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 stimuli 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., 1991). 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.

Figure 1. Experimental paradigm and the associated BOLD responses. A, A wedge-shaped stimulus was presented at random intervals for 17 ms in the lower-left visual field quadrant. Subjects were asked to press a button whenever the continuously present central fixation cross turned green, which occurred at unpredictable intervals and was uncorrelated with peripheral stimulation. B, Regions activated in response to the wedge-shaped stimulus. C, Regions activated during the reported color change of the fixation cross. Activations in B and C are displayed at p < 0.001, uncorrected (second-level analysis, general linear model).
(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 MPRAJE; 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 MPRAJE; 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 fMRI 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, offsets 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 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.

Results
All subjects performed close to ceiling on the incidental behavioral task involving the central fixation point. They detected their 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.
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 response (Fig. 4D,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**

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 and Becker, 2009).
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 homogeneous 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 above 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, 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.

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In line with this, recent studies have reported that both perceptual performance on perliminal stimuli (Busch et al., 2009; Mathewson et al., 2009) and perception of phosphenes evoked by transcranial magnetic stimulation depend on alpha phase (Romei et al., 2008b). The modulation of the BOLD response by alpha phase was strongest in putative BA17, and was present to a lesser extent in BA18, which suggests that alpha phase modulates the neural response in early sensory cortices, providing a neural substrate for the perceptual effects described above. A potential caveat is that source location for alpha oscillations is not sufficiently precise to assign the component we analyzed to specific visual areas. Furthermore, the BOLD response is not fast enough to distinguish the initial stimulus-driven neural response from feedback-related activity (e.g., whether the alpha component reflected activity in areas providing such feedback instead of in V1/V2). Our results show that stimuli presented at the trough of the alpha cycle produce a larger BOLD response compared with those presented at the peak. If alpha oscillations yield cyclic inhibition, then this inhibition appears to occur at the peak for the posterior EEG alpha rhythm. Such a “neuronic shutter” (Nunn and Osselton, 1974) or “rhythmic pulsing” (Mazaheri and Jensen, 2010) could provide a functionally appealing mechanism for clearing and updating of incoming information. In a recent study, we have argued that sustained alertness could rely on such a windshield-wiper effect of large-scale, synchronized alpha activity, which in turn might be orchestrated by central cognitive control regions (Sadaghiani et al., 2010).

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.
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.

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