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DISSERTATION

**A spatiotemporal characterization of the relationship
between ongoing and evoked activity in the human brain**

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Introductory remarks

This summary of the studies conducted within the scope of this thesis will refer to the respective publications as follows:

Study 1: Ritter, P., **Becker, R.**, Graefe, C., Villringer, A., 2007. Evaluating gradient artifact correction of EEG data acquired simultaneously with fMRI. Magn Reson Imaging 25, 923-932.

Study 2: Freyer, F., **Becker, R.**, Anami, K., Curio, G., Villringer, A., Ritter, P., 2009. Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction. Neuroimage 48, 94-108.

Study 3: Becker, R., Ritter, P., Villringer, A., 2008. Influence of ongoing alpha rhythm on the visual evoked potential. Neuroimage 39, 707-716.

Study 4: Ritter, P., **Becker, R.**, 2009. Detecting alpha rhythm phase reset by phase sorting: caveats to consider. Neuroimage 47, 1-4.

Study 5: Reinacher, M., **Becker, R.**, Villringer, A., Ritter, P., 2009. Oscillatory brain states interact with late cognitive components of the somatosensory evoked potential. J Neurosci Methods 183, 49-56.

Study 6: Becker, R., Reinacher, M., Freyer, F., Villringer, A., Ritter, P., 2010. Evidence for interaction between ongoing neuronal oscillations and evoked fMRI activity: linear superposition and beyond. (submitted)

Abstract

The combined use of electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) aims at non-invasively acquiring spatially *and* temporally highly resolved neuronal signatures in the human brain. In this respect, one promising field of EEG-fMRI research concerns the issue whether ongoing activity as reflected by spontaneously fluctuating EEG rhythms such as alpha or mu rhythm may affect evoked responses, or, in other words, whether it influences processing of sensory input. In addition to animal studies, recent EEG and fMRI studies in humans have increasingly challenged the notion of spontaneous, ongoing activity as just being noise by showing that ongoing *EEG* activity covaries with evoked activity in EEG and with fMRI background activity during rest. Furthermore this background (or ongoing) fMRI activity has been demonstrated to delineate functionally connected systems and to be linearly superimposed on the evoked fMRI response. Further, both EEG and fMRI ongoing activity has been demonstrated to be related to behavior.

Ongoing - or intrinsic - activity is thought to reflect top-down processes such as attention, vigilance, motivation or preparedness, which explains why it covaries with evoked responses and behaviour. Concerning EEG, several concepts about the interaction of ongoing and evoked activity exist, ranging from strict independence (i.e. no interaction) to indirect modulations to strong and even causative relationships as realized for example by a phase reset of ongoing rhythms generating evoked potentials (EPs).

In the present thesis, I will demonstrate how ongoing EEG activity interacts with evoked activity in EEG and fMRI. To this end, methodological issues such as the artifacts arising from the combination of these two imaging techniques, namely the ballistocardiogram (BCG) and the MR image acquisition artifact (IAA), will be approached. I will demonstrate the necessary steps for developing a robust EEG-fMRI setup capable of performing online monitoring of ongoing EEG activity and selective triggering of stimulation in the MR environment. Also, I will delineate how integrating different methodological approaches contributes to the resolution of a single question: How do ongoing EEG rhythms and evoked responses relate to each other in terms of their spatio-temporal properties? The methodological approaches comprise 1) empirical EEG studies that investigate how ongoing alpha and mu rhythms relate to EPs, 2) theoretical modelling and comparison of model predictions to real data and 3) multimodal imaging using EEG-fMRI.

Evidence was obtained for an interaction between ongoing and evoked activity with regard to variation of prestimulus alpha- and mu-activity in EEG. A phase reset of ongoing EEG activity as a possible mechanism was discarded, demanding for other mechanisms of interaction which are discussed. Furthermore, results from the EEG-fMRI study supported the theory of a *neuronal* origin of fMRI stimulus response variability. Summarizing, this thesis demonstrates the diverse mechanisms of how large-scale ongoing neuronal activity explains stimulus-response variability in both EEG and fMRI, supporting the concept of a functional role of ongoing activity in the human brain.

Introduction

One of the puzzling findings of neuroscientific research is that neuronal responses to a constant stimulus vary considerably, with single trial fluctuations as high as the actual response itself (Arieli et al., 1995). Instead of being simply noise, this variance has been considered to originate from neuronal activity, which is apparently not explained by the experimental protocol, but is nevertheless present and possibly of functional relevance. Several studies have pioneered in elucidating the role of such spontaneous activity and its tight link to evoked activity (Arieli et al., 1996; Azouz and Gray, 1999; Tsodyks et al., 1999). These exciting observations down to the single-cell level were made invasively, in animals and under anaesthesia. Hence it is not clear whether these results are directly applicable to man. A window into the working intact human brain, in turn, can be offered by non-invasive large scale measuring techniques such as electroencephalography (EEG) or functional magnetic resonance imaging (fMRI). Using these methods, meaningful insights into the role of spontaneous activity in the human brain may be obtained. However, each method suffers either relatively poor temporal (fMRI) or spatial resolution (EEG). Ideally, non-invasive measures would be obtained with both high spatial and temporal resolution.

In EEG, neuronal activity is reflected quite directly since it mainly captures postsynaptic population activity with high temporal resolution. However, EEG is derived from the human scalp as a surface signal and tracing back its exact origin within the brain (called the 'inverse problem') is prone to ambiguity (Helmholtz, 1853) without further appropriate constraints. One of the most prominent patterns of ongoing EEG activity in the human brain is the alpha rhythm, known since the late 1920s (Berger, 1929). Oscillating with a frequency of about 10 Hz and reaching amplitudes of up to 100 µV, it dominates the typical EEG derived from posterior human scalp of healthy relaxed subjects with eyes closed. How the alpha rhythm exactly originates from the cortex and involved subcortical structures is a matter of ongoing debate (Shaw, 2003b). It was known from the beginning of its discovery that it strongly decreases during eyes opening ('Berger blockade') or during visual stimulation (event-related desynchronisation, ERD, Pfurtscheller, 1977). Thus, a tight link appears to exist between the visual system and the alpha rhythm (Shaw, 2003a), sparkling early interest in investigating the relationship between alpha rhythm and the visual EP. In general, there are two major approaches to examine an interaction between a rhythmic signal and for example, evoked activity. An oscillation can be characterized by phase and amplitude, thus both are sensible features to be investigated for their predictive value for evoked activity. In a number of studies it has already been shown, that amplitude as well as the phase of ongoing rhythms may affect processing of sensory input as reflected by EPs and accordingly by behavioural measures involving memory and perception (Barry et al., 2000; Jasiukaitis and Hakerem, 1988; Klimesch et al., 2006; Linkenkaer-Hansen et al., 2004; Makeig et al., 2002; Mathewson et al., 2009; Varela et al., 1981).

The relation of spontaneous, intrinsic activity such as the alpha rhythm to evoked activity is an important topic, since it is relevant for understanding how the brain integrates stimuli from the exterior environment and intrinsic states reflecting attention, vigilance, motivation and preparedness as well as other higher cognitive processes. However there is still a considerable controversy about functional meaning and origins of this rhythm-EP interaction. Different concepts have emerged, but general

consent regarding their validity is still missing. To mention just two popular yet opposing concepts: One assumes, that the ongoing rhythm is undergoing a phase reset during stimulation which generates part of the EP (implying a strong interaction or even causative role of alpha rhythm, see Makeig et al., 2002; Sayers et al., 1974) while the opposing concept suggests independence, i.e. a linear additivity of ongoing and evoked activity (implying no interaction, see Makinen et al., 2005; Mazaheri and Jensen, 2006; Shah et al., 2004). In order to elucidate the functional role of ongoing activity, the disentangling of existing concepts is considered essential (Shah et al., 2004; Yeung et al., 2004).

Another more general question is whether an interaction observed for one type of ongoing activity such as the alpha rhythm also holds for other rhythms. For example, the central mu rhythm, exhibits a similar frequency as the posterior alpha rhythm, but is closely related to the sensorimotor system desynchronizing during sensorimotor activity (Pfurtscheller and Aranibar, 1979). It also exhibits fMRI correlates in sensorimotor areas during rest as well as during motor tasks (Ritter et al., 2009b). It is well conceivable yet not proven that this rhythm may be characterized by similar types of interaction as the posterior alpha rhythm.

Coming back to the non-invasive neuroimaging techniques at our disposal, another highly popular technique beside EEG is fMRI. In contrast to EEG, here, neuronal activity is reflected in a more indirect way: When neuronal activity increases, e.g. due to an experimental event, local oxygen consumption as well as local cerebral blood flow and volume increase. Since the increase in blood flow exceeds the increase in oxygen consumption, a decrease in local deoxygenated hemoglobin (deoxy-hemoglobin) concentration is observed. Deoxy-hemoglobin has magnetic properties measurable with optimized MR sequences (e.g. blood-oxygen-level dependent (BOLD) sequences) allowing to estimate the underlying neuronal activity.

Also fMRI research is increasingly contributing insights into the functional importance of ongoing activity. Using fMRI it has been shown that during resting-state measurements in human subjects, meaningful patterns of BOLD activity can be revealed which delineate neuro-anatomically and functionally connected networks (e.g. motor system networks, Biswal et al., 1995). Furthermore, ongoing fMRI activity accounted for a remarkable amount of variability of single-trial responses in BOLD motor activity, implying a linear superposition of ongoing and evoked activity in fMRI (Fox et al., 2006). Notably, this superimposed ongoing activity has been demonstrated to be relevant for behaviour (Fox et al., 2007). Thus, the assumption that the origin of such ongoing fMRI activity is neuronal, is reasonable (Birn, 2007), but has not been proven so far.

Postsynaptic activity, as measured by local field potentials (LFPs, being closely related to the surface EEG) is tightly linked to activity measured by BOLD-fMRI (Logothetis et al., 2001). This was a promising result further encouraging attempts to combine the complementary techniques of EEG and fMRI. The attractiveness of combining EEG and fMRI partly stems from the idea to finally obtain a 'fused' non-invase signal offering both high temporal *and* spatial resolution. Apart from studies on epileptic activity, studies on the alpha rhythm were among the very first employing simultaneous EEG-fMRI to exploit the advantage of EEG to monitor spontaneous neuronal oscillations (Becker et al., 2009). Most of these studies found that the fMRI signal in posterior parts of the cortex correlated negatively with the amplitude of the alpha rhythm, i.e. the more pronounced the alpha rhythm was, the

more the BOLD signal decreased in the visual cortex, indicating a neuronal activity decrease (deactivation) (Becker et al., 2009; de Munck et al., 2007; Feige et al., 2005; Goldman et al., 2002; Moosmann et al., 2003), but see also (Laufs et al., 2003). These findings are in accordance with the - certainly simplifying - view of the alpha rhythm as representing an "idling" of the visual cortex reflected by down-regulation of metabolic activity observable with fMRI. How this is exactly realized, whether by an 'active inhibition' of these areas via thalamo-cortical loops or by another mechanism is uncertain. Based on previous results, an interesting follow-up experiment would expand on this desing performing stimulation during periods of pronounced ongoing alpha activity which then allows determining whether the effect on responses during stimulation is comparable to the effect observed during resting-state measurements.

Thus, with combined EEG-fMRI, questions can be answered not previously addressable with either technique alone. However, there is a number of obstacles for conducting combined EEG-fMRI studies: The MR environment is inherently hostile to EEG acquisition, since it heavily corrupts EEG data during MR gradient switching inducing currents of several mV. Physiological EEG typically reaches 100 μV at most and thus is completely obscured by this image acquisition artifact (IAA) (Ritter et al., 2009a). Fortunately, due to the artifact's technical nature, approaches are available to estimate a typical IAA pattern which subsequently is subtracted from the data (template subtraction, e.g. see Becker et al., 2005). Another MR related artifact is the ballistocardiogram (BCG), which distorts the EEG signal already in the static magnetic field. It is caused by heart-beat related pulsatile blood flow changes and subsequent movements of the skull and mainly affects lower EEG frequencies. This physiological signal is varying thus cannot be simply removed by template subtraction, however approaches such as independent component analysis (ICA) can strongly reduce this artifact, especially in MR environments with field strength of about 1.5 Tesla (Debener et al., 2008). Both types of MR related artifacts challenge proper detection of spontaneous activity such as the fluctuating alpha rhythm, which in contrast to conventional evoked potentials is - per definitionem - an unaveraged measure of neuronal activity.

Ideally, the mentioned artifacts are even removed online, i.e. during the course of the experiment, allowing a setup where, for example, stimulation is performed depending on the amplitude of the ongoing rhythm. This would render the experimental design more efficient, since instead of a stimulating over a continuum of pre-stimulus alpha amplitude niveaus, sensory input would only be triggered by defined states of ongoing activity.

Aims

In the present thesis, the general aim is to link findings from EEG and fMRI studies synthesizing their insights to develop subsequent EEG and EEG-fMRI studies to further decode the functional role of neural ongoing activity in the human brain.

To this end, **first**, an existing EEG-fMRI setup was advanced to minimize the still challenging artifacts typically present in EEG-fMRI (**study 1** and **study 2**). **Study 1** was centred on evaluating different artifact removal algorithms in terms of resulting data quality with the aim to identify the optimal removal approach. Apart from optimizing the software approach, **study 2** further extends the scope of advancements to the hardware-side of conducting EEG-fMRI studies. This setup was tested and its limits were explored by the attempt to recover such subtle high-frequency activity as physiological high-frequency bursts (HFBs) in the 600Hz range from EEG during MR acquisition.

A **second** aim was to disentangle discussed concepts of interaction between spontaneous and evoked activity by theoretically modeling and empirically analyzing the interaction between the ongoing alpha rhythm and the visual EP. Specifically the role of either amplitude or phase of ongoing alpha rhythm for the different types of interaction with the visual EP was examined (**study 3** and **study 4**). Similarities and differences between ongoing alpha and mu activity with respect to their impact on evoked responses and implications regarding the possible mechanisms of interaction were examined (**study 5**).

Third, the feasibility of online amplitude detection of spontaneous rhythms during EEG (**study 5**, mu rhythm) and during EEG-fMRI (**study 6**, alpha rhythm) was studied to finally master the methodological challenges associated with performing rhythm triggered stimulation in the context of simultaneous EEG-fMRI.

Finally, in **study 6**, the major aim was to link findings from previous EEG and fMRI studies on intrinsic activity and its functional role as well as from resting-state EEG-fMRI studies by examining the interaction between the online monitored spontaneous alpha rhythm and visual EPs in simultaneous EEG-fMRI. The goal was to investigate the impact of the ongoing EEG activity on BOLD fMRI evoked responses. More specifically, it was examined whether variation of evoked fMRI responses to stimuli can be accounted for by a linear superposition of a fixed evoked fMRI response and fMRI-baseline modulations dependent on fluctuations of the ongoing EEG alpha rhythm.

Methods

Here, a short overview is provided about the methods used for the studies within the scope of this thesis. For a detailed description, the reader is referred to the method section of each respective article as cited subsequently. In general, **study 1** (Ritter et al., 2007) and **study 2** (Freyer et al., 2009) deal with methodological issues arising from the combination of EEG-fMRI, while **study 3** to **study 6**, via empirical and theoretical research, deal with the question of interaction between spontaneous rhythms and evoked activity. Of these publications, **study 3** (Becker et al., 2008b), **study 5** (Reinacher et al., 2009), and **study 6** (Becker, 2010) are based on empirical data, while **study 4** (Ritter and Becker, 2009) reflects model-theoretical issues and is based on synthetic data.

Subjects and experimental design

Healthy subjects participated throughout all empirical studies. Prior to investigation, subjects gave written consent according to the declaration of Helsinki. Studies were approved by the local ethics committee.

In **study 1** and **2**: Subjects were lying relaxed in a supine position in the bore of the MR scanner and were instructed to pay attention to the stimulus and remain awake. Visual checkerboard (**study 1**) or somatosensory median nerve stimulation (**study 2**) was performed both during MR scan periods and during non-scan periods to enable a systematic comparison of evoked responses with and without MR artifacts.

In **study 3**, subjects had to passively view two kinds of stimuli and to remain awake while lying relaxed. One stimulus, a black-and-white circular checkerboard was perceived with eyes open, while the other, a white uniform flash stimulus was delivered during eyes closed. **Study 5** and **6** had an 'oddball' design to supervise and maintain vigilance, i.e. subjects had to detect the 'oddball' stimulus out of standard stimuli (probability: 20 percent) and react accordingly. Response times were recorded. In **study 5**, subjects took part in a somatosensory oddball experiment with vibrotactile stimuli. The oddball was defined by a different frequency than the standard. In **study 6**, subjects were participating in a visual oddball experiment. The standard stimulus was a circular-checkerboard pattern shown for 900 ms. The oddball was a pattern inversion of the standard stimulus after 500 ms. In both **study 5** and **study 6**, we recorded two types of runs: An alpha- or mu-rhythm triggered run type, respectively, and a control run type with stimulation independent of an ongoing rhythm.

Data acquisition

Electroencephalography. EEG was recorded in all empirical studies using a 32-channel MR-compatible EEG amplifier (BrainAmp MR Plus, Brain Products, Munich, Germany) and an MR-compatible EEG-cap (Easy cap, FMS, Herrsching-Breitbrunn, Germany), comprising ring-type sintered silver chloride electrodes with iron-free copper leads. Scalp electrodes were arranged according to the International 10-20 System with the reference located at electrode position FCz. In addition, electrooculogram and electrocardiogram activity were recorded. In all studies except **study 5**, one amplifier and corresponding EEG caps were used. **Study 5** used a 64-channel setup, requiring a

modified EEG-cap and two amplifiers. At a resolution of 0.5 µV the resulting dynamic recording range of ±16.384 mV allows capturing both low-amplitude EEG signals and large image acquisition artifacts (essential for successful removal). Sampling rate was 5 kHz. The subject's head was immobilized with a vacuum cushion to minimize movement-related EEG artifacts.

In both 'online' studies, **study 5** and **study 6**, we used an approach of "functional calibration" (previously employed in Ritter et al., 2009b) to spatially define a filter optimally extracting the spontaneous rhythm which increases signal-to-noise ratio of the signal and reduces unrelated activity in the signal such as the BCG during EEG-fMRI. This required a pre-experiment, where the spontaneous rhythm was deliberately manipulated. Using a variant of independent component analysis (ICA) we then identified the independent component best reflecting the experimentally induced modulations of the rhythm and thus, the putative source of the rhythm itself. Individually for each subject, this filter was then transferred to the main experiment for further use. An online approach for detection of ongoing activity was employed performing a real-time short term Fast Fourier Transformation (FFT) in the alpha-band and triggering stimulation when an adaptive threshold was exceeded. It allows sparse stimulation during periods of pronounced ongoing alpha rhythm helping to reduce the number of trials needed and to create an efficient design comprising only control trials and trials with strong ongoing alpha rhythm. The efficiency of an experimental design is of high relevance when long inter-stimulus intervals are required (e.g. for inert signals such as BOLD).

Functional magnetic resonance imaging. **Study 1**, **study 2** and **study 6** were combined EEG-fMRI studies. A 1.5T scanner was used (Siemens Vision, Erlangen, Germany) for fMRI. In **study 1**, a conventional T2*-weighted BOLD sensitive echo planar imaging (EPI) sequence was used. In **study 2** and **study 6**, an EEG-fMRI optimized T2*-weighted EPI-BOLD sequence ('stepping stone', Anami et al., 2003) was used which was developed for minimization of IAAs during EEG–fMRI acquisition. Additionally, the EEG clock was synchronized to the MR device clock, required for invariant sampling of the IAA, which further optimizes correction of MR gradient artifacts (Ritter et al., 2009a).

Data analysis and modeling

Electroencephalography. Depending on the specific experimental design and subsequent analysis, EEG was band-pass filtered to remove slow drifts and high-frequency noise. In the artifact evaluation studies (**study 1** and **study 2**) no low-pass filter was applied to allow a thorough evaluation of residual high-frequency noise after MR artifact removal. In general, EEG data analysis and simulation was carried out using MATLAB (The Mathworks Inc., Natick, MA, USA) and the open-source MATLAB toolbox EEGLAB (Delorme and Makeig, 2004). Data were segmented and averaged for analysis of EPs. Also, by performing wavelet-based analysis, time-frequency behaviour was examined. In **study 3**, the alpha-rhythm amplitude based sorting of EPs was done posthoc and offline. In **study 5** and **study 6**, alpha amplitude was classified online, using a custom plug-in and a predefined spatial filter from a pre-experiment to extract the spontaneous rhythm on-line and, relevant for **study 6**, to reduce BCG during EEG-fMRI.

Artifact studies. In **study 1** and **study 2**, after correction of MR artifacts, EPs of periods with MR acquisition were averaged and compared to EPs in periods without MR acquisition. Also, EEG

band-activity was compared. This allowed evaluation of MR artifact removal performance: Differences between acquisition and non-acquisition periods indicate suboptimal artifact removal. To estimate whether physiological signal was removed, EEG data from non-acquisition periods *before* correction were compared to EEG data from non-acquisition periods *after* correction. In **study 1**, different MR removal approaches - including one in-house solution (used in Becker et al., 2005) - were compared for their performance by comparing visual EPs from MR acquisition and non-acquisition periods. The approaches differed with respect to their use of interpolation of artifact periods (allowing better alignment) and use of basis sets to better approximate artifacts before subtraction. In principle, all algorithms for removal were some variant of template artifact subtraction. That is, a number of consecutive MR acquisition periods is averaged to build a representative template which in turn is subtracted from following MR acquisition periods (Allen et al., 2000). In **study 2**, it was tested whether an optimized EEG-fMRI setup and analysis approach is capable of revealing nanovolt-sized somatosensory evoked high-frequency activity in the 600-Hz range (HFBs) during MR acquisition periods. To this end, we used elaborated algorithms such as a cascaded principal components analysis (PCA) to further refine MR artifact removal by template artifact subtraction.

Interaction studies. Simulations in **study 3** and **4** were performed using MATLAB (The Mathworks Inc., Natick, MA, USA). In **study 3**, two different theoretical models for the interaction between rhythm and EP were created and compared regarding their differential predictions for sorting EPs according to prestimulus alpha rhythm amplitude. In model one, the EEG alpha rhythm directly underwent a phase reset generating the EP ('oscillatory model'). In the other model, alpha rhythm and EP were completely independent of each other ('additive model'). Each model made unique predictions regarding the effect of pre-stimulus alpha-dependent trial sorting. Analyzed parameters comprised EP amplitudes, alpha-band time-frequency behaviour and phase-locking. Having obtained these predictions, they were compared to the acquired empirical EEG data with the aim to identify the prevailing mechanism of interaction between ongoing and evoked activity.

In contrast to alpha rhythm amplitude sorting, in **study 4** an approach to investigate the influence of the alpha-rhythm *phase* on the EP was examined. Based on previous analyses from empirical data (Becker et al., 2008a), where no phase-EP interaction was found, we conducted a theoretical investigation of previous approaches from other groups (Kruglikov and Schiff, 2003; Risner et al., 2009) for possible differences to our approach. We performed simulations using their analysis strategy.

In **study 5**, the impact of ongoing mu rhythm on the somatosensory EP was examined. We used an approach to evaluate the amplitude of the ongoing mu rhythm on-line. We then compared somatosensory EPs of two groups: trials with periods of high prestimulus mu activity and trials with random prestimulus mu activity. We also compared topographies of the ongoing mu rhythm vs. topography of observed interactions.

Finally, in **study 6** we examined (similarly to **study 3**) the influence of ongoing alpha rhythm amplitude on visual evoked responses in EEG and fMRI. In contrast to **study 4**, this was done with EEG-fMRI and on-line alpha-dependent stimulation (as in **study 5**) instead of posthoc sorting, so we were able to examine possible effects on the evoked BOLD response as well.

Functional magnetic resonance imaging. In **study 6**, fMRI data pre-processing and analysis was performed using the statistical software package SPM5 (www.fil.ion.ucl.ac.uk/spm) and the freely available SPM toolbox MARSBAR (Brett, 2002). In order to find out whether previously reported alpha-dependent modulations of the fMRI baseline imply and account for alpha-dependent modulations of evoked fMRI responses, we analyzed evoked BOLD responses of all experimental conditions: (I) The 'high-alpha state stimulation condition' implied that stimuli were shown during high prestimulus alpha amplitude. (II) In the 'state-independent stimulation condition' stimuli were shown independent of alpha amplitude, yet with stimulus timings as recorded from condition I. (III) In the 'high-alpha state inter-stimulus condition' states of high-alpha amplitude (comparable to those of condition I) were identified within condition II in periods that lacked visual stimulation (i.e. in inter-stimulus periods). A design matrix was built modeling the expected hemodynamic responses to the respective experimental conditions. By correlating the observed fMRI activity voxelwise with these models, identification of involved areas was possible. Then contrasts were computed, yielding alpha-dependent effects during and between stimulation. Finally, using MARSBAR, we compared the amplitudes of these alpha-dependent responses (i.e. of stimulus response modulation vs. baseline modulation) in all identified regions. This allowed to determine whether there were regions showing linear superposition or other types of interaction to be found.

Results

Artifact studies. Study 1: Performance of the discussed artifact correction approaches differed depending on the respective frequency band. In frequencies below gamma, differences were negligible. There, interpolation was not necessary, rendering a fast and computationally undemanding removal of gradient artifacts by simple and fast template subtraction possible. For higher frequency bands, interpolation was highly beneficial for resulting EEG quality, but also computationally more demanding. Regarding preservation of the physiological signal of interest after artifact correction, the conventional template subtraction approach was shown to be unproblematic. Other more complex approaches were shown to be potentially prone to overfitting, removing physiological signal from the EEG. Our own algorithm, based on interpolation and template artifact subtraction (ITAS), performed well in all frequency bands and with respect to both artifact reduction and biosignal preservation.

Study 2: Here, it was demonstrated that using EEG-fMRI synchronization in combination with refined artifact removal approaches allows to even recover EEG activity up to ultrahigh-frequencies (600 Hz) from MR scan periods. Notably, this was achieved without use of interpolation because gradient artifacts were invariantly sampled due to EEG-fMRI synchronization on the hardware side, making further software-based alignments of artifact periods unnecessary. Minor high-frequency artifact residuals arose from sources such as slight subject movements and were successfully removed by using a similarity measure to optimize the inclusion process of artifact periods into the template and by using a cascaded PCA approach. These software optimizations, however, implied an increase in computational demand in comparison to simple template subtraction.

Interaction studies. Study 3: By examining effects of prestimulus alpha amplitude on poststimulus activity, both theoretical models (additive vs. oscillatory model) made unique predictions concerning resulting EP amplitude, time-frequency and phase-locking behaviour in the EEG alpha band. Subsequently, in our empirical data, early EP components corresponded to the 'additive model'. However, we also found an alpha-dependent up-modulation of a late evoked component (around 250 ms) not expected in either theoretical model. Importantly, this effect is incompatible with the phase reset model, since it occurred during already strongly decreased alpha rhythm event-related desynchronisation (ERD) and its frequency was below the alpha band.

Study 4: Complementary to the amplitude-sorting approach in **study 3**, **study 4** revealed issues of phase-sorting associated with the approach used in previous studies (Kruglikov and Schiff, 2003; Risner et al., 2009). The consequence of subtracting so-called resting-state trials from actual stimulation trials sharing the same prestimulus alpha-phase - prima facie correct and elegant – was that it induced errors into the analysis. These errors are caused by the lack of event-related desynchronisation of the alpha rhythm in the resting-state trials. As a result, this subtraction approach potentially leads to erroneously mimicked phase effects on the EP.

Study 5: The feasibility of using an online approach for mu-rhythm detection and subsequent stimulus triggering was demonstrated. Analogously to **study 3**, despite differences in sensory modality (visual vs. somatosensory) and amplitude sorting approach (offline and posthoc versus online sorting), a similar up-modulation of late evoked components for the (somatosensory) EP was observed. Notably, here, the site of the maximum EP modulatory effect exhibited a central origin while the site of induced mu-rhythm increase was located contralaterally to the stimulation and (as expected) in proximity of sensorimotor cortical areas.

Study 6: Similar to EEG results of **study 3**, we found an up-modulating effect of high prestimulus alpha power on the EEG-EP in the late visual evoked component. Concerning the BOLD-fMRI data, we observed alpha-dependent significant decreases in posterior, thalamic and cerebellar areas. Notably, in visual areas, where alpha-dependent baseline responses were observed, these decreases explained the observed alpha-dependent decrease of the fMRI stimulus response. This implied a linear superposition of ongoing and evoked fMRI activity. These areas were comparable in location to those reported in previous resting-state EEG-fMRI studies (Feige et al., 2005; Goldman et al., 2002; Moosmann et al., 2003). In addition to these areas with linear superposition, thalamic and cerebellar regions as well as smaller areas of occipital cortex and precuneus exhibited an alpha-dependent decrease of the evoked BOLD response only, i.e. no corresponding decrease in the alpha-dependent baseline fMRI signal.

Discussion

From **study 1** and **study 2** it was concluded, that a specific scientific question might necessitate specific removal strategies for MR related IAAs. For example, with the experimental paradigm of **study 6** in mind, the computational load of the interpolation or the cascaded PCA approach rendered both infeasible for being used in a scenario which required real-time correction of MR artifacts and subsequent real-time alpha-rhythm evaluation. However, the integration of hardware-

side optimizations from **study 2**, i.e. use of an EEG-optimized MR sequence and of EEG-fMRI synchronization was critically useful. Only due to the thereby achieved invariant sampling of artifacts, a simple and computationally undemanding template subtraction method could be employed subsequently with success. This in turn allowed proper online detection of periods of high-alpha activity in **study 6** and thus, a sparse and efficient (i.e. alpha-dependent) rather than random stimulation. The feasibility to perform online monitored rhythm-related stimulation in the first place was demonstrated in **study 5**.

Concerning the insights from the EEG interaction study (**study 3**) and from other studies (Barry et al., 2000; Jasiukaitis and Hakerem, 1988; Polich, 1997) it was concluded that there seems to be a robust amplitude-amplitude interaction between alpha-rhythm and the late visual EP. Additionally, as observed in **study 5**, it was demonstrated that this late modulation is not only a feature of the relation between posterior alpha rhythm and the visual EP, but it also characterizes the relation between the central mu rhythm and somatosensory EP. This may point to a universal principle of how spontaneous rhythms interact with evoked activity, being valid across different sensory modalities. However, this concept needs further investigation and confirmation, since in **study 5** the observed central location of the rhythm-related EP component was distinct from the location where the rhythm was modulated most strongly. This finding needs to be reconciled with the idea of the modulation being generated by desynchronisation of the rhythm itself.

Concerning phase - EP interactions, we demonstrated by theoretical analysis possible pitfalls of previous approaches without denying the general value of such phase-EP investigations. At the time of writing, the author is not aware of a published approach which successfully integrated the desychronization of ongoing rhythms into analysis of phase-dependency of EEG-EPs. First steps were made with an approach based on short term FFTs and phase shuffling (Becker et al., 2008a).

With regard to the observed and highly reproducible impact of alpha rhythm amplitude on the EP, the temporal and spectral properties of the found up-modulation of late components seem to be incompatible with a causative role of the ongoing rhythm for EP generation via a phase reset (see the detailed discussion in Becker et al., 2008b). Rather, empirical and theoretical studies have convincingly offered an alternative, namely that such a type of interaction may originate from a specific property of the ongoing alpha rhythm which is its asymmetry (Mazaheri and Jensen, 2008; Nikulin et al., 2007). This property can readily be seen in the ongoing alpha rhythm with EEG-DC recording. It means that depending on alpha rhythm strength, the mean baseline of the alpha oscillation changes. This effect is even more emphasized in the course of any event-related desynchronisation. Then, depending on prestimulus alpha amplitude, the asymmetry of the rhythm effectively leads to systematic modulation of single-trial stimulus responses in the EEG. Such a resulting modulation of EPs which is cogently correlated with stronger prestimulus alpha (or mu) rhythm, may provide an explanation for the observed effects in the EEG - EPs of study 3, 5 and 6. However, in study 5 we observed a spatial separability of somatosensory mu rhythm (located above somatosensory cortex contralteral to stimulation) and the EP effect (located at central to fronto-central sites). This obviously leaves open the question, whether and how the asymmetry property of the spontaneous rhythm is capable of modulating evoked components at spatially distinct sites. Further empirical and theoretical

studies may be needed to clarify this issue and to possibly extend this concept to reconcile it with such empirical findings.

Results from study 6 imply a linear superposition of ongoing and evoked activity as previously demonstrated in fMRI studies (Fox et al., 2006) and studies in animals (Arieli et al., 1996). Most importantly, our data link such spontaneous fMRI activity - being superimposed on a fixed evoked fMRI response - to neuronal ongoing activity as it is reflected in EEG ongoing alpha rhythm. Beyond this mechanism of linear superposition observed in visual areas (BA 18 / 19) it is noteworthy that in additional regions, i.e. thalamic, cerebellar, occipital and precuneal regions, a distinct mechanism was observed involving an alpha-dependent effect during stimulation which was not observable in-between stimulus trials. How can we explain this finding? In contrast to the areas showing linear superposition, in these areas apparently differential effects are observed depending on whether a stimulus is following or not reflecting the possibility that 'spontaneous fluctuations seem to be regulated differently than task or stimulus-evoked brain responses' (Fox and Raichle, 2007). In these areas such a finding - that the impact of ongoing EEG activity is only visible during stimulation and not during rest - may indicate regulation of (neuronal) excitability of stimulus responses.

As an outlook, there are numerous ways how to further expand the approaches presented in this thesis. Certainly, integrating behaviour or other cognitive processes (e.g. memory, perception) into EEG-fMRI studies on ongoing activity is a logical and important next step. Furthermore, also a closer look at how exactly an interaction between ongoing and evoked EEG activity finally translates into fMRI modulations is highly relevant and interesting. Another important issue is the identification of the causal relationship between ongoing and evoked signatures. Just observing a covariation of two parameters naturally does not allow any inference on the causative role of either parameter. More complex approaches such as dynamic causal modeling (DCM, Friston et al., 2003) may contribute to answer this question.

Concluding, it can be said, that the investigations conducted within the scope of the present thesis using EEG and EEG-fMRI - complementing different aspects of neuronal activity - were instructive regarding the relationship between spontaneous and evoked activity in the healthy human brain. The rich repertoire of mechanisms of interaction between ongoing and evoked activity has been explored, both with new empirical and theoretical approaches. Also methodological issues for proper investigation of such interactions were identified ranging from artifact issues arising from the use of EEG in the MR environment to issues regarding the appropriate modeling of assumptions concerning interaction between ongoing and evoked activity. Finally, the present thesis contributes one more piece to the big puzzle which has to be solved – that is the identification of the functional meaning and the mechanisms underlying the interaction between ongoing and evoked neuronal activity.

References

Allen, P.J., Josephs, O., Turner, R., 2000. A method for removing imaging artifact from continuous EEG recorded during functional MRI. Neuroimage 12, 230-239.

Anami, K., Mori, T., Tanaka, F., Kawagoe, Y., Okamoto, J., Yarita, M., Ohnishi, T., Yumoto, M., Matsuda, H., Saitoh, O., 2003. Stepping stone sampling for retrieving artifact-free electroencephalogram during functional magnetic resonance imaging. Neuroimage 19, 281-295.

Arieli, A., Shoham, D., Hildesheim, R., Grinvald, A., 1995. Coherent spatiotemporal patterns of ongoing activity revealed by real-time optical imaging coupled with single-unit recording in the cat visual cortex. J Neurophysiol 73, 2072-2093.

Arieli, A., Sterkin, A., Grinvald, A., Aertsen, A., 1996. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. Science 273, 1868-1871.

Azouz, R., Gray, C.M., 1999. Cellular mechanisms contributing to response variability of cortical neurons in vivo. J Neurosci 19, 2209-2223.

Barry, R.J., Kirkaikul, S., Hodder, D., 2000. EEG alpha activity and the ERP to target stimuli in an auditory oddball paradigm. Int J Psychophysiol 39, 39-50.

Becker, R., Reinacher, M., Freyer, F., Villringer, A., Ritter, P., 2010. Evidence for interaction between ongoing neuronal oscillations and evoked fMRI activity: linear superposition and beyond (submitted).

Becker, R., Ritter, P., Moosmann, M., Villringer, A., 2005. Visual evoked potentials recovered from fMRI scan periods. Hum Brain Mapp 26, 221-230.

Becker, R., Ritter, P., Schmidt, R., Kempter, R., Villringer, A., 2008a. The Phase of Ongoing Alpha Activity and its Relation to the Visual Evoked Potential (conference poster). 14th Annual Meeting of Human Brain Mapping, Melbourne.

Becker, R., Ritter, P., Villringer, A., 2008b. Influence of ongoing alpha rhythm on the visual evoked potential. Neuroimage 39, 707-716.

Becker, R., Ritter, P., Villringer, A., 2009. Visual System. In: Mulert, C., Lemieux, L. (Eds.), EEG-fMRI - Physiological Basis, Technique, and Applications. Springer, New York.

Berger, H., 1929. Über das Elektrenkephalogramm des Menschen. Archiv Für Psychiatrie Und Nervenkrankheiten 87, 527-570.

Birn, R.M., 2007. The behavioral significance of spontaneous fluctuations in brain activity. Neuron 56, 8-9.

Biswal, B., Yetkin, F.Z., Haughton, V.M., Hyde, J.S., 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 34, 537-541.

Brett, M., Anton, J. L., Valabregue, R., Poline, J., 2002. Region of interest analysis using an SPM toolbox. Neuroimage 16, S373.

de Munck, J.C., Goncalves, S.I., Huijboom, L., Kuijer, J.P., Pouwels, P.J., Heethaar, R.M., Lopes da Silva, F.H., 2007. The hemodynamic response of the alpha rhythm: an EEG/fMRI study. Neuroimage 35, 1142-1151.

Debener, S., Mullinger, K.J., Niazy, R.K., Bowtell, R.W., 2008. Properties of the ballistocardiogram artefact as revealed by EEG recordings at 1.5, 3 and 7 T static magnetic field strength. Int J Psychophysiol 67, 189-199.

Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134, 9-21.

Feige, B., Scheffler, K., Esposito, F., Di Salle, F., Hennig, J., Seifritz, E., 2005. Cortical and subcortical correlates of electroencephalographic alpha rhythm modulation. J Neurophysiol 93, 2864-2872.

Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci 8, 700-711.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Raichle, M.E., 2007. Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. Neuron 56, 171-184.

Fox, M.D., Snyder, A.Z., Zacks, J.M., Raichle, M.E., 2006. Coherent spontaneous activity accounts for trial-to-trial variability in human evoked brain responses. Nat Neurosci 9, 23-25.

Freyer, F., Becker, R., Anami, K., Curio, G., Villringer, A., Ritter, P., 2009. Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction. Neuroimage 48, 94-108.

Friston, K.J., Harrison, L., Penny, W., 2003. Dynamic causal modelling. Neuroimage 19, 1273-1302.

Goldman, R.I., Stern, J.M., Engel, J., Jr., Cohen, M.S., 2002. Simultaneous EEG and fMRI of the alpha rhythm. Neuroreport 13, 2487-2492.

Helmholtz, H.v., 1853. Ueber einige Gesetze der Vertheilung elektrischer Ströme in körperlichen Leitern mit Anwendung auf die thierisch-elektrischen Versuche. Annalen der Physik 89, 211-233, 353-377.

Jasiukaitis, P., Hakerem, G., 1988. The effect of prestimulus alpha activity on the P300. Psychophysiology 25, 157-165.

Klimesch, W., Doppelmayr, M., Hanslmayr, S., 2006. Upper alpha ERD and absolute power: their meaning for memory performance. Prog Brain Res 159, 151-165.

Kruglikov, S.Y., Schiff, S.J., 2003. Interplay of electroencephalogram phase and auditory-evoked neural activity. J Neurosci 23, 10122-10127.

Laufs, H., Kleinschmidt, A., Beyerle, A., Eger, E., Salek-Haddadi, A., Preibisch, C., Krakow, K., 2003. EEG-correlated fMRI of human alpha activity. Neuroimage 19, 1463-1476.

Linkenkaer-Hansen, K., Nikulin, V.V., Palva, S., Ilmoniemi, R.J., Palva, J.M., 2004. Prestimulus oscillations enhance psychophysical performance in humans. J Neurosci 24, 10186-10190.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A., 2001. Neurophysiological investigation of the basis of the fMRI signal. Nature 412, 150-157.

Makeig, S., Westerfield, M., Jung, T.P., Enghoff, S., Townsend, J., Courchesne, E., Sejnowski, T.J., 2002. Dynamic brain sources of visual evoked responses. Science 295, 690-694.

Makinen, V., Tiitinen, H., May, P., 2005. Auditory event-related responses are generated independently of ongoing brain activity. Neuroimage 24, 961-968.

Mathewson, K.E., Gratton, G., Fabiani, M., Beck, D.M., Ro, T., 2009. To see or not to see: prestimulus alpha phase predicts visual awareness. J Neurosci 29, 2725-2732.

Mazaheri, A., Jensen, O., 2006. Posterior alpha activity is not phase-reset by visual stimuli. Proc Natl Acad Sci U S A 103, 2948-2952.

Mazaheri, A., Jensen, O., 2008. Asymmetric amplitude modulations of brain oscillations generate slow evoked responses. J Neurosci 28, 7781-7787.

Moosmann, M., Ritter, P., Krastel, I., Brink, A., Thees, S., Blankenburg, F., Taskin, B., Obrig, H., Villringer, A., 2003. Correlates of alpha rhythm in functional magnetic resonance imaging and near infrared spectroscopy. Neuroimage 20, 145-158.

Nikulin, V.V., Linkenkaer-Hansen, K., Nolte, G., Lemm, S., Muller, K.R., Ilmoniemi, R.J., Curio, G., 2007. A novel mechanism for evoked responses in the human brain. Eur J Neurosci 25, 3146-3154.

Pfurtscheller, G., 1977. [Spontaneous and evoked EEG-activity during one-second light-stimulation (author's transl)]. EEG EMG Z Elektroenzephalogr Elektromyogr Verwandte Geb 8, 145-154.

Pfurtscheller, G., Aranibar, A., 1979. Evaluation of event-related desynchronization (ERD) preceding and following voluntary self-paced movement. Electroencephalogr Clin Neurophysiol 46, 138-146.

Polich, J., 1997. On the relationship between EEG and P300: individual differences, aging, and ultradian rhythms. Int J Psychophysiol 26, 299-317.

Reinacher, M., Becker, R., Villringer, A., Ritter, P., 2009. Oscillatory brain states interact with late cognitive components of the somatosensory evoked potential. J Neurosci Methods 183, 49-56.

Risner, M.L., Aura, C.J., Black, J.E., Gawne, T.J., 2009. The Visual Evoked Potential is independent of surface alpha rhythm phase. Neuroimage 45, 463-469.

Ritter, P., Becker, R., 2009. Detecting alpha rhythm phase reset by phase sorting: caveats to consider. Neuroimage 47, 1-4.

Ritter, P., Becker, R., Freyer, F., Villringer, A., 2009a. Correction of image acquisition artefacts. In: Mulert, C., Lemieux, L. (Eds.), EEG-fMRI - Physiological Basis, Technique, and Applications. Springer, New York.

Ritter, P., Becker, R., Graefe, C., Villringer, A., 2007. Evaluating gradient artifact correction of EEG data acquired simultaneously with fMRI. Magn Reson Imaging 25, 923-932.

Ritter, P., Moosmann, M., Villringer, A., 2009b. Rolandic alpha and beta EEG rhythms' strengths are inversely related to fMRI-BOLD signal in primary somatosensory and motor cortex. Hum Brain Mapp 30, 1168-1187.

Sayers, B.M., Beagley, H.A., Henshall, W.R., 1974. The mechanism of auditory evoked EEG responses. Nature 247, 481-483.

Shah, A.S., Bressler, S.L., Knuth, K.H., Ding, M., Mehta, A.D., Ulbert, I., Schroeder, C.E., 2004. Neural dynamics and the fundamental mechanisms of event-related brain potentials. Cereb Cortex 14, 476-483.

Shaw, J.C., 2003a. Alpha in Vision and Imagery. The brain's alpha rhythms and the mind. Elsevier, Amsterdam, pp. 33-53.

Shaw, J.C., 2003b. EEG generation II: modern studies and models. The brain's alpha rhythms and the mind. Elsevier, Amsterdam, pp. 245-260.

Tsodyks, M., Kenet, T., Grinvald, A., Arieli, A., 1999. Linking spontaneous activity of single cortical neurons and the underlying functional architecture. Science 286, 1943-1946.

Varela, F.J., Toro, A., John, E.R., Schwartz, E.L., 1981. Perceptual framing and cortical alpha rhythm. Neuropsychologia 19, 675-686.

Yeung, N., Bogacz, R., Holroyd, C.B., Cohen, J.D., 2004. Detection of synchronized oscillations in the electroencephalogram: an evaluation of methods. Psychophysiology 41, 822-832.

Declaration of own contribution to selected publications

The contribution of the doctoral candidate Robert Becker to the here submitted publications presents as follows:

Study 1:
Ritter, P., **Becker, R.**, Graefe, C., Villringer, A., 2007. Evaluating gradient artifact correction of EEG data acquired simultaneously with fMRI. Magn Reson Imaging 25, 923-932.

Contribution in percent: 30.
Detailed contribution: Programming of artifact removal algorithm used for comparison against other methods. Participation in planning of the analysis approach. Participation in conducting the experiments. Participation in writing the manuscript and in the review process.

Study 2:
Freyer, F., **Becker, R.**, Anami, K., Curio, G., Villringer, A., Ritter, P., 2009. Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction. Neuroimage 48, 94-108.

Contribution in percent: 30.
Detailed contribution: Participation in planning the study. Participation in analysis of data, providing removal algorithms and concepts for extension of algorithms. Participation in writing the manuscript and in following review process.

Study 3:
Becker, R., Ritter, P., Villringer, A., 2008. Influence of ongoing alpha rhythm on the visual evoked potential. Neuroimage 39, 707-716.

Contribution in percent: 70.
Detailed contribution: Essential role in planning the study. Doctoral student performed data acquisition of study and data analysis of study. Developed required theoretical models. Student participated in writing the manuscript and in the following review process.

Study 4:
Ritter, P., **Becker, R.**, 2009. Detecting alpha rhythm phase reset by phase sorting: caveats to consider. Neuroimage 47, 1-4.

Contribution in percent: 50.
Detailed contribution: Performing simulations on synthetic data. Co-writing the manuscript and essential participation in following review process.

Study 5:
Reinacher, M., **Becker, R.**, Villringer, A., Ritter, P., 2009. Oscillatory brain states interact with late cognitive components of the somatosensory evoked potential. J Neurosci Methods 183, 49-56.

Contribution in percent: 30.
Detailed contribution: Major contribution to planning study setup and experimental design. Participation in analysis of data. Participation in writing the manuscript and in the following review process.

Study 6:
Becker, R., Reinacher, M., Freyer, F., Villringer, A., Ritter, P., 2010. Evidence for interaction between ongoing neuronal oscillations and evoked fMRI activity: linear superposition and beyond. (submitted)

Contribution in percent: 70.
Detailed contribution: Planning of experiment, developing of necessary setup, participation in acquisition of data. Major part of data analysis. Writing the manuscript.

Gelected publications

Here, references to the published versions of studies 1 to 5 are provided. For study 6, the manuscript draft is inserted.

Study 1

Ritter, P., **Becker, R.**, Graefe, C., Villring er, A., 2007. Evaluating gradient artifact correction of EEG data acqui red simultaneously with fMR I. Magn Reson Imaging 25, 923-932.

Study 2

Freyer, F., **Becker, R.**, Anami, K., Curio, G., Villringer, A., Ritter, P., 2009. Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction. Neuroimage 48, 94-108.

Study 3

Becker, R., Ritter, P., Villringer, A., 2008. Influence of ongoing alpha rhythm on the visual evoked potential. Neuroimage 39, 707-716.

Study 4

Ritter, P., **Becker, R.**, 2009. Detecting alpha rhythm phase reset by phase sorting: caveats to consider. Neuroimage 47, 1-4.

Study 5

Reinacher, M., **Becker, R.**, Villringer, A., Ritter, P., 2009. Oscillatory brain states interact with late cognitive components of the somatosensory evoked potential. J Neurosci Methods 183, 49-56.

Study 6

Becker, R., Reinacher, M., Freyer, F., Villringer, A., Ritter, P., 2010. Evidence for interaction between ongoing neuronal oscillations and evoked fMRI activity: linear superposition and beyond (submitted)

Evidence for interaction between ongoing neuronal oscillations and evoked fMRI activity: linear superposition and beyond

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SUMMARY

Variability of evoked responses in functional magnetic resonance imaging (fMRI) is relevant to perception and behavior. This strongly suggests an underlying intrinsic neuronal source, yet direct evidence is missing. We examined this theory using simultaneous electroencephalography (EEG)-fMRI and real-time classification of ongoing alpha-rhythm states triggering visual stimulation. Based on previous animal work, we hypothesized a linear superposition of a fixed evoked response and fMRI activity related to ongoing large-scale neuronal activity. This was confirmed for occipital brain areas, where observed variability of evoked fMRI-signals was almost entirely explained by linear superposition of the fMRI-response to spontaneous modulations of alpha-rhythm plus a constant (alpha-independent) fMRI stimulus-response. Interestingly, we also identified subcortical and cerebellar brain areas in which the evoked response was clearly alpha-dependent, but where intrinsic fluctuations of alpha-rhythm itself did not influence the background fMRI-signal.

Hence, we identified two mechanisms translating intrinsic neuronal activity into evoked fMRI-response variability.

INTRODUCTION

A pronounced trial-to-trial variability is a universal feature of evoked functional magnetic resonance imaging (fMRI) responses. In case of motor fMRI responses, much of this variance can be explained by fluctuating background fMRI activity which is linearly superimposed on a constant evoked response (Fox et al., 2006). Since fMRI is an indirect measure of neuronal activity, it is not clear, whether the fluctuating part of the fMRI signal reflects intrinsic *neuronal* activity. The fact that also behavior is co-modulated with these fMRI signal fluctuations (Fox et al., 2007) renders a non-neuronal source - such as respiration or heartbeat - unlikely (Birn, 2007). In the neuronal domain, invasive animal recordings indicate that a high degree of variance in evoked responses is explained by neuronal background activity superimposed on a fixed evoked response (Arieli et al., 1996).

Thus, while variations of the observed evoked vascular response (fMRI) can be explained by fluctuations of the intrinsic vascular signal and variations of an observed evoked neuronal response by intrinsic neuronal fluctuation, a key question with profound implications for the interpretation of fMRI responses is how those fluctuations of the *vascular* evoked fMRI response can be related to ongoing fluctuations in the *neuronal* domain. Although a tight link between evoked neuronal activity and fMRI signal changes has been demonstrated (Logothetis et al., 2001), so far there is no data available linking variability of the fMRI stimulus response to intrinsic neuronal activity. The latter can be assessed on a large-scale by electro-

encephalography (EEG) noninvasively in humans. Hence we used the two methods, EEG and fMRI simultaneously to investigate the neuronal underpinnings of evoked fMRI response variability.

As in invasive animal and human fMRI recordings, in the EEG domain ongoing activity is linked to the evoked response. For example, the dominant background signature, the posterior alpha (8 - 12 Hz) rhythm covaries with features of the visual evoked potential (VEP) (Becker et al., 2008; Makeig et al., 2002; Mazaheri and Jensen, 2008). Also with respect to perception and behavior a functional relevance of the alpha rhythm is proposed (Klimesch et al., 2006; Mathewson et al., 2009). Concerning its relationship to hemodynamics, this rhythm is linked to a decrease of the fMRI signal in occipital areas (de Munck et al., 2007; Feige et al., 2005; Goldman et al., 2002; Moosmann et al., 2003). Whether such alpha-rhythm dependent fluctuations of fMRI background activity (i.e. of the fMRI 'baseline') are superimposed on a constant evoked fMRI response has not been investigated so far. In case of such a mechanism, the alpha rhythm would represent a neuronal substrate for fMRI trial-to-trial variability.

Hence, our working hypothesis was that the strength of ongoing (background) alpha activity accounts for variance of sensory evoked fMRI responses. Based on previous animal work (Arieli et al., 1996), we specifically expected the measured fMRI signal to be a linear superposition of the fMRI response to the spontaneously fluctuating background activity plus a constant response to sensory stimulation (subsequently termed the '*superposition*' hypothesis).

We chose an 'alpha-dependent' online stimulation approach in order to capture more high-alpha states in a given time compared to random stimulation. Subjects accomplished an event-related visual oddball task comprising two types of runs in a pseudo-randomized order: One run type represented a 'high-alpha state stimulation' condition, meaning that visual stimuli were triggered only when the alpha-amplitude exceeded a specified dynamic threshold. The other run type represented the 'state-independent stimulation', where the same temporal pattern of stimulations as in the first condition was replayed, this time irrespectively of alpha-strength. A scheme of the experimental setup is provided in **Figure 1.**

RESULTS & DISCUSSION

We first examined whether fMRI stimulus responses are altered when stimulation occurs in the high-alpha state. To this end, regions exhibiting fMRI responses to visual checkerboard stimulation under both stimulation conditions were defined as 'regions-of-interest' (termed visual ROI, **Figure 2A,** $p \leq 0.01$, corrected for multiple comparisons, map 'gray'). We succeeded in identifying reduced stimulus-response amplitudes within the visual ROI for the high-alpha state (as compared to the control condition) in thalamic, cerebellar, precuneal, and occipital areas (**Figure 2A**, **B**, **Table S1**, map 'red', $p \leq 0.05$, corrected for multiple comparisons).

In a next step, we examined the effect of the high-alpha state on the inter-stimulus fMRI signal (i.e. alpha-dependent baseline modulations). In order to do so, within the run type of state-independent stimulation a third additional condition was defined, capturing states of high ongoing-alpha amplitudes without subsequent stimulation ('high-alpha state interstimulus' condition). We found regions with reduced fMRI

baseline signal in bilateral occipital cortex (p ≤ 0.05, corrected for multiple comparisons, **Figure S2**). For subsequent analyses, these regions were masked with the visual ROI (**Figure 2A**, **B**, **Table S1**, map 'blue').

At this stage our results provided evidence of alpha-rhythm dependent fMRI baseline and stimulus-response alterations – however they did not yet address the *superposition* hypothesis which states that fMRI-baseline modulations related to the alpha rhythm add linearly on a fixed evoked response and thus were responsible for the observed stimulus-evoked fMRI response modulations.

To this end, we quantified magnitudes of alpha-dependent stimulus and baseline fMRI responses for maps 'red' and 'blue' by estimating average hemodynamic response functions. For map 'red' (**Figure 2C**), by definition, high-alpha state stimulus responses were considerably smaller than state-independent responses (p ≤ 10^{-4}, Student's t-test). Notably, in contrast to the distinct stimulus-response modulation, no alpha-dependent baseline modulation was present (with p = 0.70, difference between stimulus response and baseline modulation p ≤ 0.005). This contradicts linear superposition which would require equal baseline and stimulus-response modulation amplitudes. In map 'blue' (**Figure 2D**), stimulus responses for both alpha states are present and show a clear amplitude difference (p ≤ 0.05). Here, the alpha-dependent baseline modulation (p ≤ 0.005) *is* equaled by the stimulus-response modulation (no difference, p = 0.88), indicating a linear superposition of alpha-dependent baseline modulation and stimulus response.

Summarizing, the study linked fMRI variance and ongoing neuronal activity in a two-fold manner: 1) Alpha states covary with amplitude modulations of the ongoing fMRI

baseline and these baseline fluctuations add linearly to a constant stimulus-evoked fMRI response. This finding supports the 'superposition' hypothesis. 2) Alpha states were linked to stimulus-evoked fMRI response modulations yet lacked alpha-dependent fluctuations of the fMRI baseline. This type of interaction is further referred to as 'modulation'. These mechanisms were pinpointed to spatially distinct areas: The superposition mechanism was restricted to occipital cortex only, while the modulation mechanism acted in a network of thalamic, cerebellar, precuneal and occipital structures.

In more general terms, we identified two mechanisms that both link variability of evoked fMRI responses to intrinsic neuronal activity, specifically to states of the ongoing alpha rhythm. The first, superposition mechanism provides a neuronal substrate of previous findings of ongoing fMRI fluctuations which account for evoked-fMRI response variability (Fox et al., 2006). The second, spatially distinct, modulation mechanism, in contrast, identified another link between intrinsic neuronal activity and evoked fMRI responses – 'bypassing' fMRI-baseline modulations. Regarding the areas characterized by such a mechanism, the observed variance in fMRI stimulus responses can not be explained by background fMRI being on top of an otherwise constant fMRI response. Rather, the evoked response itself is changed depending on alpha rhythm strength. Conceptually, the identification of different mechanisms confined to distinct brain structures helps understanding the origins of evoked fMRI response variability.

How compatible are these results with our current knowledge of neurophysiology and neuroanatomy? On a single-cell level, the broadband ongoing membrane potential correlates linearly with the evoked postsynaptic membrane potential in the cat visual

cortex (Azouz and Gray, 1999). In line, yet without excluding nonlinear scenarios, the present finding of a *superposition* mechanism are compatible with linear superposition of ongoing and evoked activities on a neuronal level and subsequent linear transformation of the summed neuronal signal into the hemodynamic response.

Concerning the second, *modulation* mechanism, the co-variation between alpha rhythm and stimulus-evoked fMRI responses without affection of the fMRI baseline may imply a remote down-modulation of stimulus-related excitability in these structures mediated by cortical alpha-rhythm generators. This is supported by the existence of reciprocal connections from visual cortex to thalamus (Steriade and Llinas, 1988) and cerebellum (Graybiel, 1974), recurrent connections within the visual cortex as well as connections between visual cortex and precuneus (Colby et al., 1988). In general such a remote down-modulation of excitability could be either mediated by long-range inhibitory neurons or, more likely, by excitatory connections with terminals ending at inhibitory interneurons. While long range inhibitions exist (McDonald and Burkhalter, 1993), the majority of connections across cortical areas is excitatory (as shown for visual areas, Buhl and Singer, 1989), suggesting the latter scenario. Another alternative is the existence of excitatory connections being active during low alpha states. Notably, independent of the underlying causal relation, ongoing neuronal activity - visible in the EEG but not in the local fMRI baseline - may predict the course of processing of information in the respective structures.

But what exactly may state-dependent stimulus processing as demonstrated here be useful for? The integration of bottom-up (sensory information from the environment) and top-down (predictions based on memory of previous inputs) processes in line with the theories of predictive coding and hierarchical inference (Friston, 2003) may

represent one possible functional role. This is also compatible with the theory of 'preferred cortical states' (Tsodyks et al., 1999), implying the existence of intrinsic facilitative neuronal modes.

Potentially, other neuronal signatures – already known to contribute to fMRI-signal variability under resting condition (Laufs et al., 2003; Mantini et al., 2007; Nir et al., 2007) – are capable of further explaining fMRI evoked-response variability. Thus, in the wake of future studies, we may be able to appreciate additional forms of integration of intrinsic neuronal context, sensation and action constituting the foundation of adaptive behavior.

EXPERIMENTAL PROCEDURES

Experimental Design and Procedure

Simultaneous EEG-fMRI was acquired from 16 healthy subjects (seven females, mean age 26.8 ± 2.9 years), with the final sample consisting of 12 subjects (see exclusion criteria in Supplementary methods). Written informed consent according to the declaration of Helsinki was obtained from each subject prior to the investigation and the study was approved by the local ethics committee.

Pre-experiment

Prior to the main experiment, all subjects underwent an EEG experiment in the static magnetic field of the magnetic resonance (MR) system designed to modulate the amplitude of the posterior alpha rhythm in a controlled fashion. Alpha rhythm was modulated by a block-wise eyes open / eyes closed task (12 blocks each consisting of 20 s eyes closed and 20 s eyes open, total duration 8 min). On these data, we performed independent component analysis (ICA) using a method termed 'temporal

decorrelation source separation' (TDSEP, Ziehe et al., 2000) which is a computationally efficient method for extraction of independent components (ICs). The method has been successfully employed for the separation of ongoing EEG rhythms (Ritter et al., 2009). We examined the degree of correlation between the alpha-band (8 - 12 Hz) amplitudes of each resulting IC and the experimental protocol. In each subject we identified the IC best representing the posterior alpha rhythm responsive to eyes opening / closing as indicated by a maximum correlation coefficient. Subsequently, the resulting individual demixing matrices were transferred to the following main experiment and the IC representing the posterior alpha rhythm (further termed 'alpha IC') was extracted. Thus we were able to optimize our real-time alpha-rhythm triggered stimulation approach by maximizing sensitivity to the signal of interest – the posterior alpha rhythm – while at the same time rejecting noise, such as the heartbeat related ballistocardiogram (BCG) artifact in EEG-fMRI recording (Debener et al., 2008).

Main experiment

The main experiment consisted of a visual oddball task, requiring subjects to remain awake with eyes open, paying attention to the stimulus while focusing on the constantly shown fixation cross and responding to deviant stimuli with the right-hand index finger. Visual evoked responses were elicited by projecting a circular black-and-white checkerboard onto an acrylic screen in the bore of the MR system. Duration of the stimulus was 0.9 s. In 20 % of the cases a deviant stimulus was shown, consisting of a contrast reversal of the circular checkerboard after 0.5 s.

We used three experimental conditions in this experiment: (I) In the 'high-alpha state stimulation condition' stimuli were applied during a state of high alpha-rhythm amplitude, achieved by the method described below. (II) In the 'state-independent

stimulation condition', stimuli were applied independent of alpha-rhythm amplitude, with stimulus timings replayed from condition I. (III) In the 'high-alpha state inter-stimulus condition', states of high-alpha amplitude similar to those of condition I were identified post-hoc within runs of condition II in between stimuli, i.e. during periods that lacked visual stimulation (**Figure 1**).

The experiment was split into eight blocks of seven minutes each. Stimulus conditions (I or II) were pseudo-randomly assigned to these blocks to avoid order effects. Also the sequences of stimulus triggers from high-alpha state stimulation runs being replayed in the state-independent stimulation runs were assigned in a pseudo-random fashion. Constraints for randomization were as follows: The first run had to be a high-alpha state stimulation run. Each high-alpha state stimulation run had to be used no more than twice for replay. The number of high-alpha state and state-independent stimulation runs had to be equal (i.e. four runs each).

EEG-FMRI

Simultaneous EEG-fMRI poses the challenge that the two major types of artifacts in the EEG, related to gradient-switching during image acquisition and to heartbeat in the magnetic field, may critically interfere with real-time alpha-rhythm evaluation. Thus, we carefully minimized those artifacts in real-time before alpha-rhythm evaluation and used strict validation criteria to ensure adequate reduction of artifacts. For detailed procedures and validation of the EEG online approach including evaluation of vigilance or attention effects, see **Supplemental Experimental Procedures** and **Figure S1**.

FMRI data acquisition

For fMRI recordings we used a 1.5 T Siemens Vision MR scanner with a modified T2*-weighted blood oxygen level-dependent (BOLD) sensitive echo planar imaging

(EPI) sequence ('stepping stone') which was specifically developed for EEG-fMRI acquisition (Anami et al., 2003). Recording parameters were as follows: repetition time (TR) = 3000 ms, acquisition time (TA) = 2090 ms, echo time (TE) = 39.29 ms, 22 slices covering the whole brain and acquired aligned to the anterior / posterior commissure, 135 volumes per run (eight runs per subject), voxel size of 3×3×5.5 mm (0.5 mm gap), a flip angle of 90°, a matrix of 64 x 64 and a field-of-view of 192 x 192 mm.

FMRI preprocessing

The fMRI data were preprocessed and analyzed using the software package SPM5 (www.fil.ion.ucl.ac.uk/spm). Preprocessing consisted of realignment for motion correction, normalization to the brain template of the Montreal Neurological Institute (MNI) supplied with SPM, resampling data with a resolution of 2×2×2 mm and subsequent spatial smoothing with a kernel of 6 mm full-width-at-half-maximum. A temporal high-pass filter with a cut-off of 128 s was applied. The first five scans of each run were discarded to account for spin-saturation effects.

FMRI statistical analysis

Statistical analysis comprised a regression analysis based on the general linear model as implemented in SPM5 for each individual subject. A hemodynamic model, i.e. a design matrix was calculated by convolving onset vectors modeled by stick-like Dirac functions with the 'canonical' hemodynamic response function exhibiting a delay of 5 s relative to event onset. Additional temporal and dispersion derivatives of this response function were added. Onset vectors comprised 1) high alpha-state stimuli, 2) state-independent stimuli, 3) high-alpha state inter-stimulus events and 4) deviant responses. The convolved design vectors for the high-alpha state inter-stimulus condition were orthogonalized to the state-independent stimulus vector of

the same session (using recursive orthogonalization from the SPM toolbox). The design matrix was complemented by adding the set of six translational and rotational realignment parameters and a constant offset. The hemodynamic model was fitted to the fMRI data and based on the estimated parameter values (effect sizes), contrast images were calculated for the three conditions of interest: (I) high alpha-state stimulation, (II) state-independent stimulation and (III) high-alpha state inter-stimulus condition.

We calculated a group-level random-effects analysis over the contrast images of single subjects, allowing inferences on the whole population. Group-analyses were computed for high-alpha state and state-independent stimulus responses, their difference (i.e. the 'alpha-dependent stimulus response modulation') as well as high-alpha state inter-stimulus responses (the 'alpha-dependent baseline modulation').

For the alpha-dependent stimulus response modulation within the visual ROI, we employed a statistical threshold of $p \leq 0.05$, corrected for multiple comparisons based on false discovery rate, (FDR, peak threshold $z = 3.1$, extent threshold 40 voxels).

For the alpha-dependent baseline modulation, we used a statistical threshold of $p \leq 0.05$, corrected for multiple comparisons based on cluster-level analysis, peak threshold $z = 2.3$, extent threshold 400 voxels). Since we were interested in alpha-dependent modulations of the stimulus response, this map (map 'cyan', **Figure S2**) was masked by the visual ROI (map 'blue').

Maps of significant baseline (map 'blue') and stimulus-response modulations (map 'red') lying within the visual ROI were overlaid on a glass-brain and on a T1-weighted spatially normalized high-resolution single-subject anatomical template (**Figure 2A**,

B) provided by the Montreal Neurological Institute (MNI). **Table S1** lists all z-values, coordinates and corresponding anatomical labels for both maps.

FMRI response functions

By using the MARSBAR toolbox for SPM we obtained estimated fMRI response functions for maps 'red' and 'blue'. This was done by first averaging fMRI time-series of all voxels for map 'red' and 'blue' respectively, performed individually for each subject. Then, these mean time-series were fitted to the canonical hemodynamic response function (see above) and averaged across subjects. This resulted in representative fMRI response curves for high-alpha state stimulus response, state-independent stimulus response, their difference, i.e. alpha-dependent stimulus response modulation and the alpha-dependent baseline modulation (**Figure 2C**, **D**). Statistical tests on peak activity (5 s after event onset) were performed using Student's t-test.

ACKNOWLEDGMENTS

REFERENCES

Anami, K., Mori, T., Tanaka, F., Kawagoe, Y., Okamoto, J., Yarita, M., Ohnishi, T., Yumoto, M., Matsuda, H., Saitoh, O., 2003. Stepping stone sampling for retrieving artifact-free electroencephalogram during functional magnetic resonance imaging. Neuroimage 19, 281-295.

Arieli, A., Sterkin, A., Grinvald, A., Aertsen, A., 1996. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. Science 273, 1868-1871.

Azouz, R., Gray, C.M., 1999. Cellular mechanisms contributing to response variability of cortical neurons in vivo. J Neurosci 19, 2209-2223.

Becker, R., Ritter, P., Villringer, A., 2008. Influence of ongoing alpha rhythm on the visual evoked potential. Neuroimage 39, 707-716.

Birn, R.M., 2007. The behavioral significance of spontaneous fluctuations in brain activity. Neuron 56, 8-9.

Buhl, E.H., Singer, W., 1989. The callosal projection in cat visual cortex as revealed by a combination of retrograde tracing and intracellular injection. Exp Brain Res 75, 470-476.

Colby, C.L., Gattass, R., Olson, C.R., Gross, C.G., 1988. Topographical organization of cortical afferents to extrastriate visual area PO in the macaque: a dual tracer study. J Comp Neurol 269, 392-413.

de Munck, J.C., Goncalves, S.I., Huijboom, L., Kuijer, J.P., Pouwels, P.J., Heethaar, R.M., Lopes da Silva, F.H., 2007. The hemodynamic response of the alpha rhythm: an EEG/fMRI study. Neuroimage 35, 1142-1151.

Debener, S., Mullinger, K.J., Niazy, R.K., Bowtell, R.W., 2008. Properties of the ballistocardiogram artefact as revealed by EEG recordings at 1.5, 3 and 7 T static magnetic field strength. Int J Psychophysiol 67, 189-199.

Feige, B., Scheffler, K., Esposito, F., Di Salle, F., Hennig, J., Seifritz, E., 2005. Cortical and subcortical correlates of electroencephalographic alpha rhythm modulation. J Neurophysiol 93, 2864-2872.

Fox, M.D., Snyder, A.Z., Vincent, J.L., Raichle, M.E., 2007. Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. Neuron 56, 171-184.

Fox, M.D., Snyder, A.Z., Zacks, J.M., Raichle, M.E., 2006. Coherent spontaneous activity accounts for trial-to-trial variability in human evoked brain responses. Nat Neurosci 9, 23-25.

Friston, K., 2003. Learning and inference in the brain. Neural Netw 16, 1325-1352.

Goldman, R.I., Stern, J.M., Engel, J., Jr., Cohen, M.S., 2002. Simultaneous EEG and fMRI of the alpha rhythm. Neuroreport 13, 2487-2492.

Graybiel, A.M., 1974. Visuo-cerebellar and cerebello-visual connections involving the ventral lateral geniculate nucleus. Exp Brain Res 20, 303-306.

Klimesch, W., Doppelmayr, M., Hanslmayr, S., 2006. Upper alpha ERD and absolute power: their meaning for memory performance. Prog Brain Res 159, 151-165.

Laufs, H., Krakow, K., Sterzer, P., Eger, E., Beyerle, A., Salek-Haddadi, A., Kleinschmidt, A., 2003. Electroencephalographic signatures of attentional and cognitive default modes in spontaneous brain activity fluctuations at rest. Proc Natl Acad Sci U S A 100, 11053-11058.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A., 2001. Neurophysiological investigation of the basis of the fMRI signal. Nature 412, 150-157.

Makeig, S., Westerfield, M., Jung, T.P., Enghoff, S., Townsend, J., Courchesne, E., Sejnowski, T.J., 2002. Dynamic brain sources of visual evoked responses. Science 295, 690-694.

Mantini, D., Perrucci, M.G., Del Gratta, C., Romani, G.L., Corbetta, M., 2007. Electrophysiological signatures of resting state networks in the human brain. Proc Natl Acad Sci U S A 104, 13170-13175.

Mathewson, K.E., Gratton, G., Fabiani, M., Beck, D.M., Ro, T., 2009. To see or not to see: prestimulus alpha phase predicts visual awareness. J Neurosci 29, 2725-2732.

Mazaheri, A., Jensen, O., 2008. Asymmetric amplitude modulations of brain oscillations generate slow evoked responses. J Neurosci 28, 7781-7787.

McDonald, C.T., Burkhalter, A., 1993. Organization of long-range inhibitory connections with rat visual cortex. J Neurosci 13, 768-781.

Moosmann, M., Ritter, P., Krastel, I., Brink, A., Thees, S., Blankenburg, F., Taskin, B., Obrig, H., Villringer, A., 2003. Correlates of alpha rhythm in functional magnetic resonance imaging and near infrared spectroscopy. Neuroimage 20, 145-158.

Nir, Y., Fisch, L., Mukamel, R., Gelbard-Sagiv, H., Arieli, A., Fried, I., Malach, R., 2007. Coupling between neuronal firing rate, gamma LFP, and BOLD fMRI is related to interneuronal correlations. Curr Biol 17, 1275-1285.

Ritter, P., Moosmann, M., Villringer, A., 2009. Rolandic alpha and beta EEG rhythms' strengths are inversely related to fMRI-BOLD signal in primary somatosensory and motor cortex. Hum Brain Mapp 30, 1168-1187.

Steriade, M., Llinas, R.R., 1988. The functional states of the thalamus and the associated neuronal interplay. Physiol Rev 68, 649-742.

Tsodyks, M., Kenet, T., Grinvald, A., Arieli, A., 1999. Linking spontaneous activity of single cortical neurons and the underlying functional architecture. Science 286, 1943-1946.

Ziehe, A., Muller, K.R., Nolte, G., Mackert, B.M., Curio, G., 2000. Artifact reduction in magnetoneurography based on time-delayed second-order correlations. IEEE Trans Biomed Eng 47, 75-87.

FIGURE CAPTIONS

Figure 1. Scheme of the experimental setup. **A**. First, image acquisition artifacts (IAAs) were removed online. **B**. Then we used the demixing matrix obtained from the pre-experiment as a spatial filter. **C.** Thus we extracted the posterior alpha rhythm and minimized signatures from other sources, such as heartbeat-related BCG. **D**. On these data a short-term Fast Fourier Transform (FFT) was applied to calculate alpha-band amplitude online. **E**. In the high-alpha state condition, stimulation was triggered when the current alpha-band amplitude exceeded an adaptive threshold (condition I). The red line indicates the threshold. **F**. Trigger timings from high-alpha state stimulation condition (condition I) were recorded and used for the state-independent stimulation condition (condition II). **G**. Interspersed with the state-independent stimulus triggers, inter-stimulus events were identified where alpha-band amplitude exceeded a given threshold. These high alpha state events, however, did not trigger stimulation (condition III).

In order to validate our experimental online setup we analyzed the recorded EEG data post-hoc (**Figure S1**). We confirmed typical posterior spatial distribution of the alpha source used for triggering visual stimulation (**Figure S1A**), reproduced previously reported alpha-dependent modulation of EEG-EPs (Becker et al., 2008; Mazaheri and Jensen, 2008) (**Figure S1B**), demonstrated proper alpha-state dependent triggering (**Figure S1C**) and efficient reduction of BCG (**Figure S1D**).

Figure 2. Two types of interaction between ongoing neuronal activity and the stimulus-evoked fMRI signal. **A.** Regions with alpha-dependent fMRI baseline modulations are depicted in blue (map 'blue'), regions with alpha-dependent fMRI stimulus-response modulations are depicted in red (map 'red'). All regions are subsets of a defined region of interest (ROI), showing visual activation (termed visual ROI, map 'gray'). **B.** Same as (A), overlaid on an anatomical standard image (visual ROI not shown). **C**, **D.** Estimated response functions of the average fMRI signal from the above maps: Evoked fMRI responses for high-alpha state (light gray) and state-independent stimulation (dark gray), for their difference, i.e. the alpha-dependent fMRI stimulus-response modulation (dashed lines), and for alpha-dependent fMRI-baseline modulations (black). Error bars indicate standard error of mean (SEM) across subjects. **C.** FMRI response functions of map 'red', exhibiting alpha-dependent stimulus-response modulations within the visual ROI. Here, stimulus responses differ significantly, however, no alpha-dependent baseline modulation is observed. Hence, superposition of alpha-dependent baseline modulations and stimulus response cannot explain the observed stimulus response modulation. **D.** FMRI response functions of map 'blue', exhibiting the alpha-dependent baseline modulations within the visual ROI. Here, the observed alpha-dependent baseline modulation accounts for the difference in stimulus responses, indicating a linear superposition of alpha-dependent baseline modulation and the evoked stimulus response. Coordinates and z-values as well as anatomical labels of identified regions are shown in **Table S1**. Unmasked alpha-dependent baseline-modulated regions are depicted in **Figure S2**.
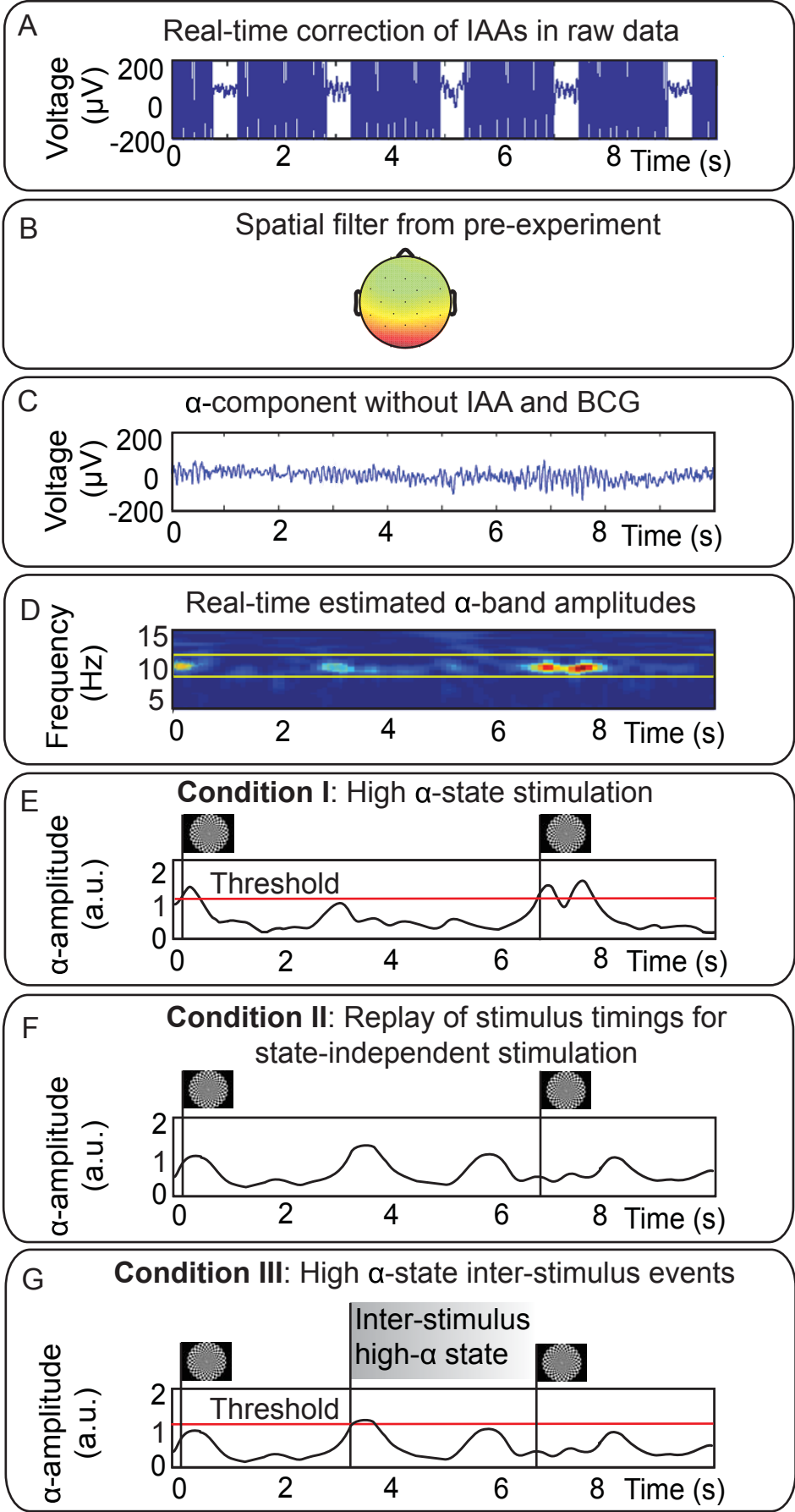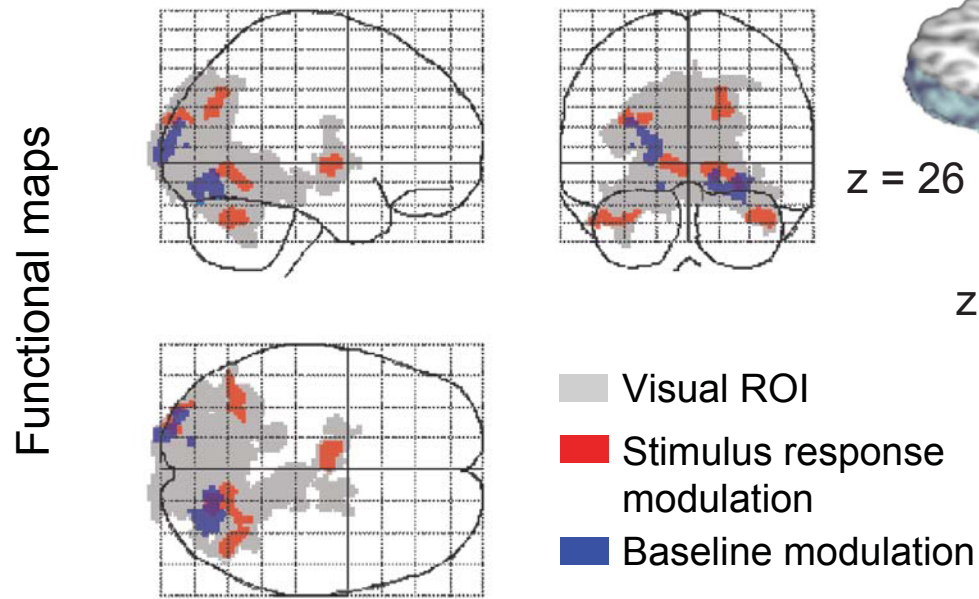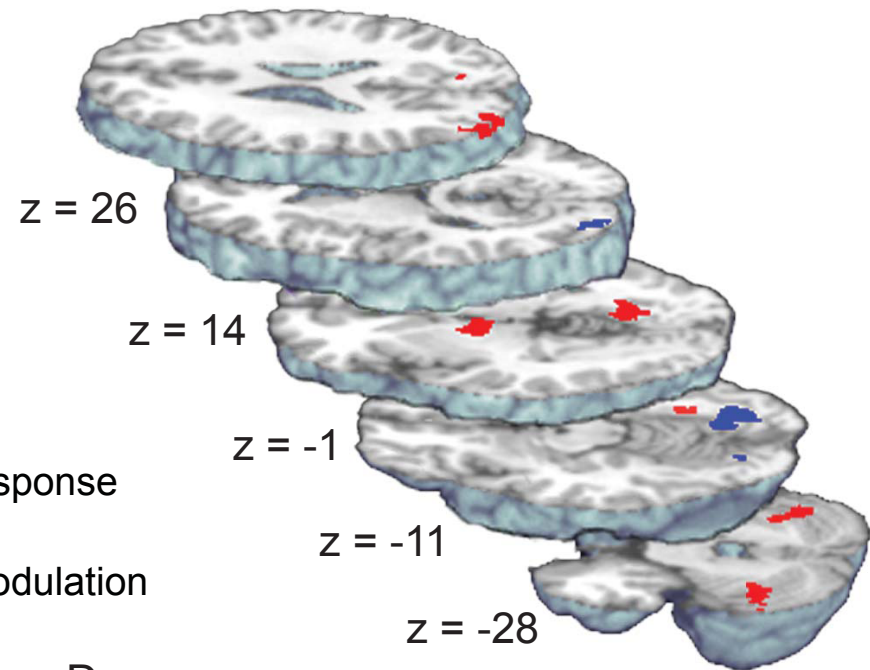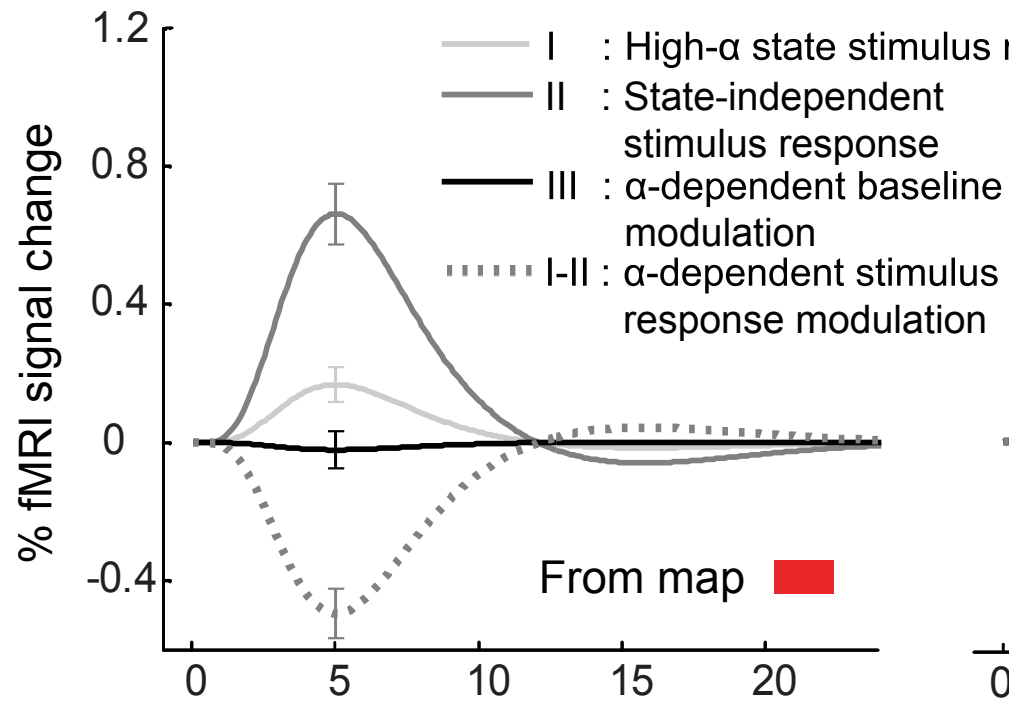
Figure 1.

A — Real-time correction of IAAs in raw data

B — Spatial filter from pre-experiment

C — α-component without IAA and BCG

D — Real-time estimated α-band amplitudes

E — **Condition I**: High α-state stimulation

F — **Condition II**: Replay of stimulus timings for state-independent stimulation

G — **Condition III**: High α-state inter-stimulus events

Figure 2.

A — Functional maps

- Visual ROI
- Stimulus response modulation
- Baseline modulation

B
z = 26
z = 14
z = -1
z = -11
z = -28

C

% fMRI signal change vs Time (s)

- I : High-α state stimulus response
- II : State-independent stimulus response
- III : α-dependent baseline modulation
- I-II : α-dependent stimulus response modulation

From map ▬ (red)

D

From map ▬ (blue)

Supplemental Information

Supplemental Information contains: Two Supplemental figures (**Figures S1** and **S2**, relating to **Figures 1** and **2**, respectively) and one supplemental table (**Table S1**, relating to **Figure 2**) and **Supplemental Experimental Procedures.** The latter provides a detailed overview on the used EEG online approach and its validation as well as behavioral data excluding possible confounds.

Supplemental Data

Figure S1 (related to Figure 1).
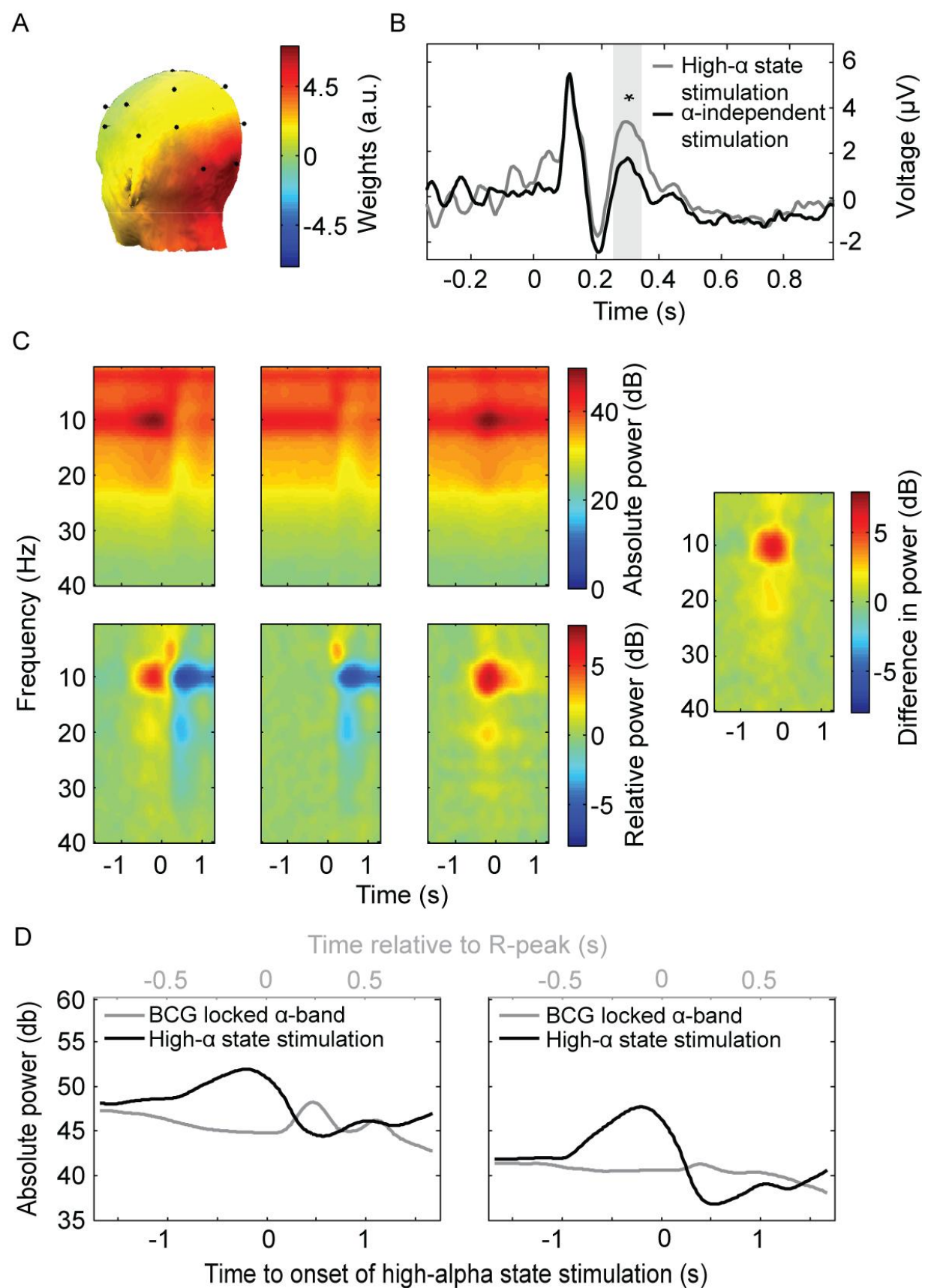
Figure S1. A. Scalp distribution of alpha-associated independent component (IC) used for triggering stimulation. This picture represents an average of all alpha ICs defined subject-wise. Color bar represents weightings (arbitrary units). **B.** Grand average (n = 12) EPs of the alpha IC for high-alpha state and state-independent stimulation (for display purposes notch filtered (45 - 55 Hz) to remove helium-pump noise), showing an amplitude increase of the late (250 - 350 ms, gray box) evoked component for the high-alpha state stimulation condition (* significant at p ≤ 0.05). **C.** Grand-average (n = 12) time-frequency plots of EEG data for stimulation and inter-stimulus conditions. Top and bottom row images depict same data, top row without baseline correction and bottom row with baseline correction (-2 to -1 s). *First column*: State-independent stimulation: Increases in spectral-band amplitudes below the alpha band reflect the evoked potential (EP). Decreases in alpha-band amplitude indicate the classical event-related desynchronization (ERD) of the alpha rhythm due to stimulation. *Second column*: High-alpha state stimulation: Equally to the first column there are amplitude increases below the alpha band reflecting the EP and decreases in the alpha band reflecting alpha-rhythm ERD. In contrast, amplitude-increases preceding stimulation are restricted to the alpha band, verifying that stimulation was effectively triggered by high-alpha states. *Third column*: High-alpha state inter-stimulus condition (no stimulation). Here, similar to the high-alpha state stimulation condition, high-alpha state events were captured; however, no stimulus was triggered. This implies that neither event-related amplitude increases nor decreases follow these events. *Fourth row*: Difference of time-frequency behavior obtained from high-alpha state and state-independent stimulation conditions. **D.** Validation of alpha-rhythm extraction and BCG reduction. Grand averages (n = 12) of EEG alpha-band amplitude of IAA-corrected data locked to either high-alpha state stimulation (black) or to the heartbeat-related R-peak (gray) before (left box) and after (right box) applying the spatial filter from the pre-experiment, extracting the alpha IC. Alpha rhythm is maintained in the alpha IC while BCG is strongly reduced. Note the different time scales for the two averages.
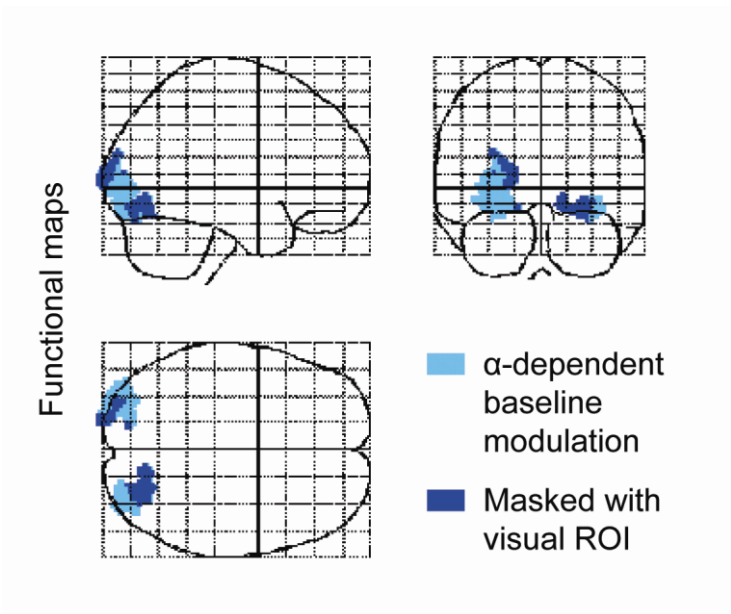
Figure S2 (related to Figure 2).

Figure S2. Regions of alpha-dependent fMRI-baseline modulations with (blue) and without masking (cyan) by the visual ROI.

Table S1 (related to Figure 2).

Anatomical region	Hemi-sphere	Coordinates			z-value
		x	*y*	*z*	
Alpha-dependent stimulus-response modulation (map 'red')					
Anterior thalamus	L	-10	-6	0	3.83
Cuneus (BA 19)	L	-32	-92	26	3.71
Cerebellum (Posterior lobe)	R	42	-64	-30	3.71
Cuneus / Precuneus (BA 7/31)	R	20	-72	28	3.66
Cerebellum (Posterior lobe)	L	-34	-60	-30	5.08
Lingual gyrus (BA 18/19)	R	16	-66	-4	4.29
Alpha-dependent baseline modulation (map 'blue')					
Fusiform gyrus / Lingual gyrus (BA 18/19)	R	22	-73	-12	3.04
Lingual gyrus (BA 18)	L	-16	-85	-13	2.50
Middle occipital gyrus / Cuneus (BA 18 /19)	L	-20	-97	9	3.75

Table S1. FMRI results of random-effects group analyses (n = 12) for alpha-dependent stimulus response modulation (map 'red') and alpha-dependent baseline modulation (map 'blue'). BA: Brodmann area.

Supplemental Experimental Procedures

EEG system and recording parameters

EEG recordings were conducted with a 32-channel MR-compatible EEG system (BrainAmp MR Plus, Brain Products, Munich, Germany) and an MR-compatible EEG cap (Easy cap, FMS, Herrsching-Breitbrunn, Germany), which comprised ring-type sintered silver chloride electrodes with iron-free copper leads. 21 scalp electrodes were arranged according to the International 10-20 System with the reference located at electrode position FCz. In addition, one electrooculogram (EOG) and two electrocardiogram (ECG) channels were recorded. Impedances of all electrodes were kept below 10 kΩ using an abrasive electrolyte gel (Abralyt 2000, FMS, Herrsching-Breitbrunn, Germany). The EEG amplifier's recording range was $\pm$ 16.38 mV at a resolution of 0.5 µV, capturing both low-amplitude EEG and high-amplitude image acquisition artifacts (IAAs) without reaching saturation. EEG sampling rate was 5 kHz. A hardware low-pass filter of 250 Hz was applied. To allow acquisition of the entire frequency spectrum of the physiological EEG as well as of IAAs, we enabled DC recording (Ritter et al., 2009a).

Real-time EEG alpha-state classification and stimulus triggering

For the necessary first step of online removal of IAAs in the EEG, we used the BrainVision RecView v1.0 software package (Brain Products, Munich, Germany). The artifact correction plug-in calculates a template based on a number of preceding IAA epochs (here: n = 3) and then subtracts this template from each following artifact epoch. This computationally undemanding approach allows online removal of IAAs, yet requires time-invariant sampling of the IAA for optimal results. We ensured this by synchronizing the EEG-sampling clock to the gradient-switching clock of the MR scanner (Anami et al., 2003; Freyer et al., 2009).

For the high-alpha state stimulation condition, we extracted the alpha IC from the IAA corrected raw EEG data by applying the TDSEP-derived spatial filter from the pre-experiment. This was done to maximize sensitivity to the posterior alpha rhythm and reduce BCG and other sources of noise.

Alpha-band (8 - 12 Hz) amplitude was calculated in real-time by an in-house developed RecView plug-in based on a real-time short-term Fast Fourier Transform

(FFT) approach. IAA corrected and spatially filtered data segments of approximately 100 samples, corresponding to 20 ms, were continuously transmitted to the plug-in which maintained a buffer corresponding to the FFT window size of 4096 sample points (819.2 ms). For each data segment, the plug-in calculated the average spectral amplitudes within the alpha band. The resulting value was compared to an adaptive baseline, which was updated once per minute to account for slower shifts in average alpha-rhythm fluctuations. This ensured a homogeneous distribution of triggers. The average alpha amplitude of the preceding one-minute time window served as the adaptive baseline. The plug-in delivered a signal to the stimulation computer when the current alpha amplitude exceeded two standard deviations (SD) of the adaptive baseline, which then triggered visual stimulation. After each stimulus a dead time of 4 - 6 s (i.e. no stimulation was allowed during that window) ensured sufficiently long inter-stimulus intervals. The resulting median inter-stimulus interval (ISI) for both high-alpha state stimulation and state-independent stimulation conditions amounted to 10.5 s (median absolute deviation: 4.9 s for high-alpha state, 4.7 s for state-independent stimulation). For each condition, deviant stimuli (independent of ongoing alpha amplitude) were triggered in 20 percent of total stimulations.

Data analysis

EEG preprocessing

For subsequent analyses, EEG data were offline corrected for IAAs and filtered (band-pass 0.5 – 40 Hz) using BrainVision Analyzer v1.05 (BrainProducts, Munich, Germany) software. Data were exported from the BrainVision Analyzer environment and all following analyses were carried out using routines written in MATLAB v7.3 (The Mathworks Inc., Natick, MA, USA) and routines from EEGLAB v5.03 (Delorme and Makeig, 2004). For handling in EEGLAB, data were down-sampled to 200 Hz.

EEGLAB-based movement-artifact rejection was carried out using an exclusion threshold of 100 µV for EEG/EOG channel data and improbability criteria, such as joint-probability and kurtosis-of-activity as estimated with EEGLAB preset defaults. Out of a total amount of 105 ± 12 (mean ± SD) trials for the high-alpha state stimulation and 107 ± 11 trials for the state-independent stimulation, on average 98 ± 15 and 99 ± 14 trials were retained after artifact correction.

For all subsequent EEG analyses we extracted the alpha IC using the demixing matrix from the pre-experiment. By back-projecting alpha ICs to occipital electrode position O2, polarity information and scaling in microvolt was regained. The average distribution of sources (normalized to absolute values from each individual map before averaging) used for triggering is depicted in **Figure S2A**.

Identification of high-alpha state inter-stimulus events

To identify high-alpha state inter-stimulus events, we first performed a time-frequency analysis of EEG data from the state-independent stimulation condition. Comparable to the online approach, alpha-band amplitudes exceeding a specified threshold were identified. Alpha-band amplitudes were calculated by a wavelet analysis (Morlet wavelet, five cycles). High-alpha inter-stimulus events were required to have a distance to stimuli exceeding 3 s to account for the post-stimulus resynchronization period of up to 2 s (Klimesch et al., 2006). The chosen threshold resulted in average alpha amplitudes corresponding to those of the high-alpha state stimulation condition (**Figure S2C**). On average, we obtained 70 ± 7 (mean ± SD) events per subject, amounting to 58 ± 10 events after movement-artifact rejection. Between events of the high-alpha state inter-stimulus condition the distance was 14.1 s (median absolute deviation 10.4 s).

Validation of real-time stimulus triggering and of extraction of posterior alpha rhythm

To validate the real-time stimulus triggering approach, we analyzed the time-frequency behavior of peri-stimulus data using a short-term FFT (window length 800 samples). In all subjects, a pronounced and frequency-specific increase of pre-stimulus alpha-band activity for the high-alpha state stimulation condition was observed (**Figure S2C**).

To assess the efficiency of the employed spatial filter for the reduction of heartbeat-related BCG contamination in our signal, we segmented data in a dual manner: (1) relative to the heartbeat-related R-peak and (2) relative to high-alpha state stimulation. Calculation of event-related spectral perturbations was performed within the alpha band using a short-term FFT time-frequency analysis and grand averages across all subjects were computed and compared (**Figure S2D**). Ideally, there should be no increase in alpha-band amplitude locked to the BCG (as defined by

segmenting data relative to R-peak). In contrast, an increase of alpha-band amplitudes prior to high-alpha state stimulation should be present. BCG-locked increases in alpha–band amplitude far below those preceding high-alpha state stimulation ensure stimulus-trigger release due to posterior alpha-rhythm increase rather than due to BCG. It has been pointed out, that BCG amplitudes increase with field strength and that the residual distortions after ICA-based BCG reduction may be less severe at 1.5 T compared to 3 T and 7 T MR environments (Debener et al., 2008). Nevertheless, also at 1.5 T, sufficient alpha-rhythm amplitude is essential to exclude erroneous stimulus triggering caused by residual BCG. Hence, our criteria for subject inclusion were: For the alpha IC, we defined a distance criterion, with respect to the difference between maximum pre-stimulus alpha-amplitude increase in the high-alpha state stimulation condition and the maximum alpha-band increase locked to the BCG. A distance criterion of 3.5 dB ensured exclusion of any subjects exhibiting a systematic relation between the timing of BCG (as reflected by timing of the R-peak) and high-alpha state stimulation. Such a relation was tested by a subject-wise chi-square test of deviation from uniform distribution of R-peaks relative to high alpha-state stimulation onset (significance threshold $p \leq 0.05$). Four subjects failed to fulfill the distance criterion and were discarded, resulting in a final sample of 12 subjects (mean 26.8 ± 3.4 years). The average reduction of BCG after denoising of data by ICA is depicted in **Figure S2D** for the remaining 12 subjects, indicating efficient reduction of BCG activity after use of the spatial filter from the pre-experiment and extraction of the alpha IC.

Analysis of evoked potentials

The grand-average evoked potential (EP) of the alpha IC was calculated for each stimulation condition. As expected from previous EEG and magnetoencephalography (MEG) studies (Becker et al., 2008; Jasiukaitis and Hakerem, 1988; Mazaheri and Jensen, 2006), late evoked-component amplitudes were significantly increased in the high-alpha state stimulation condition (paired Student's t-test over the average in the time window of 250 - 350 ms, $p \leq 0.05$, **Figure S2B**).

Behavioral data

Response times for deviant stimuli

We analyzed the response times for deviant stimuli to exclude differences in vigilance and attention between the high-alpha state and state-independent

stimulation. Response times of all subjects were compared across conditions using Student's t-test. Response times were 490 ± 182 ms (mean ± SD) for the high-alpha state stimulation condition and 499 ± 172 ms for the state-independent stimulation condition indicating similar levels of vigilance and attention across conditions (no significant difference, p = 0.52).

Supplemental References

Anami, K., Mori, T., Tanaka, F., Kawagoe, Y., Okamoto, J., Yarita, M., Ohnishi, T., Yumoto, M., Matsuda, H., Saitoh, O., 2003. Stepping stone sampling for retrieving artifact-free electroencephalogram during functional magnetic resonance imaging. Neuroimage 19, 281-295.

Becker, R., Ritter, P., Villringer, A., 2008. Influence of ongoing alpha rhythm on the visual evoked potential. Neuroimage 39, 707-716.

Debener, S., Mullinger, K.J., Niazy, R.K., Bowtell, R.W., 2008. Properties of the ballistocardiogram artefact as revealed by EEG recordings at 1.5, 3 and 7 T static magnetic field strength. Int J Psychophysiol 67, 189-199.

Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134, 9-21.

Freyer, F., Becker, R., Anami, K., Curio, G., Villringer, A., Ritter, P., 2009. Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction. Neuroimage 48, 94-108.

Jasiukaitis, P., Hakerem, G., 1988. The effect of prestimulus alpha activity on the P300. Psychophysiology 25, 157-165.

Klimesch, W., Doppelmayr, M., Hanslmayr, S., 2006. Upper alpha ERD and absolute power: their meaning for memory performance. Prog Brain Res 159, 151-165.

Mazaheri, A., Jensen, O., 2006. Posterior alpha activity is not phase-reset by visual stimuli. Proc Natl Acad Sci U S A 103, 2948-2952.

Ritter, P., Becker, R., Freyer, F., Villringer, A., 2009a. Correction of image acquisition artefacts. In: Mulert, C., Lemieux, L. (Eds.), EEG-fMRI - Physiological Basis, Technique, and Applications. Springer, New York, 153-71.

Ritter, P., Moosmann, M., Villringer, A., 2009b. Rolandic alpha and beta EEG rhythms' strengths are inversely related to fMRI-BOLD signal in primary somatosensory and motor cortex. Hum Brain Mapp 30, 1168-1187.

Curriculum vitae

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Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht mit veröffentlicht.

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Complete list of publications

Articles in peer-reviewed journals

Becker, R., Ritter, P., Moosmann, M., Villringer, A., 2005. *Visual evoked potentials recovered from fMRI scan periods.* Hum Brain Mapp 26, 221-230.

Ritter, P., **Becker, R.**, Graefe, C., Villringer, A., 2007. *Evaluating gradient artifact correction of EEG data acquired simultaneously with fMRI.* Magn Reson Imaging 25, 923-932.

Becker, R., Ritter, P., Villringer, A., 2008. *Influence of ongoing alpha rhythm on the visual evoked potential.* Neuroimage 39, 707-716.

Freyer, F., **Becker, R.**, Anami, K., Curio, G., Villringer, A., Ritter, P., 2009. *Ultrahigh-frequency EEG during fMRI: pushing the limits of imaging-artifact correction.* Neuroimage 48, 94-108.

Reinacher, M., **Becker, R.**, Villringer, A., Ritter, P., 2009. *Oscillatory brain states interact with late cognitive components of the somatosensory evoked potential.* J Neurosci Methods 183, 49-56.

Ritter, P., **Becker, R.**, 2009. *Detecting alpha rhythm phase reset by phase sorting: caveats to consider.* Neuroimage 47, 1-4.

Becker, R., Reinacher, M., Freyer, F., Villringer, A., Ritter, P., 2010. *Evidence for interaction between ongoing neuronal oscillations and evoked fMRI activity: linear superposition and beyond.* (submitted)

Review articles

Nierhaus, T., Schon, T., **Becker, R.**, Ritter, P., Villringer, A., 2009. *Background and evoked activity and their interaction in the human brain.* Magn Reson Imaging 27, 1140-1150.

Book chapters

Becker, R., Ritter, P., Villringer, A., 2009. *Visual System.* In: Mulert, C., Lemieux, L. (Eds.), EEG-fMRI - Physiological Basis, Technique, and Applications. Springer, New York.

Ritter, P., **Becker, R.**, Freyer, F., Villringer, A., 2009. *The image acquisition artifact.* In: Mulert, C., Lemieux, L. (Eds.), EEG-fMRI - Physiological Basis, Technique, and Applications. Springer, New York.

Selected abstracts

Becker, R., Moosmann, M., Ritter, P., Villringer, A., 2004. *Analysis of single-trial visual-evoked potentials during simultaneous fMRI* (conference poster). 10th Annual Meeting of Human Brain Mapping, Budapest.

Becker, R., Ritter, P., Villringer, A., 2005. *VEPs, time course of event related desynchronisation and recovery of spontaneous alpha during eyes closed and eyes open* (conference poster). 11th Annual Meeting of Human Brain Mapping, Toronto.

Becker, R., Ritter, P., Villringer, A., 2007. *Ongoing alpha activity: Epiphenomenon, generator or covariate of the visual evoked potential?* (conference poster). 13th Annual Meeting of Human Brain Mapping, Chicago.

Becker, R., Ritter, P., Schmidt, R., Kempter, R., Villringer, A., 2008. *The Phase of Ongoing Alpha Activity and its Relation to the Visual Evoked Potential* (conference poster). 14th Annual Meeting of Human Brain Mapping, Melbourne.

Becker, R., Reinacher, M., Freyer, F., Villringer, A., Ritter, P., 2009. *Spontaneous neuronal EEG signatures of the human brain account for variability in evoked fMRI responses* (conference poster). 15th Annual Meeting of Human Brain Mapping, San Francisco.

Eidesstattliche Erklärung

Ich, Robert Becker, erkläre hiermit, dass ich die vorgelegte Dissertation mit dem Thema: „A spatiotemporal characterization of the relationship between ongoing and evoked activity in the human brain" selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.

15. April 2010   -----------------
                        Robert Becker

Acknowledgments