Single-epoch analysis of interleaved evoked potentials and fMRI responses during steady-state visual stimulation

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A B S T R A C T
Objective: Aim of the study was to record BOLD-fMRI interleaved with evoked potentials for single-epochs of visual stimulation and to investigate the possible relationship between these two measures.

Methods: Sparse recording of fMRI and EEG allowed us to measure BOLD responses and evoked potentials on an epoch-by-epoch basis. To obtain robust estimates of evoked potentials, we used blocks of contrast-reversing visual stimuli eliciting steady-state visual evoked potentials (SSVEPs). For each block we acquired one volume of fMRI data and we then tested for co-variations between SSVEPs and fMRI signals. Our analyses tested for frequency-specific co-variation between the two measurements that could not be explained by the mere presence/absence of the visual stimulation.

Results: Condition-specific single-epoch SSVEPs and fMRI responses were observed at occipital sites. Combined SSVEPs–fMRI analysis at the single-epoch level did not reveal any significant correlation between the two recordings. However, both signals contained stimulation-specific linear decreases that may relate to neuronal habitation.

Conclusions: Our findings demonstrate robust estimation of single-epoch evoked potentials and fMRI responses during interleaved recording, using visual steady-state stimulation.

Significance: Single-epochs analysis of evoked potentials and fMRI signals is feasible for interleaved SSVEPs–fMRI recordings.

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1. Introduction

The aim of this work was to record single-epoch evoked potentials (EPs) interleaved with fMRI responses associated with steady-state visual stimulation. We also tested whether the variability of the steady-state visual evoked potentials (SSVEPs) predicted the variability of the fMRI responses, on epoch-by-epoch basis.

Simultaneous acquisition of evoked potentials and fMRI data is an attractive tool in order to investigate cognitive brain functioning. The high spatio-temporal resolution achieved via multi-modal imaging can undoubtedly give new insight in the study of complex neural networks (Debener et al., 2005; Benar et al., 2007); simultaneous intracortical-EEG and fMRI recordings of evoked activity have allowed the investigation of the neuro-vascular coupling, in terms of the neuro-physiological correlates of the BOLD signal (Logothetis et al., 2001; Logothetis, 2008). Nonetheless the simultaneous EPs–fMRI measure is particularly challenging, considering that EPs amplitudes are several orders of magnitude smaller than artifacts induced by the MR environment on the EEG signals (Allen et al., 1998, 2000).

The added value of concurrent EEG–fMRI recording, as compared with performing two separate recordings, has been demonstrated to improve the localization of unpredictable spontaneous activity, such as epilepsy paroxysms (Warach et al., 1996; Lemieux, 2004) and spontaneous brain rhythms (Moosmann et al., 2003; Laufs et al., 2006; Horovitz et al., 2008). In these situations the EEG recording is critical to identify the events of interest (e.g. inter-ictal epilepsy spikes, fluctuations in alpha rhythm, delta activity) and to accurately time-lock the analyses of the fMRI data. Nonetheless, it should be noted that the EEG signals associated with spontaneous activity and epileptic events are of several orders of magnitude larger than the typical amplitudes of evoked potentials, and hence easier to detect even in an MR environment.

A few recent studies measured simultaneous EP and fMRI during visual stimulation (Bonmassar et al., 1999, 2001, 2002;
Sommer et al., 2003; Kruggel et al., 2000), auditory stimulation (Liebenthal et al., 2003; Scarff et al., 2004; Mulert et al., 2005), somato-sensory stimulation (Christmann et al., 2002) or motor tasks (Lazeyras et al., 2001; see also Foucher et al., 2004; Deben
er et al., 2005; Benar et al., 2007, for simultaneous recordings during higher cognitive tasks). Nevertheless, the advantages of concurrent EPs–fMRI measurement with respect to performing separate acquisitions in two distinct sessions has only been partially demonstrated in these previous studies that mainly fo
cused on extracting session-averaged evoked responses (but see Debener et al., 2005; Benar et al., 2007). In this circumstance, separate measurements of EPs and fMRI will be adequate, assuming the overall response reproducibility can be guaranteed between two distinct sessions. In contrast, simultaneous record
ing of the two signals is necessary when stimulus-related activity has a certain degree of unpredictability, e.g. for cognitive experiments where the experimental design introduces time
dependent effects such as learning, priming or performance monitoring (Debener et al., 2005).

More generally, multiple repetitions of the same stimulus will trigger some variability in the evoked activity, because of stoch
castic contributions to the neuronal response (e.g. see Lannetti et al., 2005, showing latency jitters and amplitude variability in single trial evoked potentials). In BOLD-fMRI, habituation/adaptation effects (Grill-Spector et al., 2006) and trial-by-trial variability (Dunn et al., 2002; Fox et al., 2006) of response amplitudes, also in sensory areas, has been demonstrated as well. In general, both low-level mechanisms as well as higher order mechanisms may contribute (see Faisal et al., 2008; for a comprehensive review) as possible sources of trial-to-trial variabil
ty that is typically observed both at single neuron level and at macroscopic level (e.g. EEG and fMRI). At the lower (cel
tural) level, modelling work has shown that the probability of neuronal firing relates to complex excitatory and inhibitory cir
uits producing non-deterministic behaviors of neurons and neu
rons’ ensemble (e.g. Salinas and Sejnowski, 2000; Smith and Sherman, 2002; see also Destexhe and Contreras, 2006 for review). At the macroscopic level, higher-order factors such as attention and arousal are thought to play a key role. Fluctuation over time of these modulatory influences can introduce trial-to
trial variability, like modulation of the amplitude of cyclic neuronal response to periodic stimulation (e.g. changes in the ampli
tude of SSVEPs responses, in phase with SSVEPs stimulation and at the stimulation frequency, see Muller et al., 1998). Previ
ous studies have also linked these modulatory effects (on a time scale of tens of seconds) with behavioral performance (e.g. Fox et al., 2007; see also McIntosh et al., 2008). It is reasonable to expec
t that at least some of these factors (e.g. fluctuation of attention) will affect both EEG and fMRI responses, hence pro
cucing some co-variation between these two measures on a trial-by-trial (or epoch-by-epoch basis, depending on the fre
quency of the fluctuations). Furthermore, we should note that non-deterministic, stimulus-unrelated variance is critically used in fMRI analyses of functional and effective connectivity (Penny et al., 2005; Horwitz et al., 2005), making the investigation of these processes interesting beyond any direct implication for combined EEG–fMRI recording.

Therefore, as for the study of spontaneous activity, the concurrent EPs–fMRI approach can also be advantageously employed in order to improve the modelling of unpredictable changes in evoked activity. For example, EPs can be used to predict the variability of the BOLD response across stimulus repetitions, with the ultimate goal of localizing with improved significance hemodynamic changes associated with neuronal events (Benar et al., 2007). EPs simultaneously recorded with fMRI can also be employed to investi
gate the neuro-vascular coupling, in order to improve the predic
tion of neural activity from fMRI signals (Logothetis et al., 2001; Logothetis, 2008); and the combination of EEG and fMRI signals may improve the performance of brain–computer interface devices (Hinterberger et al., 2004).

Multi-modal analysis of single-trial EP responses requires the extraction of reliable signals from – usually noisy – single trials. This has been previously achieved using de-noising procedures like Independent Component Analysis, ICA (Makeig et al., 2004) which however requires an a priori knowledge and the validity of several assumptions to select/discard interesting/artifactual components. Here we adopted an alternative approach, attempting to evoke steady-state responses that have a larger signal-to-noise ratio and should allow us to reliably measure brain activity during a short epoch of a few seconds without any a priori assumptions. Only few simultaneous EEG–fMRI studies focused on single trial analysis (Debener et al., 2005; Eichele et al., 2005; Benar et al., 2007) and there is not any previous report on single-epoch analysis.

Critically we made use of the hemodynamic delay of the BOLD response to obtain MR-imaging artifact-free EEG traces and improve the quality of the measured evoked potentials, as previously reported (Bonmassar et al., 1999, 2001, 2002; Sommer et al., 2003; Otzenberger et al., 2005). In order to obtain robust estimates of stimulus-related EP waveforms, we used blocks of contrast reversing visual stimuli that elicited steady-state visual evoked potentials (SSVEPs). With respect to the classic transient VEPs in which slow rate of stimulation allows the sensory pathways to recover or “reset” before the next stimulus appears, the SSVEPs require a rapid stimulus repetition faster than 4 Hz (in our case with 6 and 7.5 Hz) to prevent the evoked neural activity from returning to baseline state so the elicited response becomes continuous reaching a steady-state (Di Russo et al., 2002).

The SSVEPs give less temporal information compared to transient VEP, but its fast stimulus repetition enables the collection of a high number of responses in a short time and with a high sig
nal-to-noise ratio for each epoch (see also Allison et al., 2008; for the use of SSVEP in the context of brain–computer communica
tion). Accordingly for each epoch of stimulation we measured the amplitude of the associated SSVEPs and of the peak of the fMRI re
sponse at about 17.5 s (range 16.1–18.81 s) after the beginning of the stimulation (Fig. 1B). This allowed us to correlate on an epoch-by-epoch basis the two signals. Critically, our analyses tested for frequency-specific co-variation between the two measure
ments related to stochastic changes in evoked neuronal activity that could not be explained by the simple presence/absence of the visual stimulation.

2. Methods

2.1. Participants

Nine healthy, right-handed volunteers (6 males, age 22–43 yrs) participated in the study. All gave their informed consent accord
ing to guidelines reviewed and approved by local ethics authorities for work involving humans.

2.2. Stimuli and task

To elicit SSVEPs we used a black/white horizontal Gabor patch (see Fig. 1A, foveal position, diameter: 3 deg, spatial frequency 1.33 cycle/deg sinusoidal modulated in time with phase reversals of 180 deg (pattern reversal stimulation) at 6 Hz (condition 1: Stm6) or at 7.5 Hz (condition 2: Stm7.5). Note that previous find
gings indicate that for small Gabor presented at the fovea the reti
nocortical gain (which is proportional to the amplitude of visual evoked potentials) is highest for frequencies around 1 cycle/deg (see Mari et al., 2001). The patch was presented against an isoluminant grey background. The two conditions were presented in epochs of 15 s, followed by 5 s of fixation. A third condition (Rest) consisted of central fixation only (20 s) and it was introduced to estimate the overall effect of the visual stimulation in the fMRI data. Each condition was repeated 10 times during each experimental-run, in a randomized order. Each subject underwent three runs, resulting in total of 30 repetitions for each condition/epoch-type.

We exploited the hemodynamic delay between neural activation and the associated BOLD response (approx. 5 s for 1.5 s stimulus duration, Miezin et al., 2000) to acquire EEG-data during the visual stimulation and fMRI data just afterwards (“sparse-sampling” imaging method, Ives et al., 1993; Bonmassar et al., 1999, 2001, 2002; Sommer et al., 2003; Otzenberger et al., 2005). Each fMRI volume (acquisition time, AT = 2.71 s) was acquired after each stimulation epoch, starting 1.1 s after the end of the stimulation block. Accordingly, for each 15-s epoch (Stm6, Stm7.5 or Rest) we obtained 15 s of EEG recording and one MR-volume (Fig. 1B).

The stimulus presentation and the synchronization between the visual stimuli, the MR acquisition and the EEG recording were controlled using Cogent (http://www.vislab.ucl.ac.uk/Cogent/index.html). The computer received synchronization pulses from the MR scanner and it tagged the EEG acquisition system on each stimulus cycle (i.e. at 6 or 7.5 Hz). The visual stimuli were projected via mirroring to a back projection screen using a LCD video projector (Model VPL-351QM, Sony Corp., Tokyo).

2.3. fMRI acquisition and pre-processing

We employed a Siemens Vision 1.5 T MR system (Siemens Medical Systems, Erlangen, Germany), equipped with a circular polarized volume head coil to acquire functional MR images. Each subject underwent three acquisition runs, each run consisting of 32 BOLD-sensitive gradient-echo-planar images. Each volume consisted of 24 transversal planes, acquired starting caudally and stretching to the vertex. Each plane was excited with a 90 deg slice selective RF pulse followed by echo-planar blipped trapezoidal read-out gradients (bandwidth: 1953 Hz/pixel). The acquisition parameters were TR = 20 s, TE = 60 ms, matrix: 64 × 64, FOV = 256 mm, slice thickness = 4 mm and gap between slices = 1 mm. The first two volumes were discarded from subsequent analysis. The fMRI pre-processing was performed using SPM2 (http://www.filion.ucl.ac.uk/spm/software/spm2). This included correction for motion during scanning, normalisation to MNI (Montreal Neurological Institute) template...
and spatial smoothing by a Gaussian isotropic kernel (full-width at half maximum, FWHM = 8 mm).

2.4. EEG acquisition

We used an MRI-compatible EEG-amplifier (EBNeuro, Florence, Italy) with 19 Ag–AgCl conic scalp-electrodes mounted according to the 10–20 system montage. The electrodes, pre-arranged on a cap of amagnetic material, were filled with conductive gel and placed on the scalp of the participants, prior to positioning in the bore of the MR scanner. The reference electrode was positioned on the AFz position (halfway between FPz and Fz) whereas the ground was positioned on FCz (halfway between Fz and Cz). Moreover, ECG signal and tags indicating the end of each stimulus cycle were registered in two separate EEG channels. The EEG recording unit was placed inside a shielded box to get rid of any RF disturbance on the MR images. The unit amplified the signal and performed A/D conversion and multiplexing. The digital signal was transferred via optical-fiber connection to a host computer located outside the magnet room for de-multiplexing, data acquisition, processing, and storage. The ADC sampling rate (fc) and dynamic range were set equal to 4096 Hz and 65 mV (at 16 bit), respectively, in order to adequately sample and to avoid saturation of EEG signals during fMRI.

2.5. EEG-data analysis

The aim of the EEG analysis was to obtain the amplitude of the SSVEP for each 15 s epoch of visual stimulation. The raw-data were first band-pass filtered (0.5–40 Hz) and sub-sampled to 256 Hz. We used the ECG signal to identify QRS-complexes and the ballistocardiogram BCA was then removed from each EEG channel by an improved version of Allen’s (Allen et al., 1998) strategy developed in our lab (NPX Lab 2008 software, http://www.brainface.com/cms/). The efficiency of BCA removal was evaluated in terms of the amount of residual BCA power with respect to the original power in each channel (Niazy et al., 2005). For each channel, 1.7 s long epochs (starting 0.5 s before each QRS-peak) were averaged, for QRS-peaks pertaining only to stimulation periods. The mean power of such averaged epoch (equal to the mean of the squares of each averaged epoch) was computed for both data before and after BCA removal and their ratio (%) evaluated (see also Fig. 1C).

2.6. Estimation of SSVEP amplitudes

For each condition and experimental-run, 10 epochs, each lasting 15 s, were extracted. The estimation of the SSVEP amplitude for each 15 s epoch was performed in the time-domain, considering three frequency of interest (12 Hz, 15 Hz, related to the visual stimulation frequency, plus 13.75 Hz as an additional control) and included four steps (see also Muller et al., 1998):

(1) Averaging. For each 15 s EEG-epoch, we used a 1 s window to compute average SSVEP amplitudes at three frequencies of interest (12, 15 or 13.75 Hz). Depending on the frequency of interest that we wished to estimate, the window was shifted in different steps (i.e. 166 ms, 133 ms or 148 ms; for 12 Hz, 15 Hz and 13.75 Hz, respectively). E.g. for the estimation of SSVEP amplitudes at 12 Hz, the window was shifted in steps of 166 ms. If the EEG-epoch was acquired during visual stimulation at 6 Hz, this corresponds to time-locking the average to each reversal onset (i.e. stimulus-related amplitude: \(A_{Stm6.12Hz}\)). If the EEG-epoch was acquired during 7.5 Hz stimulation, this corresponds to a control frequency (i.e. \(A_{Stm7.5.12Hz}\)). Before averaging, artifact rejection was performed excluding any segment containing values greater than 70 \(\mu V\). This computation produced an averaged SSVEP response (1 s segment) for each epoch and frequency of interest.

(2) Data fitting using sines and cosines. The 1 s segment (\(y\)) was fitted with a multiple regression model that included three predictors: a sine and a cosine (Fourier set) at the frequency of interest (e.g. 12 Hz), plus a constant (e.g. \(y = b_0 + b_1(2\pi \cdot f_{12Hz} + \cos(2\pi \cdot f_{12Hz})) + b_2 + e\), with \(b_1\) and \(b_2\) the sine and cosine parameter amplitudes, \(b_0\) the constant term, \(f = (1/fc:1/fc:1)\), and \(e\) the error term). The fitting of the model yielded to three parameter estimates (\(b_1, b_2, b_0\)).

(3) Calculation of the amplitude. The amplitude at the frequency of interest (e.g. 12 Hz) was then computed as the square root of the sum of squares of the sine and cosine parameter estimates (e.g. \(\sqrt{b_1^2 + b_2^2}\)).

(4) Adjustment using amplitude at 13.5 Hz. We removed residual stimulus-unrelated variance subtracting the 13.5 Hz amplitude from both 12 and 15 Hz amplitudes.

Thus, for each 15-s epoch we obtained two amplitude values (A12 Hz, A15 Hz; see Fig. 2C) corresponding to the actual stimulation frequency and to the control frequency. Note that the SSVEP amplitudes were computed at twice the frequency of the visual stimulation (i.e. 6 Hz, 7.5 Hz), because for pattern reversal most of the cortical response occurs at twice the stimulation frequency (Di Russo et al., 2002, 2007).

2.7. Prediction of BOLD signal changes from SSVEP amplitudes

SSVEPs amplitudes estimated from the entire (15 s) epoch of stimulation (as described above) were used as predictor of the measured BOLD signal changes (SSVEP-covariateA). Convolution of the SSVEPs amplitudes with the hemodynamic response was not performed since the acquisition of fMRI data was not time jittered with respect to stimulation timing. For our data, the correlation coefficient between the regressor obtained after convolution of SSVEPs amplitudes with the hemodynamic response function (HRF, modelled by the combination of two gamma-variate functions with a first positive peak at 5 s, followed by an under-shoot at 16 s after the beginning of the stimulation, default in SPM2) and the regressor of unconvolved SSVEP amplitudes was equal to 0.9997.

Nevertheless, considering the epoch of 15 s of continuous stimulation and the sluggishness of the HRF, the expected BOLD response at the time of the MR acquisition (in the range 16.1–18.81 s after the start of the stimulation epoch) will combine different effects. The first half of the stimulation will have little effect on the expected BOLD response; while the second half will contribute most. Because of this, we computed a second predictor of BOLD signal changes (SSVEP-covariateB), estimating the SSVEP amplitudes using shorter (2.5 s) traces and convolving these amplitudes with the HRF. The procedure described above to extract SSVEP amplitudes was hence applied for six consecutive segments of 2.5 s within each stimulation epoch, instead of the entire (15 s) epoch of stimulation. For each segment we computed the amplitude at the stimulation frequency and at the control frequency. We then convolved these amplitudes with the HRF and we sampled the expected amplitudes (stimulus and control frequency) at the time of the fMRI acquisition. Thus, we obtained two new amplitude values for each epoch (A12 Hz and A15 Hz, but now convolved with the HRF).

We used these SSVEP covariates to compute the number of subjects showing a reliable (statistically significant) effect of stimulation vs. control frequency. In particular, we identified subjects...
having stimulus-related SSVEP effects (SSVEP-covariateA, SSVEP-covariateB) larger than stimulus-unrelated SSVEP effects, by computing a one-sample t-test of the difference (\([\text{Stm6}_A12 \text{ Hz} + \text{Stm7.5}_A15 \text{ Hz}] / C0 [\text{Stm7.5}_P12 \text{ Hz} + \text{Stm6}_P15 \text{ Hz}]\)), across 30 repetitions per subject, for average amplitudes obtained from occipital electrodes O1 and O2 (SSVEP-covariateA). Error-bars display the amplitude average ± SE over 30 repetitions. Except for two subjects (subject N. 5 and subject N. 9), the SSVEP amplitude was stimulus-frequency-specific, i.e. with larger amplitudes for the stimulation frequency than the other (control) frequency that was also computed for each epoch.

2.8. Combined SSVEPs–fMRI analyses

For all SSVEP–fMRI correlation analyses (see below), we only included data of subjects who had reliable SSVEP effects (e.g., seven subjects for SSVEP-covariateA, see Fig. 2C). Three regression models were employed: the first to test epoch-by-epoch SSVEPs–fMRI correlations on a voxel-by-voxel basis, the second for epoch-by-epoch SSVEPs–fMRI correlations on average fMRI signals, and the third to verify the significance of epoch-by-epoch SSVEPs–fMRI correlations on average fMRI signals without modelling linear drifts.

Model 1: epoch-by-epoch SSVEP–fMRI correlations on a voxel-level basis. Covariates per run: effects of condition (two covariates: \(\text{Stm6}\) and \(\text{Stm7.5}\)), main effect of fMRI-run (constant modelling the mean signal of each run), four SSVEP covariates: \(\text{Stm6}_A12 \text{ Hz}\), \(\text{Stm7.5}_A15 \text{ Hz}\), \(\text{Stm7.5}_P12 \text{ Hz}\), \(\text{Stm6}_P15 \text{ Hz}\), and linear drift over time within each run.

These eight covariates were modelled separately for each run and each subject, resulting in a total of: 8 covariates \(\times 3 \text{ runs} \times 7 \text{ subjects} = 168\) predictors in this regression model. A linear compound (contrast) was used to compare the estimated parameters for stimulus-related amplitudes (\(\text{Stm6}_A12 \text{ Hz} + \text{Stm7.5}_A15 \text{ Hz}\)) minus control amplitudes (\(\text{Stm7.5}_P12 \text{ Hz} + \text{Stm6}_P15 \text{ Hz}\)), across runs and subjects. In this model the statistical inference is based on between-scans variance (i.e. fixed effects multiple regression analysis, SPM2, http://www.fil.ion.ucl.ac.uk/spm/). Model 1 was run using SSVEP-covariateA, and in a separate analysis SSVEP-covariateB.

Aim of this analysis was to test the hypothesis that stochastic changes of stimulus-related brain activity may result in a correlation between the SSVEP amplitude and BOLD single-voxel
time-courses. Thus, we used the epoch-to-epoch variance of the SSVEP amplitude as a predictor for the fMRI analyses. It should be noted that the inclusion of the main effect of condition in all our analyses, ensured that we specifically tested for the co-variance between SSVEP and BOLD, over and above any average signal change related to the stimulation (i.e. presence/absence of the visual stimulus, or differential responses depending of the frequency of the pattern reversal). In terms of the parameter estimates relative to SSVEP covariates, in SPM this is equivalent to include in the regression model the main effect of condition and the SSVEP covariate orthogonalized to the regression model. The employed contrast (see above) will selectively highlight only voxels where the co-variance between SSVEP and BOLD was specific for the frequency of the visual stimulus. Because we expected any such co-variance to occur within brain areas activated by the visual stimulation, we assigned corrected p-values using a small volume correction procedure (Worsley et al., 1996). We identified the volume of interest (ROIstim) using the fMRI data and comparing the two stimulation conditions (Stm6 and Stm7.5) vs. Rest (fixed effects analysis, p < 0.05, corrected for family-wise errors (PWE) over the whole brain; see also Fig. 3A).

Model 2: epoch-by-epoch SSVEP–fMRI correlations averaging BOLD data in visual cortex. Same covariates as Model 1, but the model was used to fit fMRI averaged data for the whole visual cortex (volume of interest ROIstim, see above and Fig. 3A) and separately for each subject. The average fMRI time-series across ROIstim was employed in this analysis in order to reduce thermal noise variability. For each subject, we compared the parameter estimates for stimulus-related vs. control SSVEP amplitudes. These differences were then entered in a one-sample t-test for group-level statistical inference, now based on between-subjects rather than between-scans variance (random effects model). Model 2 was run using SSVEP-covariateA only.

Model 3: epoch-by-epoch SSVEP–fMRI correlations averaging BOLD data in visual cortex without modelling the linear drifts. Same covariates and random effect inference as Model 2, but now without the predictors modelling linear drifts over time. Accordingly, any change over time that was present both in the EEG and in the fMRI data will be now affect the parameter estimates associated with the SSVEP covariates. Model 3 was run using SSVEP-covariateA only.

It should be noted that all models included the effect of condition as a predictor in the multiple regression model, thus effectively testing for correlations between BOLD and SSVEP amplitude using only variance unique to the SSVEP predictors.

2.9. Analysis of BOLD and SSVEP amplitude over time

Since Model 2 and Model 3 gave substantially different results (see Section 3) we hypothesized that both BOLD and SSVEP signals may change over time. Accordingly, we tested whether BOLD and SSVEP changes over time were specific for the stimuli presented to the subject (i.e. stimulation vs. control frequency), as would be expected for any physiologically meaningful effect (e.g. habituation). Accordingly, for each subject and stimulation condition (Stm6, Stm7.5 or Rest), we co-varied separately the fMRI signal in visual cortex (ROIstim) and the SSVEP amplitudes over occipital electrodes (O1/O2) with a time vector corresponding to the timing of the data acquisition (i.e. from 16.1 to 596.1 s for fMRI; and from 0 to 580 s for EEG). The slope of the fit between fMRI/SSVEP signals and the time vector were then assessed by least square fitting. For group-level statistical inference, we compared the slope of these linear changes over time for the stimulation frequency vs. the control frequency using a paired t-test (both for fMRI and for SSVEPs data).

3. Results

3.1. EEG

Before any data analysis, we quantified the efficiency of the preprocessing procedures that were applied to the EEG traces in order to remove any artifact associated with the EEG measurement inside the MR scanner. Sparse recording of EEG and fMRI allowed us to obtain MR-artifact-free EEG-epochs, which where however degraded by nuisance heartbeat-related voltage changes (ballistocardiac artifact: BCA). In particular, the heartbeat produces large BCA in EEG recorded in the scanner, due to (cardiac) pulse-driven head nodding motion, pulsatile blood flow and pulse-driven scalp-expansion. The percentage of residual BCA power was used to evaluate the efficiency of the filtering procedure (see Section 2). This is shown in Fig. 1C for electrodes O1 and O2, for all subjects. Overall, the residual BCA power for these two electrodes was around 0.5%, with only one subject with the residual power above 1%. Considering previous findings (Niazy et al., 2005), these results demonstrate a highly efficient removal of the ballistocardiac.

Next, we turned to the analyses of the SSVEPs elicited by the pattern-reversal visual stimulation.

Fig. 2 shows the averaged SSVEP waveforms for one subject (subject N. 7), considering the entire stimulation-epoch length (SSVEP-covariateA), separately for visual stimulation at 6 and at 7.5 Hz (Fig. 2A and B, respectively). The scalp distribution of the SSVEPs highlights the expected predominance over posterior electrode sites, consistent with generators in the visual cortex. The frequency of the EEG response was twice the frequency of the pattern reversal, as typically observed for this type of visual stimulation (Di Russo et al., 2007).

Fig. 2C summarizes the SSVEP effects for all subjects (average ± SE over 30 repetitions), according to stimulation condition (6 Hz/7.5 Hz), on average across electrodes O1 and O2 (SSVEP amplitudes at the two electrode positions were not significantly different), considering the entire stimulation-epoch length (SSVEP-covariateA). This demonstrates reliable frequency-specific EEG activity in seven out of nine subjects (subjects N. 1–4, 6–8). Each of these seven seven subjects showed significantly larger SSVEP amplitudes for the stimulated frequency compared to the control frequency (p < 0.05). This stimulus-frequency-specific effect was also significant at the group level (t(6) = 3.09, p < 0.011; for these seven subjects). Considering every single stimulation epoch across these seven subjects, SSVEP amplitudes drawn from the entire 15 s epochs at the stimulation frequency were larger than those at the control frequency in 85.7% of the trials/epochs (i.e. about 360 out of a total of 420 epochs).

For SSVEP amplitudes estimated from 2.5 s traces and convolved with the HRF (SSVEP-covariateB), only five out of nine subjects (subjects N. 3–4, 6–8) showed reliable SSVEP effects. Furthermore, considering the seven subjects who showed reliable effects for SSVEP-covariateA, the percentage of epochs with greater effects for stimulation vs. control frequency now considering SSVEP-covariateB dropped from 85.7% to 68.6% only. Accordingly, the “weighting” (i.e. hemodynamic convolution) of different short-segments of stimulation on the expected BOLD response at the time of the MR acquisition reduced the reliability of single-epoch, condition/frequency-specific SSVEP.

3.2. fMRI and combined SSVEPs–fMRI

Using the fMRI data, we first identified brain regions responding to the visual stimulation. We compared the two stimulation conditions vs. Rest, in the group of seven subjects. This revealed activa-
tion of the primary visual cortex at the occipital pole (i.e. the foveal representation), plus adjacent extra-striate occipital regions (see Fig. 3A). We also directly compared the two stimulation conditions (6 vs. 7.5 Hz, and vice-versa), but this did not reveal and significant effect of stimulation frequency in the fMRI data (note that the two frequencies were actually very close).

We then asked whether frequency-specific SSVEP amplitudes explained any additional fMRI signal-variance on an epoch-by-epoch basis, beyond any overall effect of the visual stimulation. Thus the effects of condition, the main effect of fMRI-run (i.e. constant modelling the mean signal of each run), and linear trends within each run were also included in the statistical model as covariates of no interest (see Section 2, Models 1 and 2). We tested for co-variation between the SSVEP and the residual BOLD signal (i.e. after accounting for all variance explained by the covariates of no interest, see above) utilising all 15 s stimulation epochs of the seven subjects (for SSVEP-covariateA) or five subjects (for SSVEP-covariateB) who were retained after the initial EEG analyses (see above). This did not revealed any significant frequency-specific co-variation between the two measures, neither the voxel-level (Model 1) nor averaging the fMRI signal (Model 2) in all voxels responding to the visual stimulation (ROIstim, see Fig. 3A).

Fig. 3. (A) fMRI activation relative to the overall effect of visual stimulation (ROIstim) at the group level (fixed effects on seven subjects, p < 0.05, corrected for family-wise errors). fMRI activation is overlaid on (left) a 3D rendering and (right) two axial sections of the MNI-template (SPM2). As expected for visual stimulation, both primary and extra-striate visual areas are robustly activated. (B) Slow changes of BOLD signal over time; and (C) SSVEP amplitudes over time. BOLD-fMRI data are shown (B1) for an example dataset (subject N. 2) over time within a run, per each condition (Stim6, Stim7.5, Rest), and with repetition across three runs (solid lines represent the linear least square data fit). SSVEP amplitudes during stimulation conditions (Stim6 and Stim7.5 together) are shown (C1) for an example dataset (subject N. 2) over time within a run, with repetition across runs (solid lines represent the linear least square data fit). SSVEP amplitudes are estimated at stimulus-frequency (Stm6_A12 Hz and Stm7.5_A15 Hz, in red) and at control frequency (Stm6_A15 Hz and Stm7.5_A12 Hz, in blue). The slopes of the linear fit (linear drift of fMRI/SSVEPs data over time) for each of the seven considered subjects (B2/C2) and the averages of these slopes at the group level (B3/C3, mean ± SE, n = 7) are also shown.
3.3. Analysis of BOLD and SSVEP amplitude over time

Nonetheless, a closer examination of the two datasets revealed that both fMRI and SSVEP amplitudes decreased linearly over epochs, within each run. Indeed, significant correlation between the BOLD in occipital cortex and SSVEP amplitude at occipital sites was found by simply omitting the linear trends from the epoch-by-epoch analysis (p < 0.05, Model 3). The interpretation of these drifts over time may be difficult (see Section 4), but interestingly here we were able to show that these decreases occurred selectively during visual stimulation in the fMRI data (see Fig. 3B; compare red and green vs. yellow); and they were specific for the stimulation frequency in the SSVEP data (Fig. 3C, compare red vs. blue).

Fig. 3B1 shows linear decreases of the fMRI signal over time, for one subject (subject N. 2). Note that this decrease was more pronounced for fMRI signal associated with stimulation blocks (green and red in Fig. 3B1) compared to that relative to Rest (in yellow), suggesting that this effect was not merely due to hardware instability. Fig. 3B2 reports the slopes of the regression lines of fMRI signal over time for the seven considered subjects (subjects N. 1–4 and 6–8, according to the numbering of the whole group of subjects), separately for the three conditions. In most of the subjects the slopes were more negative in the two stimulation conditions than in the Rest condition. Formal comparison of the slopes in the different conditions supported this observation also at the group level (7.5 Hz vs. Rest: t(6) = -2.02, p < 0.045, one-tailed; 6 Hz vs. Rest: t(6) = 1.26, p < 0.13; see also Fig. 3B3).

The EEG-data showed an analogous pattern over time. Fig. 3C1 displays the decrease of the SSVEP amplitudes (SSVEP-covariateA) over time, for one subject (subject N. 2). The amplitude of the stimulation frequency (A12 Hz for Stim6, and A15 Hz for Stim7.5; plotted in red) tended to decrease more rapidly (i.e. with steeper slopes) than the amplitude of the other/control frequency (i.e., A15 Hz with Stim6, and A12 Hz for Stim7.5; plotted in blue). Also, note that in this case the two amplitudes (i.e. at the stimulus-frequency and control frequency) were actually measured within the same epoch of pattern-reversal visual stimulation. Fig. 3C2 show that this effect was consistent across subject (five out of seven showed this pattern). At the group level, the comparison of the two slopes did not reach full significance but a statistical trend was present (stimulation vs. control frequency: t(6) = -1.34, p < 0.11; see Fig. 3C3).

4. Discussion

Aim of the present study was twofold: to prove the feasibility of recording reliable single-epoch SSVEPs in the MR environment, and to test if improved prediction of fMRI signals variability across stimulus repetitions can be obtained using interleaved SSVEPs recordings of cortical responses.

4.1. Feasibility of average and single-trial SSVEPs in the MR environment

Previous studies have indicated that the recording of EPs simultaneously with fMRI does not affect latencies and amplitudes of average EP waveforms, as well as the scalp distribution (Bonmassar et al., 1999, 2002; Sommer et al., 2003; Kruggel et al., 2000; Iannetti et al., 2005). With regard to average steady-state EPs, our study shows the feasibility of detecting reliable response amplitudes utilising 15-s epochs of stimulations and only 30 repetitions. With our data acquisition procedure (sparse sampling), the main limitation to obtain good quality SSVEPs was related to the removal of the ballisto-cardiogram artifact. Compared with other MR-induced artifacts (e.g. radio-frequency and gradient pulse sequence), the BCA has a smaller amplitude, but it is unstable and unpredictable and is one of the most relevant sources of noise in EEG recordings in an MR environment (Allen et al., 1998; Niazy et al., 2005). Here the removal of the BCA was achieved using a modified version of Allen’s algorithm (Allen et al., 1998) that was found to compare favorably with previously developed methods (cf. Niazy et al., 2005, see also Fig. 1C). Our result adds strength to the feasibility of recording good quality averaged SSVEPs within an MR-scanner environment (Sammer et al., 2005). However, when the interest is focussed on standard averaged data and for experiments with a high reproducibility, EP and fMRI data could in principle be combined from separate acquisition’s sessions.

In contrast, when the multi-modal analysis is focused on exploring unpredictable changes of neural activity evoked by the same repeated stimulus, the use of concurrent EPs and fMRI and the extraction of single trial/epoch responses are required. Indeed a single-trial/epoch approach permits the assessment of between-trial variations of electrophysiological responses and their relationship with other parameters (e.g. stimulus intensity, psychophysical performance and fMRI responses); this may ultimately increase the power of statistical fMRI analysis and allow within-subject comparisons. A few recent studies suggested that it is possible to estimate evoked potential on a single event basis, during concurrent EPs–fMRI recording: this was achieved either by the use of ICA de-noising (Debener et al., 2005; Eichele et al., 2005) or with standard EPs pre-processing only (Iannetti et al., 2005; Benar et al., 2007). Here we report for the first time reliable single-epoch steady-state EPs during fMRI. In seven out of nine subjects, we obtained reliably larger SSVEP amplitudes for the stimulation frequency compared with the control frequency (cf. Fig. 2C). Considering every single stimulation epoch across these seven subjects, we found larger SSVEP amplitudes for the stimulation frequency compared to the control frequency in 85.7% of the trials/epochs.

Considering the gain in spatio-temporal resolution and the complementary specificity of each technique to neuronal activity, our results show that multi-modal imaging of evoked activity is a promising tool to investigate brain function in healthy subjects. Moreover, our findings suggest that multi-modal brain–computer interfaces (BCIs) for patients with severe motor deficits might be developed. For example, the combination of EEG and fMRI (and particularly so when recorded concurrently within a single subject) may improve the performance of BCI paradigms based on covert selective attention (e.g. when subjects choose covertly, without eye movements, one of two stimuli flickering at different frequencies; Allison et al., 2008). In particular, fMRI could help to identify specific cortical regions that show attentional modulation at the single subject level. This additional information could be used to improve dipole modelling and/or to weight EEG signal measured at different electrodes, enhancing the sensitivity and selectivity of BCI algorithms. SSVEP recordings may provide a valuable approach for this, enabling the estimation of SSVEP and fMRI activation (and possibly any attentional modulation thereof) in relatively short epochs of just few seconds, useful for online control of some external device. Moreover, the use of interleaved SSVEPs–fMRI recordings will also provide EEG-feedback inside an MR-scanner environment and will also enable direct comparison of the performances of EEG– and fMRI-based BCIs.

4.2. Predictability of the variability of BOLD responses by SSVEPs

Having achieved good quality SSVEPs at the single-epoch level, we tested whether stochastic variation of BOLD-fMRI response across stimulation epochs could be predicted using the variability of the SSVEPs amplitude, for the same epochs. It should be noted...
that the inclusion of the mean effect of condition in all our analyses, ensured that we specifically tested for the co-variance between SSVEP and BOLD-fMRI, over and above any average signal change related to the stimulation (i.e. stimulus-frequency and presence/absence of the visual stimulus). This type of analysis was performed in a few previous studies (Eichele et al., 2005; Benar et al., 2007) demonstrating the ability to localize fluctuations of evoked activity, yet with a quite high inter-subject variability (see Benar et al., 2007). With regard to the epoch-to-epoch variability, our study did not show any significant correlation between SSVEP amplitudes and fMRI responses in visual cortex. But note that this was obtained by including in the model both the expected (i.e. stimulus-frequency) and unexpected (i.e. control frequency) SSVEP amplitudes, as well as the effects of condition, fMRI-run specific offsets and linear drifts.

Our analyses question the ability to localize fMRI activation with improved significance using interleaved EEG recordings. Nevertheless, we should point out that the sparse-sampling paradigm entails relatively long stimulation epochs (15 s) with just one fMRI measurement associated with each epoch. Accordingly, this method can detect only SSVEP amplitude’s variations that are stable for the entire duration of the epoch and that yield to a change of BOLD signal precisely time locked with the fMRI acquisition (cf. also Fig. 1B). For changes of the stimulus-related signal amplitudes faster than those hypothesized in the current approach, another design, namely with more but shorter epochs, could be favorably employed. This would increase the temporal resolution of the analysis, with a higher number of SSVEP observations and recorded MR-volumes. Nevertheless, the SSVEP amplitude estimation may be less reliable (see our results for SSVEPcovariateA using 15 s data-segments; vs. SSVEPcovariateB using 2.5 s data-segments). Future studies should systematically manipulate the duration of each epoch to reveal the boundary conditions (e.g. minimum duration) that allow measuring reliable SSVEP on a single-epoch basis. Other EEG–fMRI methods (i.e. with continuous acquisition of both modalities) permit the investigation of the relationship between evoked potentials and fMRI responses utilising running-average techniques (e.g. Debener et al., 2005), and thus avoiding this limitation. However, continuous EEG–fMRI acquisition produces larger MR-induced artifacts on the EEG traces (e.g. radio-frequency and gradient pulse sequence artifacts) that can compromise single-epoch/trial evoked-potential analyses, when employing inappropriate artifact removal strategies.

Another reason that may have concealed any co-variation between SSVEP amplitude and BOLD signal in the current study concerns stimulus-unrelated variability which might have contributed to fMRI signal fluctuations (as in Fox et al., 2006, 2007) but not to changes in SSVEP amplitudes; this is the case for slow changes in ongoing spontaneous fluctuations with EEG effects at frequency or phase other than those of the stimulation cycle (Arieli et al., 1996; Makeig et al., 2002). Indeed, ongoing EEG activity at the stimulation frequency but with a phase other than that of the stimulation cycle was reduced by about an order of magnitude by time locked averaging of EEG traces to stimulus onsets; thus the influence of those stimulus-unrelated fluctuations of ongoing electrophysiological signals on the variability of the fMRI signal over and above any stochastic variations of the evoked responses was reduced. Moreover, electrophysiological changes subtler than those induced at the stimulation frequency may be more inherently coupled with the hemodynamic response. For example, sensory stimulation can induce phase-unlocked synchronization/desynchronization in specific frequency bands (e.g. alpha, beta and gamma band, see Fries et al., 2008), and intracortical recordings demonstrated that the BOLD response to visual stimuli can be predicted by local-field potentials in the 40–130 Hz frequency range (i.e. mainly in the gamma band; Logothetis et al., 2001). Nevertheless, gamma activity is not easily recorded using EEG or MEG scalp recordings (see Hoogenboom et al., 2006) due to attenuation at scalp sites and to the overlap with small muscle artifacts (Fries et al., 2008).

4.3. Decreases of BOLD and SSVEP amplitude over time

In our EEG–fMRI dataset we also investigated slow components and we found that both signals contained significant linear decreases over time during a run. A component of linear drifts in BOLD signals can be ascribed to instability of the static magnetic field, related to hardware stress of the MRI system (e.g. switching gradients result in heating of resistive parts of the MRI scanner, see El-Sharkawy et al., 2006). On the other hand, electrode heating and displacement might be the origin of slow changes in EEG recorded signals and account for linear decreases of EEG signals over time.

However, in our experiment the linear decreases appeared to be specific for the presence of visual stimuli in the fMRI data (cf. Fig. 3B), and were specific for the amplitude of the steady-state evoked potentials at the stimulation frequency (cf. Fig. 3C). Moreover, linear decreases where observed for SSVEP amplitudes after removal of very slow fluctuations of raw EEG signals by high-pass filtering at 0.5 Hz (see Section 2). This may be consistent with some adaptation/habituation at the neural level related to the repetition of the visual stimulation. Repetition suppression (adaptation) is a phenomenon described in both fMRI end ERP studies (Grill-Spector et al., 2006). A previous study employing simultaneous EEG–fMRI acquisition also found habituation effects to repeated painful stimulation (Christmann et al., 2007). As in the current study, both the BOLD signal and the evoked potentials decreased over time. Nonetheless this previous study could estimate signal decreases only after averaging data within experimental blocks, thus investigating habituation using three time-points only. Here, using SSVEP we were able to track these changes on a much shorter time scale, estimating BOLD and SSVEP amplitude for each single 15-s epoch (see Fig. 3). Overall the results of both studies are consistent with the proposal that neuronal habituation might be the most likely source of concomitant decreases of both fMRI signals and electrophysiological responses. Our results extend previous work showing such effects in the visual cortex and at a better temporal resolution, using interleaved EEG–fMRI measurements of SSVEP.

In conclusion this study demonstrates the feasibility of single-epoch analysis of evoked potentials and BOLD-fMRI responses. Specifically, the use of sparse sampling SSVEPs–fMRI acquisition resulted in good quality evoked potentials and fMRI signals elicited by short epochs of visual stimulation.

Conflict of interest

We do not have any conflict of interest with the subject matter of this paper.

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References
