Behavioral/Systems/Cognitive

Modulation of Visually Evoked Cortical fMRI Responses by Phase of Ongoing Occipital Alpha Oscillations

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Using simultaneous electroencephalography as a measure of ongoing activity and functional magnetic resonance imaging (fMRI) as a measure of the stimulus-driven neural response, we examined whether the amplitude and phase of occipital alpha oscillations at the onset of a brief visual stimulus affects the amplitude of the visually evoked fMRI response. When accounting for intrinsic coupling of alpha amplitude and occipital fMRI signal by modeling and subtracting pseudo-trials, no significant effect of prestimulus alpha amplitude on the evoked fMRI response could be demonstrated. Regarding the effect of alpha phase, we found that stimuli arriving at the peak of the alpha cycle yielded a lower blood oxygenation level-dependent (BOLD) fMRI response in early visual cortex (V1/V2) than stimuli presented at the trough of the cycle. Our results therefore show that phase of occipital alpha oscillations impacts the overall strength of a visually evoked response, as indexed by the BOLD signal. This observation complements existing evidence that alpha oscillations reflect periodic variations in cortical excitability and suggests that the phase of oscillations in postsynaptic potentials can serve as a mechanism of gain control for incoming neural activity. Finally, our findings provide a putative neural basis for observations of alpha phase dependence of visual perceptual performance.

Introduction

The dominant oscillatory electroencephalography (EEG) activity during resting wakefulness is observed to be ~10 Hz and is commonly referred to as the alpha rhythm (Berger, 1929). The amplitude of alpha activity has been suggested to play an important role in gauging the brain’s capacity to process information (Klimesch et al., 2007; Mazaheri and Jensen, 2010). Evidence for this has been found in a number of studies that have shown a reduction in the amplitude of alpha activity in regions processing task-relevant stimulus features, accompanied by an increase in alpha amplitude in regions not required for the task (Thut et al., 2003, 2006; Jokisch and Jensen, 2007; Medendorp et al., 2007; Rihs et al., 2007; Romei et al., 2008a,b). Further studies have shown that this latter increase in posterior alpha activity reduces visual discrimination abilities (van Dijk et al., 2008; Zhang et al., 2008).

Compared with the effect of the amplitude (or power) of alpha activity, the evidence regarding an impact of oscillation phase on neural processing and perceptual performance has remained rather sparse. Several studies have successfully linked differences in the shape or power of poststimulus activity to the prestimulus phase of ongoing alpha activity (Bechtereva and Zontov, 1962; Dustman and Beck, 1965; Makeig et al., 2002; Barry et al., 2003). However, this relationship has remained controversial because of the difficulties in unambiguously separating evoked responses from the ongoing activity (Kruglikov and Schiff, 2003; Shah et al., 2004; Yeung et al., 2004; Mazaheri and Jensen, 2006; Klimesch et al., 2009; Risner et al., 2009; Ritter and Becker, 2009).

Even though it was suggested nearly 80 years ago that cortical responsiveness undergoes cyclic changes (Bishop, 1932), only a few studies since then have shown an influence of the phase of the ongoing activity on the visual processing of stimuli (Callaway and Yeager, 1960; Dustman and Beck, 1965; Nunn and Osselton, 1974; Varela et al., 1981). Two recent EEG studies using perliminal stimuli (Busch et al., 2009; Mathewson et al., 2009) found a link between the phase of the prestimulus alpha activity and conscious perception (for review, see Wyart and Sergent, 2009). However, a direct demonstration of phase-dependent local cortical response properties has not yet been sufficiently well achieved. In the present study, we used simultaneous EEG (as a time-resolved measure of ongoing oscillatory activity phase) and functional magnetic resonance imaging (fMRI) (as a spatially resolved bulk measure of the local stimulus-driven response) to examine how the evoked cortical blood oxygenation level-dependent (BOLD) response is affected by the amplitude and phase of ongoing alpha activity at the onset of a brief visual stimulus. For amplitude, but not for phase, it was important to model pseudo-trials without visual stimulation to account for the intrinsic, nonstimulus-driven relationship between occipital alpha activity and the BOLD signal that has been demonstrated in a number of studies (Goldman et al., 2002; Gonçalves et al., 2006;
Laufs et al., 2006; Ritter et al., 2009; Scheeringa et al., 2009; Yuan et al., 2010).

Materials and Methods

Subjects

Eighteen subjects (14 female; 4 male; mean age, 25 years; range, 18–65), with no history of psychiatric or neurological disorders, participated in the simultaneous EEG/fMRI sessions. All had normal or corrected-to-normal vision. Before the start of the experiment, written informed consent was obtained from each subject. The experiment was approved by a local ethical committee (CMO region Arnhem-Nijmegen, The Netherlands). One female subject was excluded from further analyses because of anxiety and consecutive excessive movement during scanning.

Experimental paradigm

To ensure subjects’ cooperation and alertness, they were asked to fixate a central fixation cross (width, 0.75°) and report by a button press whenever it turned green. During each session, the fixation cross changed to green for 300 ms eight times at pseudorandom moments. Not related to this central incidental task, short (17 ms), wedge-shaped, black-and-white checkerboard visual stimuli were presented in the lower left corner of the visual field (angle of wedge, 32.7°; visual angle width, 10.4°; starting 3.8° outside the center of fixation). These peripheral stimuli required no subject response. The stimulus and fixation mark are depicted in Figure 1A. In addition to the short but strong peripheral visual stimuli as experimental trials of interest, we designed pseudo-trials without such stimulation to estimate the intrinsic correlation of alpha amplitude with the occipital BOLD signal. For these trials, no visual stimulus was presented apart from the central fixation cross that was always present. Forty trials of each condition occurred at random intervals ranging from 3 to 7 s according to a uniform chance distribution. Six sessions of ~6.5 min were recorded with this paradigm.

EEG data acquisition

EEG data were recorded with an MRI-compatible cap equipped with carbon-wired Ag/AgCl electrodes (EasyCap) from 61 scalp positions according to the international 10–10 system. One additional electrode was placed under the right eye to record eye movements. The reference electrode was placed at FCz. Two MRI-compatible EEG amplifiers (BrainAmp MR, Brain Products) with a built-in 250 Hz low-pass analog hardware filter were used. The EEG was recorded with a 10 s time constant and continuously sampled at 5 kHz. EEG recordings were performed with Brain Vision Recorder software (Brain Products).

EEG preprocessing

The EEG data were corrected for gradient and pulse artifacts along the lines described by Allen et al. (1998, 2000) using Vision Analyzer (Brain Products). To denoise the EEG data further, we applied Infomax (standard, not extended) independent component analysis (ICA) (Bell and Sejnowski, 1995) on all of the 8–12 Hz bandpass-filtered (fourth-order Butterworth filter) concatenated trials (2 s long) using the algorithm implemented in EEGLab 5.03 (Delorme and Makeig, 2004). For each subject, a single alpha component was identified based on right-lateralized topography and the presence of an alpha peak in the power spectrum when the unmixing weights were applied to unfiltered data. To quantify the amount of ICA-related reduction of noise and other neural sources in the mixed sensor signal, we calculated the percentage of variance explained by the selected component in the data (filtered in the alpha band) for the channel selected for calculating the prestimulus phase and amplitude. On average, the selected component explains 14% of the biologically generated variance, indicating that ICA application results in a greatly enhanced selectivity for the source of interest. Our logic in data analysis closely followed the suggestions outlined for EEG/fMRI investigations of trial-by-trial variability in Debener et al. (2006). The accordingly identified single components from all subjects were projected back to channel level. To approximately indicate where the source of this alpha component was localized, we conducted a dipole analyses in BESA2000 (v 5.2; BESA) using a four-shell elliptical head model (Berg and Scherg, 1994). For each individual subject, a genetic algorithm (standard in BESA) was used to fit a single dipole to the scalp topography of the subject’s alpha component’s mixing weights.

EEG analysis

Instantaneous phase and amplitude estimates. Phase was calculated by discrete Fourier transforms (DFTs) for the subjects’ dominant alpha frequency. The DFTs were calculated by using three-cycle-long data segments before stimulus onset (e.g., 10 Hz results in a 0.3 s window). Each data segment was Hanning tapered before the DFT was calculated. We estimated phase as close as possible to stimulus onset with a window of three cycles to give sensible frequency resolution under the assumption of phase continuity until frequency onset, since poststimulus-evoked activity would confound and contaminate the phase estimate. The trials in each subject were then binned according to where in the alpha cycle the stimulus onset occurred. These bins formed the basis for regressors used in the integrated EEG/fMRI analysis. Instantaneous amplitude was estimated based on the same prestimulus time windows used for the individual alpha frequency for both the real and the pseudo-trials.

Effects of phase on power. For the effect of phase on poststimulus power, we first computed the poststimulus power effects related to the stimulus presentation. Significant time–frequency clusters of increases or decreases were further tested for effects of phase using a one-way ANOVA. Time–frequency analysis was based on 0.5 s Hanning tapered windows in a 2–30 Hz frequency range in 0.05 s time steps up to 0.8 s poststimulus. Poststimulus power estimates were normalized by computing the logarithm of the ratio relative to a prestimulus baseline. The average power value in the period between 350 and 250 ms before stimulus onset served as a baseline.

fMRI data acquisition

fMRI data were acquired using a 3.0-T whole-body MRI scanner (Magnetom TIM Trio; Siemens) and a custom-built eight-channel array coil.
(Stark Contrast) covering the occipital cortex for functional imaging. Twenty-one slices positioned parallel to the calcarine sulcus were recorded using a BOLD gradient echo EPI sequence [repetition time (TR), 1400 ms; echo time (TE), 30 ms; 60° flip angle; 3.0 mm slice thickness; 0.5 mm gap; voxel size, 3.5 × 3.5 × 3.5 mm; with bias field correction filter].

After the functional scans, two anatomical scans of the entire brain were acquired, one with the eight-channel occipital array coil (3D MPRA; TE; 3.5 ms; TR; 2300 ms; 10° flip angle; 192 slices per slab; voxel size 0.8 × 0.8 × 0.8 mm; with bias field correction filter) and one with an eight-channel phased array head coil (3D MPRA; TE; 3.0 ms; TR; 2300 ms; 8° flip angle; 192 slices; voxel size 1.0 × 1.0 × 1.0 mm; with bias field correction filter). The anatomical image from the occipital array coil was acquired because the bias field in sensitivity is similar to the functional images and as a result provides a better match for coregistration.

Image preprocessing
We preprocessed the (f)MRI data using statistical parametric mapping software (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software); preprocessing involved realignment, correction for motion and differences in slice acquisition time, spatial normalization, and smoothing with an isotropic Gaussian kernel of 6 mm full-width at half-maximum. Anatomical normalization to MNI space was performed by coregistration of the functional images, first with the anatomical T1 scan acquired with the eight channels occipital array coil, which has the same bias field as the functional images. Second, this anatomical image was coregistered with the anatomical scan obtained with an eight-channel head coil, keeping the functional images in alignment. Parameters for the normalization to MNI space were estimated by normalizing this scan to the T1 MNI template provided by SPM8, and were subsequently applied to all anatomical and functional images.

Stimulus-related BOLD response
For the standard analysis, a general linear model was constructed. Stick functions convolved with the canonical hemodynamic response function from SPM8 were used to model the stimulus onset, onsets of reported color changes of the fixation stimulus, and fixation change misses. The contrast estimate images for the wedge-shaped visual stimulation onsets and the color change of the central fixation were subjected to a single sample t test at group level.

Integrated EEG-fMRI analysis
Influence of alpha amplitude on the evoked BOLD response. The effect of prestimulus alpha amplitude on the evoked BOLD signal was assessed using a finite impulse response (FIR) model as implemented in SPM8. Activity levels were estimated at a resolution of 1.4 s (one value per TR) in a time range starting 2.8 s prestimulus and ending 16.8 s poststimulus (14 time points in total). Based on a median split, all visual stimulation trials were sorted into two conditions with either high or low prestimulus alpha amplitude. Since occipital alpha power has been reported to show a (negative) intrinsic correlation with BOLD signal in visual areas, we also modeled pseudo-trials without stimulation after applying the same median split. The FIR model estimates were extracted and averaged for right hemispheric regions of interest that we defined by the overlap of stimulus-related activity (p < 0.05, uncorrected at group level) with Brodmann areas 17, 18, and 19 from the WFU Pick Atlas (Maldjian et al., 2003, 2004). We then tested for an interaction between the factors trial type (two levels: real and pseudo) and amplitude (two levels: high and low). Such an interaction would implicate a genuine effect of alpha amplitude on the evoked BOLD response. In practice, this interaction effect was tested by first subtracting the FIR time courses for high and low pseudo-trials from high and low real trials, respectively, followed by a cluster-based nonparametric statistical test based on a dependent samples t test (see Statistical inference, below).

Influence of alpha phase on the evoked BOLD response. A similar FIR model as for amplitude was constructed for the phase effect. In this case, four phase bins were constructed for the estimated alpha phase at stimulus onset, as follows: −π to −0.5π (rough rise, bin 2), 0 to 0.5π (peak rise, bin 3), and 0.5π to π (peak fall, bin 4). This model did not contain pseudo-trials. Further model specifications were the same as for the amplitude effect. Significance was assessed using a cluster-based nonparametric statistical test based on the F statistic obtained by a one-way ANOVA (phase with four levels).

For comparison of the results with analysis on channel level, we constructed a model based on channel-level alpha phase estimates. For this analysis, phase was estimated for each subject at the right posterior channel with the largest absolute mixing weight for the selected right posterior alpha component. The other steps in the analysis were the same as for the analysis based on the right posterior alpha component.

Statistical inference
The effects of alpha phase and amplitude on fMRI and EEG responses were assessed using a nonparametric cluster-based randomization procedure that effectively controls the type I error rate in a situation involving multiple comparisons (Maris and Oostenveld, 2007). This randomization procedure allows for the construction of user-defined test statistics tailored to the effect of interest within the framework of a cluster-based randomization test. It works by calculating the sum of a statistic of choice over all data points within a cluster that exceeds an arbitrary predefined threshold. Each cluster sum is then compared with a distribution of maximum cluster sums obtained by randomly permuting the labels of the independent variable. The number of randomizations for computing the reference distribution was 1000.

For the effect of alpha amplitude on the BOLD response, we used the t value obtained by the dependent-samples t test between the high and low alpha amplitude BOLD time courses (after subtraction of the pseudo-trials). Clusters were defined as adjacent data points exceeding an uncorrected p value of 0.1 for this t test (e.g., data points that fall within the upper or lower 5% of the t distribution).

For the phase effect on the BOLD response, we used the F value obtained from a repeated-measures ANOVA with four levels as a test statistic. Clusters were defined as data points that exceeded an uncorrected threshold of 0.1 for the data points ranging between 0 and 10 s poststimulus. Post hoc differences between phase bins were assessed with t tests on the average BOLD response within the significant clusters.

Clustering for the power analysis was performed over both time and frequency dimensions and for the event-related potential (ERP) only over the time dimension. For the time frequency analysis of power and the ERP, a two-tailed one-sample t test against baseline served as the test statistic. Clusters were defined as adjacent data points passing a p = 0.1 uncorrected threshold. Randomization was performed by randomly multiplying the single subject effects by 1 or −1. For the ERP, separate one-tailed t tests were performed for predefined intervals for the P1 (80–120 ms poststimulus) and N1 (150–200 ms poststimulus). The ERP was root-mean-square normalized from 200 ms before stimulus to 1000 ms poststimulus before statistical tests were performed.

Results
All subjects performed close to ceiling on the incidental behavioral task involving the central fixation point. They detected its color change in at least 97.9% of occurrences, which means they made maximally one mistake across the six task blocks. Average reaction time was 771 ms with a SD across subjects of 94 ms. Neither alpha phase (F(3,45) = 1.69, p = 0.18) nor amplitude (F(15) = 0.57, p = 0.57) had any significant effect on reaction times to the fixation change.

For 16 of the 17 subjects, a right lateralized posterior alpha component could be identified from the recordings of ongoing EEG activity. The grand average power spectrum is depicted in Figure 2A and contains a distinct peak in the alpha frequency range. The average scalp topography of the mixing weights (Fig. 2B) shows a clear right posterior lateralization of the modeled activity. Dipole fits for these 16 subjects (Fig. 2C) tentatively link this component to activity in right hemispheric early visual areas. Phase and amplitude of this component were analyzed trial by trial to probe their impact on evoked responses.
Evoked fMRI responses
The peripheral experimental 17 ms visual stimulus in the left lower quadrant of the visual field yielded a significant BOLD response in predominantly right hemisphere visual regions (Fig. 1B), whereas the central fixation change resulted in bilateral activation throughout a large part of the visual system (Fig. 1C). We extracted data from three regions of interest [Brodmann area (BA) 17–19] to test for effects of prestimulus alpha amplitude and phase on fMRI signal related to the peripheral stimulus. For amplitude, clear differences between high and low amplitude were present in the estimated FIR time courses (Fig. 4A–C). Yet, these differences could be attributed to a negative intrinsic relation between alpha amplitude and the BOLD signal that is already present without external stimulation. After subtracting the FIR model estimates for the high and low alpha amplitude pseudo-trials from the high and low amplitude real trials, there was no significant effect of alpha amplitude on the evoked BOLD response (Fig. 4D–F).

Contrary to alpha amplitude, a significant effect of alpha phase on the evoked BOLD response was observed for Brodmann area 17 (p = 0.013, corrected for multiple comparisons) and a trend for Brodmann area 18 (p = 0.063, corrected) (Fig. 5A–C). Subsequent t-tests for differences between the different phase bins showed that visual stimuli presented at the trough of the alpha cycle produce a larger BOLD response than stimuli presented at its peak (Fig. 5D, E). This demonstrates that the strength of the neuronal response, as expressed in the amplitude of the BOLD response, is dependent on the phase of the ongoing alpha rhythm at the onset of stimulation.

To illustrate the effect of selecting the right posterior alpha independent component, we performed the same analysis for phase estimated at the channel level with the maximal absolute mixing weight. For this channel, however, no significant modulation of the BOLD signal by alpha phase could be observed (Fig. 5F–H). This suggests the ICA decomposition greatly improves estimation of the right posterior alpha signal.

Discussion
The objective of our study was to test whether ongoing alpha oscillations generate cyclic gain changes of evoked neural responses. We used the technique of simultaneously recording EEG and fMRI (Laufs et al., 2008) to address this question, because the BOLD signal permits a well localized assessment of the amount of synaptic activity induced by a stimulus (Logothetis, 2008). We analyzed signal from stimulus-sensitive regions of interest in the right hemisphere and observed a relatively weaker evoked BA17 (and perhaps BA18) BOLD response when the ultra-short stimulus in the peripheral left hemifield was presented at the peak of the alpha wave compared with its trough. We conclude from these findings that the position in the phase cycle of the posterior alpha oscillation impacts on the overall strength of the neural evoked response, as indexed by the BOLD signal.

In addition to investigating the effect of alpha phase on the evoked BOLD response, we also interrogated our data for potential effects related to alpha amplitude. Although trials preceded by higher alpha amplitude were indeed, as one might predict, associated with lower evoked BOLD responses, we believe this effect to be accounted for by an intrinsic negative correlation between posterior alpha power and occipital BOLD signal (Goldman et al., 2002; Gonçalves et al., 2006; Laufs et al., 2006; Ritter et al., 2009).
2009; Scheeringa et al., 2009; Yuan et al., 2010). When we removed effects from this intrinsic correlation, we retained no evidence for an effect of alpha amplitude on the strength of the evoked response.

At first glance, it might seem somewhat surprising that we observed an effect of phase but not of amplitude. One possible explanation is that alpha amplitude is more variable across the cortex than alpha phase, especially in the presence of a central attention-demanding behavioral task. If posterior alpha oscillations are coordinated by synchronous thalamic drive or thalamocortical feedback, then alpha phase could be expected to be largely homogenous across the visual cortex. Conversely, the actual strength of the alpha oscillations at different places across the visual cortex might be more variable because of local intervening processes, which may be poorly reflected in the component signal we used to determine amplitude. An alternate possible explanation is a (nonlinear) threshold effect of alpha amplitude on evoked responses. This possibility could be tested in future studies by using a range of stimuli with varying strengths and it might also be relevant for the relative size of the effect that phase has on the evoked response.

Interestingly, this effect of alpha phase on the evoked BOLD response was not reflected in a modulation of EEG power changes in the lower frequencies. This could suggest that the BOLD response covers synaptic activity changes that are not captured in the surface EEG signal. Residual gradient artifacts present in simultaneous EEG/fMRI measurements prevented us from testing for potential effects in frequencies /H11022 30 Hz that might carry such information (Logothetis, 2008). The effects of alpha phase on the conventional ERP response are hard to assess because the sorting and averaging based on phase introduces phase effects in the ERP even in absence of a stimulus (Klimesch et al., 2009; Risner et al., 2009; Ritter and Becker, 2009). We can therefore only speculate that no such relationship was present in our data given that there was no effect of prestimulus alpha phase on the power of the poststimulus frequencies (i.e., the spectral representation of the ERPs).

Our observation that alpha oscillations reflect periodic variations in cortical excitability and hence neural response strength is compatible with existing views that alpha oscillations serve the cyclic inhibition of task-irrelevant regions (Klimesch et al., 2007; Mazaheri and Jensen,
The question of the physiological mechanism behind an alpha cycle-related modulation of the BOLD response arises. We are not aware of any evidence suggesting that such an effect could be considered an artifact related to a cyclic modulation of the neurovascular coupling function. In other words, we consider our findings, which are grounded in the BOLD signal, to indicate that neural responses to stimulation are modulated by the alpha cycle. GABAergic feedback from interneurons has been strongly implicated in the physiological mechanism generating the alpha rhythm (Lopes da Silva et al., 1976; Crunelli and Leresche, 1991; Jones et al., 2000; Liley et al., 2002). Thus, it is possible that rhythmic neuronal activity generating the alpha oscillations is a consequence of a GABAergic inhibitory feedback paced by neocortical or thalamic rhythm generators (Hughes and Crunelli, 2005; Lörincz et al., 2008, 2009; Liley et al., 2010). We suggest that this GABAergic feedback could function to directly dampen processing by providing shunting inhibition to pyramidal neurons or reduce the efficacy of incoming excitatory input. The phase of the alpha oscillations then reflects the cyclic state of this inhibition.

More generally, our findings demonstrate that the phase of oscillations in postsynaptic potentials can potentially serve as a mechanism of gain control for incoming neural activity, as put forward by theories on neural communication (Fries, 2005). Our study illustrates how such phase-related gain effects on neural processing can be studied noninvasively in human subjects by means of simultaneously recording EEG and fMRI. Methodolog-
ically, this study provides a proof of principle, since this technique can be adapted easily to investigate other combinations of frequency, scalp site, and brain regions of interest across a range of different cognitive states.

References
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