Single-Trial Analysis of Oddball Event-Related Potentials in Simultaneous EEG-fMRI

Christian-G. Bénar,1,2* Daniele Schön,3 Stephan Grimault,1 Bruno Nazarian,1 Boris Burle,4 Muriel Roth,1 Jean-Michel Badier,2 Patrick Marquis,2 Catherine Liegeois-Chauvel,2 and Jean-Luc Anton1

1CNRS IFR131, Centre d’IRMf de Marseille, Marseille, France
2INSERM, U751, Marseille, France; Université Aix-Marseille, Marseille, France
3CNRS, UMR6193, Marseille, France; Université Aix-Marseille, Marseille, France
4CNRS, LIMR6155, Marseille, France; Université Aix-Marseille, Marseille, France

Abstract: There has recently been a growing interest in the use of simultaneous electroencephalography (EEG) and functional MRI (fMRI) for evoked activity in cognitive paradigms, thereby obtaining functional datasets with both high spatial and temporal resolution. The simultaneous recording permits obtaining event-related potentials (ERPs) and MR images in the same environment, conditions of stimulation, and subject state; it also enables tracing the joint fluctuations of EEG and fMRI signals. The goal of this study was to investigate the possibility of tracking the trial-to-trial changes in event-related EEG activity, and of using this information as a parameter in fMRI analysis. We used an auditory oddball paradigm and obtained single-trial amplitude and latency features from the EEG acquired during fMRI scanning. The single-trial P300 latency presented significant correlation with parameters external to the EEG (target-to-target interval and reaction time). Moreover, we obtained significant fMRI activations for the modulation by P300 amplitude and latency, both at the single-subject and at the group level. Our results indicate that, in line with other studies, the EEG can bring a new dimension to the field of fMRI analysis by providing fine temporal information on the fluctuations in brain activity. Hum Brain Mapp 28:602–613, 2007.

Key words: simultaneous EEG-fMRI; auditory oddball; evoked potentials; single-trial analysis

INTRODUCTION

The feasibility of recording the electroencephalogram (EEG) within an MRI scanner was proven by Ives et al. [1993], despite the difficult conditions arising from placing conductive wires within large magnetic fields. This breakthrough was followed by several methodological studies investigating patient safety [Lemieux et al., 1997], EEG recording, and filtering techniques [Allen et al., 1998; Goldman et al., 2000; Muri et al., 1998], image quality [Bonnassar et al., 2001; Krakow et al., 2000; Krakow et al., 1999; Seeck et al., 2003a], and EEG quality [Bénar et al., 2003; Salek-Haddadi et al., 2003a].

The simultaneous recording of EEG and fMRI opened a new path by enabling the analysis of spontaneous EEG events during functional MRI (fMRI) scanning [see Salek-Haddadi et al., 2003b, for a review]. In epilepsy, the simultaneous EEG recording is in fact the only way to know when epileptic discharges take place during fMRI acquisition [Al-Asmi et al., 2003; Jackson and Opdam, 2000; Krakow et al., 1999; Seelke et al., 2003; Warach et al., 1996]. The timing of the discharges can then be used in the analysis of the fMRI images, and the resulting statistical maps provide information on the brain areas involved in the generation of these discharges, with high spatial resolution.

*Correspondence to: Christian-G. Bénar, INSERM U751, Faculté de Médecine de la Timone, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 05, France.
E-mail: Christian.benar@medecine.univ-mrs.fr
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A further step from using the timing of discrete events is the computation of the \textit{fluctuations in the EEG} in terms of power in given frequency bands and their correlation with the fMRI signal. This permitted investigation of the brain areas involved in alpha rhythm [Goldman et al., 2002; Laufs et al., 2003a], but also more generally the joint fluctuations of signals in the resting state [Laufs et al., 2003b]. These findings could help refine the definition of an fMRI “baseline,” and permit the use of EEG as a marker of brain state in fMRI studies.

Recently, there has been a growing interest in the use of simultaneous EEG-fMRI for \textit{evoked activity} in cognitive paradigms, thereby obtaining functional datasets with both high spatial and temporal resolution. Thus, several groups have shown that it is possible to observe event-related potentials (ERPs) in the MR scanner that are similar to those obtained in regular conditions [Bonmassar et al., 1999; Kruggel et al., 2000; Liebenthal et al., 2003; Mulert et al., 2004; Otzenberger et al., 2005]. These first studies used interleaved EEG and fMRI recordings; more recently, a demonstration was done that showed that the ERPs could be obtained during actual scanning periods, after removal of scanning-related artifacts [Becker et al., 2005; Brandeis et al., 2003; Comi et al., 2005; Iannetti et al., 2005; Sammer et al., 2005].

A first interest in the simultaneous recording for evoked activity is the possibility of obtaining ERPs and functional MR images in the exact same environment and conditions of stimulation. This could be important, for example, in auditory paradigms, where the loud noise of the scanner can influence brain response. This effect was shown by Novitski et al. [2003], who used tape-recorded fMRI acoustic noise and reported modified amplitudes of the auditory ERPs compared to a noise-free environment for both early and late components.

A second interest of simultaneous EEG-fMRI is that it enables the observation by the two modalities of the brain in the same state of arousal, attention, and habituation. Indeed, reports have been made of variability of EEG responses to rare events depending on the time of day or the time spent on the task [Polich and McIsaac, 1994; Rovden and Polich, 1999; Wesensten et al., 1990]. In terms of fMRI response, Matsuda et al. [2002] have shown, thanks to simultaneous EEG-fMRI, that the level of arousal has an effect on the pattern of fMRI activation.

In the present study, we wanted to investigate a third opportunity offered by simultaneous recording, namely, the possibility of tracking the trial-to-trial changes in event-related EEG activity, and of using this information as a parameter in fMRI analysis. Such trial-to-trial variability has been reported both for EEG [Effern et al., 2000; Jung et al., 2001] and fMRI [Duann et al., 2002; Kruggel and von Cramon, 1999]. This variability is typically not taken into account in classical analysis procedures that rely on averaging, as done for ERPs, or on fitting a model with constant amplitude, as performed for fMRI statistical maps. This variability can be a consequence of variations in the design and can be difficult to quantify, or it can reflect different brain states, possibly unrelated to the protocol. In either case, the extraction of single-trial features such as amplitude or latency of certain brain waves could provide a new source of information for refining the analysis of fMRI data.

We used a classical auditory oddball paradigm for which considerable information exists in the EEG literature, and for which results have already been obtained for simultaneous EEG-fMRI recordings [Liebenthal et al., 2003; Mulert et al., 2004]. This paradigm consists of alternating frequent tones and rare (“target”) tones. It is known for robustly eliciting a positive EEG response to the rare tones, often referred to as the “P300” or “P3” wave, more prominent on the midline electrodes and occurring at a latency of around 300 ms. We used a varying target-to-target interval (TTI), as this parameter has been described as an important factor in the modulation of P300 amplitude [Gonsalvez and Polich, 2002].

Our specific goals were the following. First, we wanted to assess whether P300 features such as amplitude and latency could be retrieved on a trial-by-trial basis, despite heavy distortion of the EEG obtained during scanning. Second, we wanted to test whether the extracted features could be used for parametric modulation in the analysis of the fMRI signals. Our study resembles in some aspects that of Nagai et al. [2004], who measured the trial-by-trial energy of the contingent negative variation (CNV) on EEG in the scanner. However, while the CNV is a slow potential, with no precise temporal signature, the P300 is a rather fast ERP component. Parametric analysis was proposed in an oddball paradigm by Horovitz et al. [2002] in order to link ERPs and fMRI signals; however, they recorded EEG outside the scanner and grouped the trials by subsets. Calhoun et al. [2006] used a joint ERP-fMRI analysis based on independent component analysis in order to identify the joint variations of ERP and fMRI components across subjects. Recent studies have reported single-trial analysis of the amplitude of the error negativity [Debener et al., 2005a] and N1, P2, P3 waves [Eichele et al., 2005], using simultaneous EEG-fMRI. To our knowledge, our study is the first attempt at parametric analysis of fMRI comparing modulation by both amplitude and latency of the single-trial EEG waves. We also used a fully simultaneous paradigm (continuous scanning), contrary to Eichele et al. [2005], who used sparse imaging. We think it is important to show the feasibility of EEG single-trial analysis in continuous acquisition, as this gives more flexibility to the protocol and higher fMRI statistical power. Moreover, we present both group and single-subject results in order to assess the intersubject...
variability, contrary to previous studies reporting group analysis only. Indeed, similar to the single-trial EEG analysis that permits exploring intertrial variability, single-subject analysis permits revealing single-subject patterns that could be hidden in the averaging procedure.

In order to meet our goals we performed simultaneous EEG-fMRI on a group of 12 healthy subjects. After filtering the EEG signal, we extracted trial-by-trial amplitude and latency of the P300 wave and tested the correlation of these features with the TTI and the reaction times (RTs). We then used these features in a parametric analysis of the fMRI signals, which resulted in significant activations in fMRI statistical maps.

**SUBJECTS AND METHODS**

**Subjects**

We recruited 12 healthy subjects with no history of neurological disturbance or hearing dysfunction (mean age 32 years, two females). All subjects were right-handed and had previous experience with fMRI scanning. We selected mainly male subjects for technical reasons (size of the EEG cap). The subjects signed an informed consent form according to the rules of the local ethical committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, CCPRPB Marseille 2).

**EEG Recording**

The equipment used for the EEG recording consisted of a 30-channel EEG cap, plus two ECG channels (Micromed SRL, Mogliano Veneto, Italy), and a 32-channel MR-compatible amplifier (SD-MRI). When placing the cap, we made sure that the Cz electrode was at the vertex. We injected conductive gel (Micromed SRL) at each electrode; the quality of the contact was assessed by visual inspection of the EEG traces. Two ECG electrodes were placed on the chest. The subjects wore earplugs with 27 dB attenuation, as well as Mrconfon headphones (Magdeburg, Germany) providing further attenuation. The head was held in a vacuum cushion (VacFix, S&S X-Ray Products, Houston, TX) in order to limit head movements [Bénar et al., 2003]. Foam was placed between the O1 and O2 electrodes in order to avoid having the subject’s head lying directly on the electrodes. The subject was instructed to keep his/her eyes open during scanning, and the light in the room was dim. We installed a mirror reflecting the outside of the magnet for the subject’s comfort.

The head of the subject was set at the isocenter of the bore. The EEG cable laid on foam and was covered with sandbags in order to limit vibrations induced by the scanner. The amplifier was placed as far as possible from the scanner, with the cable positioned at a right angle from the main axis of the bore. The amplifier was connected to the recording computer located outside the room by means of an optic cable.

The EEG was recorded with SystemPLUS software (Micromed SRL), concurrently with MR triggers, stimulation and response triggers, at a sampling frequency of 2048 Hz.

**Stimulation Protocol**

Auditory stimulation was provided with the help of Labview software (National Instruments, Austin, TX) and delivered with an Mrconfon headset (Magdeburg, Germany); the subject’s response was recorded as EEG digital triggers using a custom-made button. For each run we provided the subjects with pure sine wave tones (duration 75 ms, rising time of 2 ms), one at a lower frequency (either 600 or 800 Hz) for the frequent tones, and another at a higher frequency (1600 Hz or 1800 Hz, respectively) for the rare (“target”) tones. We used two different pairs of tones in order to limit habituation effects. We alternated along runs between the two pairs of tones and counterbalanced their order of presentation (600/1600 or 800/1800 first) across subjects. The frequencies of the tones were chosen in order to avoid overlapping with the dominant frequency of the scanner noise (around 1000 Hz) or its harmonics/subharmonics. The volume was set to a level that permitted both clear hearing of the stimuli and comfort of the subject.

The stimuli were delivered with a constant interstimulus interval (ISI) of 765 ms. This duration was equivalent to that of 10 MRI slices plus an offset of 5 ms. The offset was introduced in order to decorrelate the timing of the MR-induced artifacts and that of the evoked responses.

The values of the target-to-target intervals (TTIs) varied between 2 ISIs and 10 ISIs, i.e., between 1.53 s and 7.65 s, respectively. The total number of rare tones for a given TTI varied between 9 for a TTI of 2 and 5 for a TTI of 10. Each run consisted of 397 frequent tones and 61 rare tones (probability of appearance, 15.4%), and lasted ~5 min.

Subjects were instructed to respond as quickly as possible to the rare tones only with the right thumb. Subjects were also asked to avoid moving the head when pressing the button. The requirement of a response helped the subject pay attention to the tones and permitted having a behavioral parameter for the validation of single-trial EEG results.

We conducted four runs for each subject in the scanner with concurrent EPI scanning, followed by an anatomical scan, plus another run with EEG only outside the MR scanner, but still inside the Faraday cage. The total time in the scanner room was ~1 h.

**MR Scanning**

Image acquisition was done on a 3 T Bruker Medspec 30/80 Avance (Bruker BioSpin MRI, Ettlingen, Germany). Scanning was performed continuously for each run, with the acquisition of series of blood oxygenation level-dependent (BOLD)-sensitized EPI images. We acquired 30 slices with 3 × 3 mm in-plane resolution and 4 mm slice
thickness. The slice acquisition time was 76.36 ms. The repetition time of the EPI sequence was chosen to be a multiple of the EEG sampling period, equal to 4,700 samples at 2048 Hz. This synchronization between the scanning artifacts and the EEG samples improved the subtraction of the scanning artifact, even though some periodic fluctuations (with a period of 16 frames) in the artifact residuals were still visible because perfect synchronization could not be achieved.

**Processing of EEG Data**

For each EEG dataset we first performed subtraction of the scanning artifact with the SystemPLUS software using the triggers obtained from the scanner. We used a running average of 12 frames, with oversampling for better frame realignment, which was subtracted from each frame [Allen et al., 2000]. After subtraction we observed that one subject had a bad electrical contact at the reference electrode; this subject was therefore discarded. We then exported the data to the Brain Vision Analyzer software (Brain Products, Munich, Germany). We excluded bad channels, filtered the data with a 70-Hz low-pass filter (slope of 24 dB/oct), and downsampled to a rate of 256 Hz. Pulse artifacts were marked by pattern matching on a given channel; for this, we selected a channel with high artifact amplitude. The periods of EEG around the pulse artifact were averaged by groups of 10 and subtracted from the EEG [Allen et al., 1998].

The resulting data were filtered with three different filters. First, a high-pass filter at 0.5 Hz (Butterworth Zero Phase Filter, Time constant 0.3183 s, 12 dB/oct) in order to remove low-frequency drifts. Second, we used a low-pass filter at 8 Hz (Butterworth Zero Phase Filter, 48 dB/oct) in order to filter out the residual scanning artifact (frequencies at multiples of 1/0.076 ~ 13 Hz) but still preserve the P300 wave, which is in the 3–5 Hz range. This low-pass filter was not sufficient to remove completely the alpha activity and therefore a notch filter (implemented in the frequency domain) was used, centered at 10 Hz with a bandwidth of 4 Hz.

We averaged the data within a window between −0.2 s and +0.8 s around the stimuli in order to obtain event-related evoked potentials (ERPs). We verified on the averaged waves that the P300 general aspect was not modified despite the heavy filtering (see Results). We grouped the rare events in three categories based on their TTI: short (2 or 3 ISIs), medium (4–6 ISIs) and long (7–10 ISIs), in order to assess the effect of the TTI on the P300 amplitude. We examined the evoked potentials at the Fz and Cz electrode and rejected subjects for which the signal-to-noise ratio (SNR) on the average ERPs was deemed too low on visual inspection (one subject) or the ERP's were very different outside and inside the scanner due to major artifacts (one subject).

We then exported the rare events to the EEGLAB toolbox [Delorme and Makeig, 2004] within the Matlab software (MathWorks, Natick, MA). We performed raster plots of the rare events at electrode Cz, reordered based on the RT as proposed by Jung et al. [2001]. We chose this electrode as it occupies a central position among the electrodes involved in P300 (mainly Fz, Cz, and Pz), and because it had a good SNR as estimated from the raster plots. We defined visually for each subject a time window containing a positive wave around 300 ms, based on the averaged data and on the raster plots at Cz. If two positive waves were seen on the raster plot at these latencies, then we chose the wave with the longer latency. One subject was discarded because an early positive wave and the late wave seemed to intermingle for low RTs.

Eight subjects remained out of the initial 12. For each subject we searched within the P300 window for the point with maximum amplitude and retained its amplitude and time of occurrence, which were considered to be estimators of the single-trial P300 amplitude and latency, respectively. We computed the distributions of these P300 features and rejected the points with any of the following characteristics: an amplitude lying outside of 3 standard deviations (SDs) from the mean, a latency at the border of the time window, or no corresponding button-press. The number of rejected points ranged between 18 (7.4%) and 41 (16.8%) out of 244 (mean, 28.4 points or 11.7%). The amplitude and latency values of these rejected points were set to the mean value across the other nonrejected values; as the modulating parameters are mean-centered in the fMRI analysis (see next section), this means the rejected values had no impact on the fit to the data.

In order to validate the features obtained on a trial-by-trial basis, we performed linear regressions based on the TTIs and RTs, as these factors have been shown to be linked to P300 amplitude and latency, respectively [Gonsalvez and Polich, 2002; Jung et al., 2001]. We used a robust framework for regression (MM-estimator) as well as for estimation of the standard errors [Croux et al., 2003]. We tested the significance of the linear relation by computing a t-test on the slope of the regression line and its corresponding P-value.

**Processing of fMRI Data**

Statistical analysis of the functional images was done with SPM2 software (http://www.fil.ion.ucl.ac.uk/spm/software/spm2/). The images were interpolated in time to correct for different acquisition times of each slice (“slice timing”), and were spatially realigned to the first image of each session. Both anatomical and functional images were transformed to a common standard space (MNI space). We performed spatial smoothing with a Gaussian filter with full-width at half-maximum of 12 mm. The low frequency temporal variations were removed from the functional data (high-pass filter with a cut-off of 120 s).

We constructed two general linear models, one for modulation by P300 amplitude, the other for modulation by P300 latency. Each model consisted of three regressors of interest for each session. The first regressor of each session corresponded to the frequent events, the second to the rare events, and the third to the parametric modulation of the rare events by either P300 amplitude or latency. Each
regressor consisted of impulse functions corresponding to the timing of the corresponding events (frequent or rare), convolved with a canonical hemodynamic response function (HRF). For regressor two (effect of the rare events), the amplitude of the impulse functions was set to unity. For regressor three (effect of the modulation of rare events), the amplitude of each impulse function was set to the value of the modulating parameter corresponding to this trial (with a mean set to zero). We performed the following contrasts: rare events vs. frequent events (contrast \([0, 1, 0]\)), modulation by P300 amplitude, and modulation by P300 latency (contrast \([0, 0, 1]\) for testing positive responses or \([0, 0, -1]\) for testing negative responses). As we were not interested in intersession effects, we concatenated homologous regressors across the four sessions (for example, the actual contrast vector for the rare vs. frequent contrast was \([-1, 1, 0, -1, 1, 0, -1, 1, 0, -1, 1, 0]\)). Therefore, this analysis resulted in three statistical maps per subject: 1) rare versus frequent; 2) modulation by P300 amplitude; and 3) modulation by P300 latency.

Although the aim of the study was to investigate effects for each individual subject, we also performed a fixed
effect analysis in order to test for reproducible activations across our series of subjects. We obtained one map for the effect of rare vs. frequent, one for the effect of the modulation by P300 amplitude and one for the modulation by P300 latency. The modulation is a subtle effect superimposing on the large effect of processing rare stimuli. Therefore, we did not expect effects of the same amplitude, and chose different statistical thresholds for the two types of maps, as done in previous studies [Horovitz et al., 2002; Calhoun et al., 2006; Eichele et al., 2005].

RESULTS

EEG Evoked Potentials

Eight subjects were retained for further analysis. The ERPs are presented in Figure 1 for the first subject, on the midline electrodes. On Fz (midline frontal) and Pz (medial parietal), a negative wave can be seen between 100 and 200 ms, that likely corresponds to the N100. This wave is followed by a wave of opposite polarity occurring between 200 and 300 ms that likely corresponds to P300. The P300 seems to be separated into two phenomena, one frontal (maximum at Fz) and early (around 250 ms) and the other more central and late (maximum at Cz, in the 300–400 ms range). This could correspond to the distinction between P3a and P3b that has been reported in the literature [Ruchkin et al., 1987; Squires et al., 1975]. The N100 and P300 waveforms and topographies were similar between the ERPs outside and inside the scanner.

In Figure 2 we present the effect of the heavy filtering (0.5–8 Hz) that we applied to the traces. We compared this filtering to that of a more standard filter set between 0.5 and 20 Hz. It can be seen that the 0.5–8 Hz filtering did not modify significantly the ERP topography and temporal shape.

Therefore, despite the difficult conditions, we could verify that signals of good quality were obtained inside the scanner.
Figure 4 for all subjects, along with the threshold of significance ($P = 0.05$). We observed significant correlations for the regression of P300 latency vs. RT and TTI. For P300 latency vs. RT, the correlations were significant for seven subjects out of eight, with a positive sign. For P300 latency vs. TTI, the correlations were significant for six subjects out of eight, with a negative sign. By contrast, the correlation of P300 amplitude vs. TTI was never significant, and the correlation of amplitude vs. TR was significant for two subjects only (Subjects 1 and 9).

These correlations validate the detection of the P300 wave, allowing further use of the single-trial values.

**Analysis of fMRI Data**

For the fMRI data analysis, in a first step we computed statistical images corresponding to the effect of the rare stimuli vs. frequent stimuli (contrast $[-1, 1, 0]$), which permitted assessing the quality of the images despite the presence of the EEG system. We examined the activations at the group level (fixed effect analysis, single voxel threshold $P = 10^{-6}$ corrected). We obtained highly significant activation patterns comprising the temporo-parietal junction bilaterally, left pre- and postcentral gyri, cerebellum, medial frontal gyrus, cuneus/lingual gyrus, insula, and thalamus. Figure 5 shows statistical images corresponding to the group analysis.

In a second step we computed the statistical images corresponding to the effect of the modulation by the P300 features extracted from the EEG on a single trial basis (contrast $[0, 0, 1]$ for testing positive responses and $[0, 0, -1]$ for testing negative responses). This step was the ultimate goal of

**Figure 4.** Values of the t-statistics for the regression lines of the detected features vs. RT and TTI for all subjects. The thresholds of significance ($P = 0.05$) are displayed as dashed lines.

**Figure 5.** fMRI statistical map in standardized MNI space for the group analysis corresponding to rare stimuli vs. the frequent stimuli (fixed effect analysis), overlaid on the anatomical image of Subject 1. The single voxel threshold is set at $P = 10^{-6}$ (corrected). There is a clear activation around the temporo-parietal junction, which is probably related to the processing of rare targets, and another activation in the left central region, corresponding to the motor response.
the study, i.e., to verify that the single-trial EEG features can help refine the fMRI analyses. The single-subject activation maps are shown in Figure 6 (single voxel threshold $P = 0.005$ (uncorrected) and the cluster threshold is 10 voxels). There is variability in the pattern of responses; however, three broad regions seem to be involved: the parieto-occipital junction (dashed lines), the medial frontal region (dotted lines), and the lateral frontal regions (dot-dashed lines).

Figure 6. fMRI statistical maps (projected) for all subjects. The single voxel threshold is set at $P = 0.005$ (uncorrected) and the cluster threshold is 10 voxels. There is variability in the pattern of responses; however, three broad regions seem to be involved: the parieto-occipital junction (dashed lines), the medial frontal region (dotted lines), and the lateral frontal regions (dot-dashed lines).

DISCUSSION

The simultaneous recording of EEG and fMRI is advantageous in cognitive studies as it permits obtaining evoked
potentials and statistical maps of fMRI activation under the exact same conditions. However, it is only by analyzing the single-trial data that one can hope to fully extract the information present in this mixed dataset; the possibility of single-trial analysis could be in fact the strongest incentive for the simultaneous recording.

Horovitz et al. [2002] proposed a framework where they incorporated the variation of amplitude of P300 as a modulating parameter in the fMRI analysis. They found significant correlations between the P300 amplitude and the fMRI signal in the supramarginal gyri, thalamus, insula, and right medial frontal gyrus. However, their analysis was based on data acquired separately, and relied on TTI as a modulating parameter. They used averages on subsets of trials, which indirectly incorporated the TTI information into the analysis. Moreover, the averaging procedure does not permit distinguishing between amplitude variations arising from latency jitter across trials and actual single-trial amplitude variations. In contrast, we directly used the single-trial amplitude and latency information derived from the EEG recorded simultaneously with fMRI.

Such single-trial analysis was performed in previous studies [Debener et al., 2005a; Eichele et al., 2005; Nagai et al., 2004]. To our knowledge, the current study is the first attempt at a single-trial EEG analysis in fully simultaneous EEG-fMRI applied to fast cognitive evoked potentials that uses not only information on the amplitude of the waves but also their latency. The use of single-trial ERP features together with fMRI data opens a new way in the investigation of the modulation of evoked cognitive activity by enabling the combined use of the fine temporal reactivity of the EEG and the excellent spatial resolution of fMRI. Single-trial analysis is a difficult operation in classical EEG studies because of the low SNR of evoked activity; it is even more difficult in the noisy environment of the MR scanner. We therefore aimed at accumulating evidence that the features we obtained were true indicators of neural activity.

First, we confirmed that it is possible to obtain averaged ERPs during the actual scanning period [Becker et al., 2005a; Brandeis et al., 2003; Comi et al., 2005; Iannetti et al., 2005; Sammer et al., 2005]. This approach allows more flexibility in the protocols compared to a sparse imaging approach, where the stimuli are presented outside of scanning periods. It also gives more statistical power, as we accumulate more fMRI data points for a given session length.

Second, we have shown for the first time in fully simultaneous EEG-fMRI (i.e., continuous fMRI scanning) raster plots with a clear evoked activity (in the range of P300) that was response-locked, as shown by Jung et al. [2001] in regular recording conditions. We extracted automatically single-trial P300 latency, which presented significant correlation with parameters external to the EEG (TTI and RT). This correlation suggests that the single-trial P300 waves were correctly detected.

Third, we obtained significant activation in the fMRI statistical maps for both the contrast corresponding to the effect of the rare stimuli and the contrast corresponding to the parametric modulation. The effect of the rare stimuli vs. the frequent stimuli was highly significant and involved a large network, in good concordance with previously reported fMRI studies [Kiehl et al., 2005]. This is a good indication that we obtained fMRI images of good quality despite the presence of the EEG system.

For the parametric modulation, we observed significant activations both at the single subject level and at the group level. These activations comprised both positive and negative t-values, i.e., positive or negative correlation between our EEG parameters and the amplitude of the fMRI signal. These results indicate a link between the evoked potentials and the fMRI signal, which could be more or less direct. A “direct” link would occur if the modulation of the ERP reflects a modulation of neural activity that is visible in the fMRI. In this case, we would expect the region where the modulation of the fMRI signal takes place to corre-

Figure 7.

fMRI statistical map for the group study (fixed effect analysis). The single voxel threshold is set at $P = 0.005$ (uncorrected) and the cluster threshold is 10 voxels.
spond to the actual source of the EEG evoked potential. Another possibility is that both EEG and fMRI signals are jointly modulated by the same factors, for example, the level of attention. In that case, the regions of fMRI changes would not indicate the actual EEG source, but would nevertheless be informative on the mechanisms involved in the modulation of the EEG wave.

For the analysis corresponding to P300 amplitude, the positive correlation in the anterior cingulate cortex resembled that obtained by Calhoun et al. [2006] in a similar paradigm. This region has been reported to be involved in the regulation of goal-directed behaviors [Koechlin et al., 2002]. The negative correlation with P300 amplitude in the precentral region could be related to the motor response, and seems consistent with the tendency of P300 amplitude to be negatively correlated with the reaction time. Generally speaking, several recent reports indicate an anticorrelation between the BOLD response and ERP amplitude [Foucher et al., 2003] or between the BOLD response and low-frequency EEG activity in the theta range, alpha or beta range [Laufs et al., 2003b; Mukamel et al., 2005; Niessing et al., 2005]. Negative correlation has also been observed in some regions preferentially involved during intertrial periods [Raichle et al., 2001]. Studies on the influence of attention on the fMRI signal have also reported distributed patterns of both activation and deactivation [Lawrence et al., 2003].

For the analysis corresponding to the P300 latency, we observed positive correlation medially at the level of the parieto-occipital junction. This effect could be related to attention, as this broad region, including the precuneus, has been reported to be more active in the “default mode” than during goal-directed activities [Raichle et al., 2001]. The activity of the medial frontal region was negatively correlated with the P300 latency. This negative correlation was not surprising: since P300 latency correlates with the RT, this indicates that the medial frontal region activity correlates with performance. Indeed, there is a large body of evidence that this region is involved in cognitive control, with more control leading to better performance [see Ridderinkhof et al., 2004, for a review].

Despite the tendency for broad regions to be activated—confirmed by the group study—we observed a large variability in the patterns of activations across subjects. This could be due to the fact that the impact of the P300 modulation on the fMRI signal is a subtle effect in comparison with the main effect of the rare vs. frequent stimuli, leading to detection across subjects of different subsets of the activated regions. Moreover, the P300 effect involves large distributed networks [Kiehl et al., 2005; Smith et al., 1990], which could be variable across subjects, reflecting different strategies of response or different subject states.

The observations of significant fMRI activations constitute a further step in validating the relevance of the extracted single-trial features. Moreover, this shows on real data that the assumption of a simple linear relation between the EEG features and the BOLD response can be useful in the combined analysis, as was previously proposed in theoretical models [Trujillo-Barreto et al., 2001].

An interesting finding on the cognitive side is the fact that we obtained significant fMRI activations in the analysis based on the P300 amplitude at Cz, even though the amplitude could not be linked to TTI and could be linked to RT for two subjects only. This suggests that the P300 amplitude was a relevant marker of fluctuations of the brain state that could not be predicted using simple external parameters.

Further improvements of the method lie in the single-trial estimation of EEG features, for which many studies exist in the literature—often on P300 itself. Wavelet denoising has been proposed by several groups for improving single-trial estimation of EEG parameters [Benkherrat et al., 2005; Effern et al., 2000; Quian Quiroga and Garcia, 2003]. Another promising area, introduced by Debener et al. [2005a] for simultaneous EEG-fMRI recording, is that of independent component analysis (ICA), which could help improve the SNR by incorporating several channels in the analysis and by separating the activity of interest from the background EEG [Jung et al., 2001]. ICA could also help disentangle different concurrent processes that are mixed on a given electrode; for example, P200 and P300, or P3a and P3b [Debener et al., 2005b], and as a consequence obtained better markers of a given phenomenon.

In summary, we have proven the feasibility of tracking single-trial variations of both amplitude and latency of an EEG wave during fMRI scanning. This use of simultaneous EEG-fMRI can be seen as a bridge between the well-established field of evoked cognitive potentials and the fast-growing field of fMRI studies; our study contributes to this goal by linking the fluctuations of the features of a well-known ERP component to the fMRI signal. It also permits extracting new information from evoked activity, with a very high spatial resolution. Furthermore, our results indicate that, in line with other studies, EEG can bring a new dimension to the field of fMRI analysis by providing fine temporal information on the fluctuations in brain activity.

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REFERENCES


