53 ± 1	52 ± 1	53 ± 1	54 ± 1
Mod. pain	55 ± 1	57 ± 1	54 ± 1	59 ± 1	59 ± 1

Across-subjects mean percentage ($\pm$ standard error) of the phase-locked EEG single trials following the galvanic nonpainful and (moderate and slight) painful stimulations. The percentage values refer to all bands of interest (theta, alpha, beta 1, beta 2, and gamma).

TABLE III. Mean phase of single trials

Condition	Theta	Alpha	Beta1	Beta2	Gamma
No pain	220 ± 18	194 ± 28	146 ± 24	149 ± 33	132 ± 33
Slight pain	178 ± 25	166 ± 31	144 ± 14	143 ± 29	124 ± 26
Mod. pain	212 ± 26	189 ± 27	149 ± 22	180 ± 33	154 ± 31

Across-subjects mean phase ($\pm$ standard error) of phase-locked EEG single trials following the galvanic nonpainful and (moderate and slight) painful stimulations. The mean phase values refer to all bands of interest (theta, alpha, beta 1, beta 2, and gamma).

+5.4 $\mu\text{V}/\text{cm}^2$ (± 3.2 SE, phase-locked data) and +4.5 $\mu\text{V}/\text{cm}^2$ (± 3 SE, nonphase-locked data). Of note, the low amplitude and inter-subjects variability in latency made the P170 hardly detectable in the illustrated grand average SEP waveforms.

ANOVA analysis evaluated the influence on the SEP amplitude (nonpainful stimulation) of 2 Components (phase-locked, nonphase-locked) $\times$ 2 Potentials of interest (N60, P170). A main effect for the Potentials ($F(1,10) = 65.52$; $\text{MSe} = 58.03$; $P < 0.00001$) showed the largely expected difference in amplitude between N60 and P170 potentials. More interestingly, a significant interaction components $\times$ potentials ($F(1,10) = 7.1$; $\text{MSe} = 17.95$; $P < 0.02$) revealed that the potential amplitude of N60 (post-hoc test, $P < 0.003$) but not P170 was greater with phase- than nonphase-locked SEPs.

Figure 3.

Across-subjects grand average waveforms of spatially enhanced SEPs, which are located at right frontal (F4 electrode site of 10-20 system), central (C4), and parietal (PC6) leads contralateral to the nonpainful stimulations. These waveforms refer to the SEPs obtained averaging separately phase-locked (thick black traces) and nonphase-locked (thin gray traces) single trials.

Topographical mapping

Maximally across-subjects responsive frequency bands were observed at 6 Hz (theta), 10–12 Hz (alpha), 14–20 Hz (beta 1), 24–32 Hz (beta 2), and 40–48 Hz (gamma). T color maps of Figure 4 illustrate global statistical differences of the spectral ratios for the phase-locked moderate painful vs. nonpainful stimulations and for the nonphase-locked moderate painful vs. nonpainful stimulations. There were statistically stronger spectral values for the phase-locked moderate painful than nonpainful stimulations (positive t -values). The maximum positive t -values were distributed mainly in the contralateral frontal or central areas at all bands of interest. Whereas, significant negative t -values were observed only in the ipsilateral fronto-central area at beta 2 band.

Compared to these maps, the maps of the corresponding nonphase-locked data were characterized by lower and topographically diverse positive t -values, i.e., central and parietal theta-alpha responses in the contralateral hemisphere. There were also negative t -values of fronto-central alpha response in the ipsilateral hemisphere. On the other hand, the only consistent t -values for the moderate vs. slight painful stimulations were observed for the phase-locked data, i.e., negative t -values of theta band in the parietomedian area (not shown).

For the phase-locked data, final statistical comparisons for the moderate vs. nonpainful stimulations (Bonferroni corrected $P < 0.05$; 11 df) confirmed statistically significant t -values at theta band FC2 ($t = 4.54$; $P < 0.0004$), alpha band FC2 ($t = 3.25$; $P < 0.004$), beta 2 band C4 ($t = 6.46$; $P < 0.0002$) and C3 ($t = -3.92$; $P < 0.001$), and gamma band C4 ($t = 8.54$; $P < 0.000002$). For the nonphase-locked data, statistically significant results were obtained at alpha band P4 ($t = 3.0$; $P < 0.006$) and only a statistical trend was computed at alpha band C3 (-1.75 ; $P < 0.05$).

The confirmatory statistical analysis for the moderate vs. slight painful stimulations was performed at only one electrode site (Pz) and for phase-locked data,

Figure 4.

T-values color maps plotting the results of an explorative statistical comparison of spectral ratios for the phase-locked moderate painful vs. nonpainful stimulations and for the nonphase-locked moderate painful vs. nonpainful stimulations. Color scale (256 hues): maximum negative and positive t -values are coded in white

and violet, respectively. Negative t -values (white-red) indicate a lower spectral density for the moderate painful stimulation than the nonpainful stimulations. Vice-versa for the positive t -values (violet-blue).

due to the low level of t mapping values. A significant t -value was computed at theta band Pz ($t = -2.44$; $P < 0.02$).

Figure 5 plots the maps of linear correlation between phase- and nonphase-locked spectral ratio data for the nonpainful vs. painful stimulations. A correlation value close to -1 (white color) indicates that an increase (or decrease) of phase-locked EEG oscillations corresponded to a decrease (or increase) of nonphase-locked EEG oscillations. This indicated a specific phase-locking of the EEG oscillations after painful stimulations. In the figure, the correlation maps showed highly significant negative correlation maxima (-1). Their topography changed at the different

bands of interest and regarded mainly bilateral frontal, contralateral central, and medial parietal areas.

DISCUSSION

Methodological remarks

The reliability of galvanic painful stimulations and surface Laplacian transformation (EEG spatial enhancement) was discussed in detail in the companion study [Babiloni et al., 2001] and is just summarized here. Median-nerve galvanic painful stimulation might exhibit poor nociceptive specificity [Arendt-Nielsen, 1994; Bromm and Lorenz, 1998; Chen et al.,

Figure 5.

Maps illustrating the linear correlation (r -values) between phase- and nonphase-locked spectral ratio data for the moderate painful vs. nonpainful stimulations. Color scale (256 hues): maximum negative and positive r -values (± 1) are coded in white and violet, respectively.

1998d]. The following reasons, however, would support the reliability of the present results. First, the galvanic stimulation was carefully based on a subjective scale, to guarantee a stable experience of the slight and moderate painful stimulations during the corresponding recording sessions. Second, the painful but not nonpainful stimulations disclosed the typical vertex negative-positive complex observed in previous studies using highly specific painful stimulations (i.e. CO₂ laser beam) [Bromm and Lorenz, 1998]. Third, our moving grand averaging technique minimized the inter-subjects variability of EEG responses to painful stimulations.

Results of the surface Laplacian estimate should be interpreted with caution. In fact, surface Laplacian maxima could not always overlie cortical sources of EEG potentials, due to the influence of both radial and tangential cortical generators [Babiloni et al., 1996]. Furthermore, relevant cortical sources such as secondary somatosensory area and insula cortex [Kitamura et al., 1995] were not investigated, because surface Laplacian estimate is not fully reliable when computed at the border temporo-parietal electrodes [Nunez, 1995]. Finally, the medium resolution of the EEG spatial sampling (31 recording channels) permitted a description only of the more robust topographical results. It should be stressed, however, that this spatial sampling is equal or higher than that of all previous studies on EEG oscillations related to painful stimulations

Classification of phase- and nonphase-locked single trials

In this study, a simple procedure for the discrimination of the so called phase-locked and nonphase-locked (EEG) single trials was described (see Introduction for pros and cons of such a procedure). The procedure selected phase-locked single trials showing a post-stimulus polarization of the phase (FFT) within 50 bins of 7.2°. In previous studies, the frequency analysis was performed on averaged event-related potentials, whereby the phase-locked responses were enhanced and the nonphase-locked responses were attenuated [Jervis et al., 1983; Kaufman et al., 1989]. In addition, the global amount of phase-locked EEG oscillations was computed along the time axis [Kolev and Yordanova, 1997]. On the whole, there were only few studies focusing on the phase characteristics of EEG oscillations [Basar, 1998; Brandt et al., 1991; Jervis et al., 1983; Sayers et al., 1974] and no previous study addressed (i.e., trial-by-trial) the correlation between phase- and nonphase-locked EEG oscillations.

To further substantiate the present sorting procedure, we compared the amplitudes of the two SEPs generated by averaging separately phase- and nonphase-locked single trials, classified on the basis of the (FFT) phase of alpha band oscillations. Results showed that the SEPs within 100 ms post-stimulus (i.e., frontal N60) were higher in amplitude with the

phase- than nonphase-locked single trials, as expected by the fact that averaging procedure enhances phase-locked component of EEG data. These results may confirm that the present EEG phase-locked component is effectively an emerging feature of the phase-locked single trials compared to the nonphase-locked single trials. Furthermore, these results are in line with a previous influential study [Jansen and Brandt, 1991] demonstrating that EEG alpha oscillatory phase affected a negative potential (N1, about 80 msec post-stimulus) but not a subsequent positive potential (P2) of visual evoked potentials.

Cortical sources of EEG oscillations after painful stimulation

The present results would indicate that the galvanic painful stimulation determined a strong phase-locked increment of EEG oscillations in the contralateral fronto-central (theta, alpha) and central (beta 2, gamma) areas as well as a circumscribed decrement of these oscillations in the ipsilateral central (beta 2) area. With respect to the phase-locked EEG reactivity, the nonphase-locked EEG oscillations were lower in magnitude and circumscribed to the alpha band. The significant nonphase-locked alpha EEG oscillations increased in the contralateral hemisphere (i.e., central and posterior parietal areas) and decreased in the ipsilateral hemisphere (i.e., fronto-central areas).

These findings extended in spatial details (i.e., by Laplacian derivation) and phase relationships the results of preceding EEG investigations using tonic thermal and chemical stimulations [Backonja et al., 1991; Chen et al., 1989, 1998c; Chen and Rappelsberger, 1994; Veerasarn and Stohler, 1992], which have shown decreased alpha and increased beta oscillations due to the painful stimulation. The present findings extend also the results of a previous EEG study showing a statistically significant enlargement of EEG power at theta and alpha bands in response to a galvanic painful stimulation [Bromm et al., 1989]. The increment and decrement of spatially enhanced EEG oscillations may depend on the cortical areas of interest and the phase- and nonphase-locked components of the data examined.

We could indirectly compare phase-locked EEG oscillations after painful stimulations with respect to the corresponding averaged SEPs [Babiloni et al., 2001]. These oscillations were maximum in the contralateral fronto-central (theta, alpha) and central (beta 2, gamma) areas. In contrast, the averaged SEPs to painful stimulations were maximum mainly on the posterior parietal (P80) and ipsilateral central (N125, P170,

P200) areas. In other words, painful stimulation induced phase-locked EEG oscillations in cortical areas not overlapped to those generating averaged SEPs. On the other hand, as expected, the oscillatory reactivity to painful stimulation of the phase-locked data was very similar to that computed by the spectral analysis in the averaged SEPs (not shown), which are phase-locked for definition. On the whole, these results support the idea that time and frequency domain analyses of EEG data complement each other in the study of human nociception.

EEG oscillations affected by galvanic painful stimulation: novel evidence

The findings of the present study would suggest that multiple operating rhythms of the contralateral central and frontal (attentional) cortical areas increased in magnitude and were phase-locked to the painful input.

Compared to the slight painful stimulations, the moderate painful stimulation provoked a decrement of theta oscillations over the posterior parietal cortex. The specificity of the parietal phase-locked theta response in the coding of the painful intensity would be substantiated by the local negative correlation values of the phase- and nonphase-locked data (moderate vs. slight painful stimulation). Higher the parietal theta response in the phase-locked data, lower the same theta response in the nonphase-locked data. Because the theta response is considered the fingerprint of limbic/hippocampal activity [Lopes da Silva, 1992], it can be hypothesized that the higher intensity of the painful stimulation modulates limbic-cortical oscillatory networks, for the analysis of the affective-motivational components of painful information processing [Treede et al., 1999].

The present results would support the idea that reactivity of alpha oscillations after the painful stimulations occurs both in phase and not in phase with the painful stimulation. Topography of these oscillations may hint an involvement of associative posterior and frontal cortical areas rather than an activation of the contralateral primary somatosensory cortex. Whereas, the beta-gamma reactivity was phase-locked and might be more specifically related to nociceptive processes occurring in this primary cortex. This enriches the debate on local "alphas" rhythms of human brain [Basar, 1998; Kuhlman, 1978].

A striking finding of the present study was the decrement of phase-locked beta 2 oscillations in the central area ipsilateral to the movement, which was concomitant with the increased phase-locked beta

2-gamma oscillations in the contralateral central area. The increased beta 2-gamma responses may subserve the perceptual evaluation of the painful information in the contralateral primary somatosensory cortex. Whereas, the decreased beta 2 response would point to a concomitant inhibition of the ipsilateral somatosensory cortex not involved in the processing of the painful input. These original results complement well the known properties of high frequency responses in the perceptual binding of sensory stimuli [Eckhorn et al., 1988; Gray and Singer 1989] and disclose the importance of inter-hemispherical interactions in the modulation of EEG oscillations induced by a painful stimulation.

Phase- and nonphase-locked EEG oscillations

About 50% of the single trials presented the same (FFT) phase value of the EEG oscillations evoked by median nerve stimulations (phase-locked single trials), indicating a preponderant phase-locked compared to nonphase-locked component. The remaining single trials show random phase values (nonphase-locked single trials), indicating a preponderant nonphase-locked compared to phase-locked component. The phase-locked component was then extracted by the phase-locked single trials, whereas the nonphase-locked component was extracted by the nonphase-locked single trials. As aforementioned, this does not imply that in a certain single trial there is either a phase-locked component or a nonphase-locked component (see Introduction).

The maximum phase locking of the EEG oscillations was observed in the frontal-parietal scalp areas contralateral to the stimulation. The phase- and nonphase-locked EEG single trials were intermingled across the experiment, indicating that post-stimulus phase locking of the EEG oscillations would not be due to a simple linear process related to the nociceptive input. In this case, such an input would have generated phase-locked EEG oscillations in all single trials. Furthermore, the negative correlation values of the phase- and nonphase-locked EEG oscillations showed that the painful stimulation induced a power spectral increment of the phase-locked responses and a power spectral decrement of these responses in the corresponding nonphase-locked data. This confirmed the stimulus-dependence of the phase of cortical oscillators triggered by painful stimulations.

CONCLUSIONS

The present procedure for the sorting of EEG single trials allowed a separate mapping of the phase- and

nonphase-locked components of EEG oscillations after nonpainful vs. painful stimulations (see Introduction for pros and cons of such a procedure).

Phase- and nonphase-locked EEG oscillations after painful stimulations showed a different topography that arises the issue of their difference functional meaning. EEG phase-locked component was much more reactive than EEG nonphase-locked component to the painful stimulation (i.e., nonpainful stimulation as a reference).

Phase-locked EEG oscillations after painful stimulation (theta, alpha, beta 2 and gamma bands) globally increased in the contralateral hemisphere. Beta 2 and gamma oscillations increased specifically over the contralateral primary somatosensory cortex and may be strictly involved in the perceptual (binding?) analysis of the painful stimulus. In addition, there would be a (inhibitory?) reduction of these oscillations over the opposite, non-operand cortical area. Finally, the phase-locked theta responses (limbic input?) over the posterior parietal areas would modulate the coding of painful intensity.

In parallel to increased phase-locked alpha responses over the contralateral fronto-central areas, painful stimulation induced decreased nonphase-locked alpha oscillations over the ipsilateral fronto-central areas. This may suggest a multiple nature of alpha oscillations involved in nociception. These oscillations would react (increase vs. decrease) diversely in the contralateral and ipsilateral hemispheres and in the phase- and nonphase-locked components of the EEG oscillations.

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