Hemodynamic response to oscillatory EEG rhythms in the human visual cortex

DISSEPTION

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Eigenständigkeitserklärung
1. Zusammenfassung


Ziel der vorliegenden Dissertation ist es, den Zusammenhang zwischen ereigniskorrelierter oszillatorischer Aktivität im Alpha- und Gammabereich und der begleitenden hämodynamischen Antwort im visuellen Kortex des Menschen näher zu untersuchen. Es wurden experimentelle Paradigmen in drei Probandenstudien eingesetzt, um selektiv die oszillatorische Aktivität in Alpha- und Gammabereich zu induzierten, während simultan die elektrophysiologische und die hämodynamische Antwort mittels EEG und NIRS erfasst wurde.


2. **Summary**

Understanding the relationship between electrophysiological and hemodynamic signals is of superior importance to draw inferences from modern vascular based imaging techniques back to the underlying neuronal brain activity. Non-invasive studies fusing direct and indirect neuronal based methods such as electroencephalography (EEG) coupled to functional magnetic resonance imaging (fMRI) or near-infrared spectroscopy (NIRS) in human has become a significant approach to elucidate the neurovascular mechanisms and to validate invasive findings in animal. Currently, it is still unclear which electrophysiological components or which combinations of these components have the strongest influence on the hemodynamic signal. Therein, oscillatory brain activity in the alpha- and gamma-range represents fruitful neuronal predictors because of their involvement in a widespread number of cognitive tasks and the assumption of oscillatory activity as a multifunctional coding mechanism in the visual system.

The present dissertation aims to shed light on the coupling mechanisms between event-related oscillatory activity and the concomitant hemodynamic response in human. For this purpose, three
studies with different experimental designs were performed in order to selectively induce oscillatory activity in the alpha- and gamma-range in the visual cortex and to test the influence of a particular oscillatory band on the hemodynamic response. To adequately address this neurovascular relationship EEG and NIRS techniques were applied simultaneously in all studies.

The first study showed that the resonance phenomenon, a local maximum that appears when the stimulation frequency of flicker-light matches the endogenous alpha-frequency, is not accompanied by an increase in vascular parameters. Neither evoked potentials nor ongoing alpha-power or even a combination of both electrophysiological parameters predicted the magnitude of the hemodynamic response. It was therefore suggested that the resonance phenomenon is caused by a low energy demanding phase-resetting mechanism. Furthermore, it could be shown that the resonance boost observed in electrophysiological studies and the 8 Hz peak response observed with hemodynamic based techniques represent independent phenomena.

Study II provided evidence for a predictive link between resting state alpha-parameters and evoked signals during flicker-light stimulation. It was shown that resting alpha-frequency is negatively related to the amplitude of evoked potential, to the induced alpha-power and to the magnitude of the hemodynamic response upon stimulation. The results provide further support for the assumption of a functional linkage between evoked potentials and alpha-rhythm (Study I & II).

Study III showed a tight coupling between oscillatory activity in the gamma-range and the hemodynamic response during parametrical contrast variation of a visual moving grating. Also, it could be shown that during constant contrasts behavioural performance to the accompanied task was linked to the magnitude of gamma-activity and the hemodynamic response. Here, faster response times were preceded by a phasic enhancement of gamma-band activity. Thus, Study III provides further evidence for the superior role of gamma-oscillations in visual processing and behavioural performance and validates the close coupling between hemodynamic signal and gamma-activity noninvasively in human.

In conclusion, by focussing on the same task with complementary methods, the studies of the present dissertation provide a further insight into the relationship between electrophysiological components and hemodynamic signals. Herein, simultaneous assessment of EEG and NIRS techniques provide a powerful tool to study the relationship between direct and indirect neuronal signals in humans.
3. List of original publications

This dissertation is based on the following original research articles:

Study I

Study II

Study III
4. Theoretical background

4.1. Introduction

Vascular based imaging techniques such as functional magnetic resonance imaging (fMRI) allow for an insight into the brain at work. In the last two decades an extensive body of research utilized fMRI to study the functional principles of the brain with various experimental paradigms ranging from basic to clinical research. Hence, fMRI undoubtedly has vastly enlarged our knowledge on the neuronal correlates of many complex cognitive tasks and supplemented the neuropsychological knowledge by an insight into the underlying neurophysiology. Although endowed with an exquisite spatial resolution, fMRI represents an indirect approach to study the brain since the recorded Blood Oxygen Level Dependent (BOLD) signal reflects hemodynamic changes in the sampled voxel and does not allow for a direct assessment of neuronal activity per se. When using fMRI or Positron Emission Tomography (PET), inferences from the imaging signal are drawn to describe the neuronal activity underlying a specific neuropsychological process. Conceptually, this indirect assessment of neuronal activation dates back to Roy and Sherrington (1890) who proposed a close relationship between brain function and blood flow. The rationale is that the brain stores only small quantities of substrate to accommodate metabolic demand contrary to other organs (e.g. muscle). Hence an increase of neuronal activity should elicit an increase of metabolic substrate and oxygen to meet the surplus in energetic demand. This represents the basis of a close coupling between the electrophysiological, neuronal and the hemodynamic signal. However, the exact mechanisms how a neuronal response is translated into a hemodynamic change are not fully understood. Here, a number of questions have puzzled the researchers. Beyond the general agreement on the evidence of neurovascular coupling, some relevant deviations from a straight-forward translation between the vascular response and the underlying neuronal activity seem to accumulate the more we enquire into the physiological mechanisms. For the work discussed here it seems of interest to better understand (1) the type of neuronal activity that mostly contributes to the hemodynamic signal, (2) the type of relationship between neuronal and hemodynamic response, and (3) the question of potential deviations between both signals that might lead to fallacious interpretations of vascular based imaging techniques.

Different approaches have contributed to a better comprehension of the relationship between neuronal and hemodynamic signals, i.e. neurovascular coupling (Villringer and Dirnagl, 1995):
(a) Invasive measurements in animals have used implanted microelectrodes and vascular based methods such as fMRI or optical techniques (Logothetis et al., 2001; Niessing et al., 2005).
(b) Invasive measurements in patients with implanted intracranial electrodes were used to identify seizure foci for potential surgical treatment and fMRI (Mukamel et al., 2005; Nir et al., 2007) or optical techniques (Dreier et al., 2009).
Noninvasive measurements in healthy subjects with EEG/magnetoencephalography (MEG) and fMRI/NIRS used simultaneous acquisition designs (Goldman et al., 2002; Laufs et al., 2003a; Moosmann et al., 2003) or sequential designs with identical paradigms (Brookes et al., 2005; Hoogenboom et al., 2006).

An important challenge is to transfer and generalize the findings derived from animals to humans (e.g. Logothetis et al., 2001; Hall et al., 2005) and also to bridge the gap between hemodynamic data derived from humans and the field of electrophysiological recordings obtained from animals (e.g. Heeger et al., 2000; Rees et al., 2000). The latter typically focused on spiking activity which is not accessible with noninvasive EEG (but see Ritter et al., 2008). Spiking activity is associated with neuron action potentials (Nicholson and Freeman, 1975; Mitzdorf, 1985; Destexhe, 1998; Logothetis, 2002) and refers to the axonal output of single neurons. Local field potentials (LFP) on the other hand represent another partly independent electrophysiological signal that can be measured in animals (frequencies below 250 Hz of the raw field potential). LFPs are assumed to stem from the summed dendritic activity of a large number of neurons and are thought to be dominated by a current flow imputable to synaptic activity (Pesaran et al., 2002; Logothetis, 2003).

Importantly, LFPs generated by the summed activity of post-synaptic currents of thousands of pyramidal cells is considered to be constitutive for the EEG signal detected over the scalp (Mitzdorf, 1985). Relating human hemodynamic data to neuronal spiking activity as registered in animals undergoing a similar stimulation protocol has yielded widely different ratios between these two parameters (Heeger et al., 2000; Rees et al., 2000). This has been explained by the low correlation between spiking and hemodynamics in general, also additional assumptions have to be formulated. For example that the same functional principles in animals and human subjects are linked through comparable neuronal and hemodynamic effects and the premise that anaesthesia or other stabilizing substances as used in animals do not influence the measured signals.

When studying neurovascular coupling in humans several aspects need to be taken into account: One promising approach is to study patients with invasive methods. However, a general limitation is that the tissue investigated is partially diseased thus limiting the inference drawn from such studies to pathological rather than physiological principles of neuroscience. Also the number of eligible patients with intractable seizures or subarachnoid hemorrhage is limited (Mukamel et al., 2005; Lachaux et al., 2007; Nir et al., 2007; Dreier et al., 2009). Because depth electrodes or electrodes attached to the dura are locally restricted to the subject-specific focus of disease, observations are often limited to single case reports.

Noninvasive measurements in human hence represent the most relevant approach to study the neurovascular relationship over a wide range of experimental paradigms. Compared to invasive studies in animals, noninvasive recordings have a lower spatial resolution for both types of signal (EEG and MEG: cm-range vs. LFP in animal: μm range; fMRI in human: ~mm range vs. optical
imaging in animal: µm-range) but allow to study large-scale dynamics and the interactions between areas.

4.2. Brain rhythms

Berger first described the rhythmic behaviour of the human brain activity (Berger, 1929). By means of EEG Berger observed high amplitude oscillations around 10 Hz during rest with closed eyes which he termed ‘alpha rhythm’. When the subject open his eyes the amplitude of the alpha rhythm decreases (Berger effect) and a rhythmic activity of higher frequency with lower amplitude emerges, which Berger defined as beta rhythm (15-25 Hz). Some years later additional oscillatory bands were found (delta 0-4 Hz, theta 5-8 Hz, gamma > 25 Hz). It has been shown that classical EEG brain rhythms have different neural generators and are modulated by stimulus processing, cognitive tasks and motor response. Therefore, it is thought that oscillatory activity reflects different neural mechanisms and functions. Synchronizations (enhancement of the energy of the detected signal) and desynchronizations (energy suppression) in several frequency bands with specific spatial and temporal organisation have been observed repeatedly during stimulus processing, cognitive tasks and motor response (Crone et al., 1998a; Crone et al., 1998b; Foucher et al., 2003; Lachaux et al., 2005). For example, delta-, theta- as well as gamma-activity increase in amplitude during cognitive effort whereas alpha- and beta-activity usually show an amplitude reduction during active cognitive processing (Başar-Eroğlu et al., 1996; Basar et al., 2001). In sum oscillatory neuronal activity can be considered to contain relevant information on the ongoing neuronal processing in a specific brain region. Therefore, it is of great interest to understand how changes of these electrophysiological features translate into a vascular response accessible to vascular based imaging techniques, most prominently fMRI. From the neurovascular perspective it seems fruitful to investigate oscillatory EEG activity. In addition, compared to evoked potentials, event-related oscillations might be closer related to the hemodynamic response (Foucher et al., 2003). Several reasons have been postulated by Foucher and colleagues (2003) for this discrepancy: The mismatch between evoked potentials and hemodynamic response might stem from the low energetic phase resetting mechanism, provided that evoked potentials are generated by partial phase-resetting of ongoing oscillatory activity (Makeig et al., 2002). Furthermore, oscillatory activity requires an interplay between pyramid cells and inhibitory cells, whereas evoked potentials originate from pyramid cells only. Because the activity of all neural cells contributes to the hemodynamic response, it seems plausible to assume that the latter is more sensitive to oscillatory activity. However, it still remains unclear which oscillatory components of the EEG (temporal changes in the millisecond-range) have the strongest influence on the sluggish hemodynamic signal (timescale in seconds).
4.2.1. **Alpha Rhythm**

Perceptual and motor processes have frequently been associated with a desynchronization in the alpha band (Pfurtscheller, 2001). It is assumed that thalamo-cortical feedback-loops and strong thalamic interconnections are necessary prerequisites for the cortically generated alpha-rhythm (Lopes da Silva et al., 1980; Steriade, 1999; Nunez, 2000; Nunez et al., 2001). Alpha-oscillations can be observed for the visual, auditory and the sensory-motor system. The functional relevance of the alpha-rhythm remains unclear. Based on a number of studies finding a link between alpha-rhythm and a widespread number of cognitive tasks, this rhythm must be considered multifunctional. However, there is strong support for the assumption that the amplitude of the alpha-rhythm is related to the level of cortical activation: an increase in alpha-power (synchronization) is associated with cortical and behavioural deactivation or even inhibition (Ray and Cole, 1985; Klimesch, 1999; Worden et al., 2000; Hummel et al., 2002; Jensen and Tesche, 2002; Cooper et al., 2006; Thut et al., 2006; Klimesch et al., 2007; Rihs et al., 2007). Beyond this, the role of the alpha-rhythm has been also linked to specific perceptual (Ergenoglu et al., 2004; Hanslmayr et al., 2005; Thut et al., 2006), attentional (von Stein and Sarnthein, 2000; Worden et al., 2000; Sauseng et al., 2005; Thut et al., 2006; Rihs et al., 2007) and memory processes (Klimesch et al., 1997; Klimesch, 1999; Klimesch et al., 2005).

4.2.2. **Gamma Rhythm**

Neuronal activity in the 25-90 Hz frequency range has been observed in the visual cortex under a variety of stimulation paradigms (Singer and Gray, 1995; Fries, 2005). The origin of cortically generated gamma-activity is still debated. Inhibitory mechanisms however seem to play a crucial role (Whittington et al., 1998; von der Malsburg, 1999; Fries et al., 2001a; Hasenstaub et al., 2005; Niessing et al., 2005). Irrespective of whether gamma-oscillations arise from intrinsic membrane properties of interneurons or from neocortical excitatory-inhibitory circuits (Gray et al., 1990; Llinás et al., 1991), it is assumed that gamma-oscillations reflect local activity (von Stein and Sarnthein, 2000; Bruns and Eckhorn, 2004). It is thought that also gamma-band synchronization reflects a multifunctional coding mechanism and plays a central role in neural communication (Varela et al., 2001; Fries, 2005). Gamma-activity has been associated with encoding and binding of stimulus properties (Shadlen and Movshon, 1999; Singer, 1999b, 1999a) and visual awareness (Gray and Singer, 1989; Engel et al., 1999; Engel and Singer, 2001). Oscillatory activity in the gamma-range has also been related to retention and retrieval of information independent of the sensory modality (Tallon-Baudry and Bertrand, 1999; Sederberg et al., 2003; Herrmann et al., 2004b; Herrmann et al., 2004a; Kaiser et al., 2005; Kahana, 2006; Jensen et al., 2007).
4.3. **Neurovascular coupling**

About 120 years ago it has been postulated that local variations of functional neuronal activity are followed by local changes in blood flow (Roy and Sherrington, 1890). This relationship forms the theoretical basis for hemodynamic based imaging of brain activity. Studying the neurovascular coupling targets different mechanisms and aspects of coupling and encompasses experimental variations of stimulus properties (e.g. intensity, frequency, repetition and duration), but focuses also on specific electrophysiological phenomena.

4.3.1. **Neurovascular coupling studies in animals**

In the murine cerebellar cortex the relationship between neuronal activity and regional cerebral blood flow (rCBF) has been extensively studied building on the well characterized interplay between parallel and climbing fibres and the Purkinje cells, which can be considered as the output of the system. Mathiesen and colleagues (1998) measured single unit activity (spikes) and extracellular field potentials of Purkinje cells together with rCBF (laser Doppler flowmetry) during electrical stimulation of parallel and climbing fibres. During stimulation of climbing fibres that elicited spike-activity in Purkinje cells the authors found a strong relationship between LFPs and CBF. Conversely, when parallel fibres were stimulated the spiking pattern of the Purkinje cells disappeared. Interestingly, and of relevance to the issue of neurovascular coupling, the latter scenario - though eliciting an inhibition of Purkinje cells - led to an increase in both blood flow and LFP activity. Mathiesen and colleagues concluded that blood flow changes are due to postsynaptic activity rather than spiking activity (Mathiesen et al., 1998; Caesar et al., 2003). Logothetis and his group drew a very similar conclusion based on data obtained in the visual cortex of monkeys (Logothetis et al., 2001; Logothetis, 2002). They measured spiking, LFP activity and fMRI BOLD signal to checkerboard stimulation. Although spiking activity correlated with the BOLD signal, LFP activity revealed a much better prediction especially for the tonic, sustained activation over several seconds. Therefore, it was suggested that synaptic activity rather than the spiking output contributes to the BOLD response (Logothetis and Wandell, 2004). A number of studies found support for a linear coupling between hemodynamic response and synchronized synaptic activity in animals (Brinker et al., 1999; Ngai et al., 1999; Goloshevsky et al., 2008). Some studies however, reported a nonlinear relationship between electrophysiological and hemodynamic signals (Devor et al., 2003; Sheth et al., 2004; Devor et al., 2005). For example, Devor and coworkers (2003) observed a saturation of spiking and synaptic activity at higher amplitudes of whisker deflections, whereas the optically measured hemodynamic response continued to grow beyond the saturation of the electrophysiological activity. One of the most important findings for the visual system was recently reported by another group investigating neurovascular coupling in the cat. Niessing and colleagues (2005) observed a close relationship between LFPs in the gamma-range and the
hemodynamic response in the visual cortex. They used implanted microelectrodes and optical imaging simultaneously and parametrically varied the contrast of a visual grating and observed enhanced gamma-activity accompanied by a stronger hemodynamic response with increasing contrast level. The tight coupling between neuronal and hemodynamic fluctuations was also preserved when the contrast level was kept constant. Niessing and colleagues (2005) therefore demonstrated that fluctuation in the high LFP activity was mirrored in the hemodynamic signal, irrespective whether the source was a contrast change or a modulation of internal state variables. The interpretation of animal data and the implication of animal studies for the awake, conscious human brain remains restricted. For instance, it has been shown that pharmacological treatment, as necessary for anaesthesia in animals, strongly influences neuro-vascular coupling (Erchova et al., 2002; Villeneuve and Casanova, 2003; Stefanovic et al., 2007).

4.3.2. Neurovascular coupling studies in humans

From an experimental perspective neurovascular coupling studies in human can be divided into resting state studies with a focus on internal fluctuations and studies with external stimuli or tasks. The mutual influences of resting state and task-related states, is becoming a more central issue with improved combined EEG-fMRI/NIRS approaches (Fox et al., 2007).

4.3.2.1. Resting state studies

Resting state studies have nearly exclusively focussed on the alpha-rhythm, the dominant rhythm during relaxed wakefulness (Berger, 1929). Here, simultaneous EEG-fMRI or EEG-NIRS assessments (Goldman et al., 2002; Moosmann et al., 2003) have been used to explore the spontaneous amplitude fluctuations of the alpha-rhythm, i.e. the waxing and waning in a time range of seconds and a possible relationship with the hemodynamic response. One of the most relevant findings was the inverse relationship between alpha-power and hemodynamic signal in the occipital cortex: An increase in alpha-power coincides with a decrease of the hemodynamic activity in the visual cortex and vice versa (Goldman et al., 2002; Laufs et al., 2003a; Laufs et al., 2003b; Moosmann et al., 2003; Feige et al., 2005; Gonçalves et al., 2006; de Munck et al., 2007). This finding supports and extends the idling hypothesis of the alpha-rhythm stating that brain areas are more active during epochs of decreased alpha-power and less active during high alpha-power (Pfurtscheller et al., 1996; but see also Klimesch et al., 2007). Based on the hemodynamic results it has been proposed that a relatively inactive functional state of the brain, as indicated by high alpha-activity, origins presumably from widespread thalamo-cortical synchronization and represents a lower level of local brain metabolism. Recent studies however, revealed evidence for high intra- and inter-subject variability in the alpha-BOLD coupling (Gonçalves et al., 2006). This fact might indicate an involvement of other EEG rhythms (Laufs et al., 2003a; Laufs et al., 2003b) and/or
more complex coupling properties (Greicius et al., 2003; Laufs et al., 2003b) of several distinct resting state networks (‘default mode network’; Mazoyer et al., 2001; Raichle et al., 2001).

4.3.2.2. Activation Studies

A broad range of task designs have been used to study the relationship between neuronal and hemodynamic parameters upon stimulation. The linear coupling between fMRI and neuronal amplitude in the human somatosensory cortex as a function of stimulus intensity has been tested by Arthurs and colleagues (Arthurs et al., 2000; Arthurs et al., 2007). They compared somatosensory evoked potentials (SEP) and the BOLD-amplitude with varying intensities of electrical median nerve stimulation. The group found an increase in electrical and hemodynamic parameters with increasing intensity and observed a linear coupling in 4 of 5 subjects. This finding is in line with the assumption that EEG activity reflects summed synaptic activity and that synaptic activity reveals, at least within certain limits, a linear coupling with vascular based imaging data (Logothetis et al., 2001). With respect to the visual cortex Janz and co-workers (Janz et al., 2001) investigated neural adaptation during a checkerboard paradigm with different interstimulus intervals (ISI) and observed a trade-off between visual evoked potentials (VEP) adaption and fMRI-BOLD amplitude: The assumption of a linear superposition of single-event responses only held for ISIs above 2 s. For shorter ISIs however, the linear model failed to adequately predict the BOLD amplitude. This was also the case when the linear model included the habituated VEP time course. Janz and colleagues attributed the linear trade-off between BOLD and VEP magnitude during ISI change to a nonlinear mechanism between oxygen consumption; blood-volume and blood-flow. A number of studies investigated the effect of temporal frequency of a visual stimulus (flicker-light, checkerboard) on either electrophysiological parameters (Van Der Tweel and Lunel, 1965; Pigeau and Frame, 1992; Herrmann, 2001) or the hemodynamic response (PET-rCBF & fMRI-BOLD: Fox and Raichle, 1984; Kwong et al., 1992; Mentis et al., 1997; Thomas and Menon, 1998; Ozus et al., 2001). However, the coupling of EEG parameters and the hemodynamic response has not been specifically addressed for oscillations, presumably due to methodological issues (broad frequency range; EEG contamination by MR artefacts) and a complex interaction with background alpha-, beta- and gamma-oscillators that leads to resonance phenomena as observed for stimulation frequencies at 10, 20, 40 and 80 Hz (Regan, 1977; Pigeau and Frame, 1992; Herrmann, 2001).

Oscillatory activity in the gamma-range has been found in various experimental designs in animals (Eckhorn et al., 1988; Gray et al., 1989; Gray and Singer, 1989; Rodriguez et al., 1999; Fries et al., 2001a; Fries et al., 2001b; Mima et al., 2001; Henrie and Shapley, 2005) and humans (Tallon-Baudry et al., 1996; Bodis-Wollner et al., 2001; Busch et al., 2004; Brookes et al., 2005; Hoogenboom et al., 2006; Schadow et al., 2007). Though gamma-oscillations are considered a central coding mechanism across species, only a few studies specifically addressed the relationship between gamma-activity and the hemodynamic response in humans. Comparing findings from
invasive electrocorticography and fMRI (Mukamel et al., 2005; Nir et al., 2007) Mukamel and colleagues could show that single unit activity and LFPs in the auditory cortex of neurosurgical patients during a movie presentation correlated with the BOLD-contrast changes as measured in a parallel fMRI experiment (Mukamel et al., 2005). They found a strong positive correlation between the hemodynamic signal and LFP activity in the gamma-range but also a positive correlation between firing rate and high-frequency LFP (but see Logothetis et al., 2001). Moreover, Mukamel and colleagues could also demonstrate the frequency-specific coupling between BOLD signal and LFP activity. The coupling between BOLD and LFP was negative for activity in the alpha-range, and positive for the gamma-range. This finding is in line with observations from resting state studies (Goldman et al., 2002; Moosmann et al., 2003) and animal studies (Niessing et al., 2005) and supports the assumption that cognitive processing is linked to a desynchronization of lower frequencies and enhanced activity in the higher frequency spectrum.

For the visual system, Brookes et al. (2005) found an increased sustained MEG gamma-band activity to a static checkerboard stimulus in healthy humans. This gamma-band activity was co-localised to the fMRI BOLD response in the visual cortex. The experimental paradigm, however, was performed in separate MEG and fMRI sessions. Beyond gamma-activity the authors also found a sustained increase of a slow component (direct current, DC-signal) and a decrease in the alpha-band during checkerboard stimulation, which might suggest that gamma-activity is one of several electrophysiological signals with a rather unspecific contribution to the hemodynamic signal. In a similar MEG-fMRI approach Hoogenboom and group (2006) support the findings of Hall and colleagues (2005). The authors observed a good co-localization between the BOLD signal and the sustained gamma-band activity in response to a concentrically moving sine wave grating. However, they also observed a concomitant desynchronization in the alpha- and beta-range. Therefore, also Hoogenboom and colleagues (2006) could not demonstrate the specific contribution of higher oscillatory frequencies on the hemodynamic response. So far, the influence of a parametric stimulus variation and the relationship between gamma-band activity and the hemodynamic response has been tested only on animals (Niessing et al., 2005).

5. Methodological background

5.1. Electroencephalography (EEG)

EEG provides a more direct measure of neuronal activity and has a temporal resolution in the range of milliseconds but a low spatial resolution compared to MEG or fMRI. Surface EEG reflects voltage differences between electrodes positioned on the skull (Berger, 1929). The EEG signal is composed of summated activity of post-synaptic currents of thousands of pyramidal cells in the underlying cortex that have the same spatial orientation and are synchronously activated (for review see Barlow, 1983). EEG is only sensitive to currents from sources located with a radial orientation
to the skull. Because the strength of electric fields falls off with increasing distance, deep sources contribute less to the EEG signal than sources near the skull. Neuronal oscillatory activity, which can be recorded with EEG, is caused by complex interactions between inhibitory and excitatory mechanisms either on the level of single neurons mediated by intrinsic membrane properties or on the level of networks mediated by local inhibitory interneurons and feedback loops (Lopes da Silva, 1991; Singer, 1993b). Oscillatory activity can be related to functionally distinct brain rhythms that are defined by a characteristic frequency and spatial distribution. These rhythms seem to reflect different states of brain functioning and specific aspects of information processing. Whereas synchronous oscillation in the beta (15-25 Hz) and gamma (25-120 Hz) frequency range seem to reflect binding of locally distributed stimuli and memory representations (Gray et al., 1989; Singer, 1999b; Tallon-Baudry, 2003; Fries, 2005), oscillations in the theta (4-8 Hz) and alpha (8-14 Hz) frequency range have been linked to long-range thalamo-cortical and cortico-cortical connections and top-down attentional control (von Stein and Sarnthein, 2000; Klimesch et al., 2005; Palva and Palva, 2007). In addition, oscillatory activity over modality-specific sensory cortices indicates the functional state of these brain regions (Berger, 1929; Hari et al., 1997; Pfurtscheller and Lopes da Silva, 1999).

5.2. Near-Infrared Spectroscopy (NIRS) and Optical Topography

Near-infrared spectroscopy (NIRS) provides a unique tool to study non-invasively the cerebral oxygenation and hemodynamics in humans. Compared to EEG and MEG, which measure the electrophysiological signal from neurons, vascular based imaging methods such as NIRS and fMRI represent indirect techniques since they are sensitive to neuronally induced hemodynamic changes. Based on the differential spectral absorption spectra of the two mayor dynamic chromophores\(^1\), concentration changes of oxygenated (HbO) and deoxygenated (HbR) hemoglobin can be measured in the underlying tissue when light in the near-infrared range is applied. The so called 'biological window' for noninvasive spectroscopy (Cope and Delpy, 1988) denotes the near infrared range (650-950 nm) of the electromagnetic spectrum in which the absorption of light by water and hemoglobin is low enough to allow photons to penetrate biological tissue up to the cortex when applied on the adult’s head. The amount of detected light depends mainly on the absorption and scattering properties of the interrogated tissue, which is conditioned through the quantity and properties of the chromophores in the tissue. For optical spectroscopy using strongly simplified assumptions only absorption is considered (scattering is assumed to be constant). Because every chromophore has a unique absorption spectrum, several chromophores can be assessed simultaneously when the wavelength specific extinction coefficient of each chromophore is known and the number of used wavelengths corresponds or outnumbers to the assessed number of chromophores.

\(^{1}\) Chromophores refer to molecules with selective absorption properties of light, such as water, lipids, oxygenated and deoxygenated hemoglobin and cytochrome-c-oxidase.
chromophores. Compared to static absorbers (water, lipids, bone), oxygenated and deoxygenated hemoglobin (HbO, HbR) represent the most relevant chromophores for NIRS due to their dynamical variation with a stimulus. The spectral extinction coefficients (i.e. ‘colours’) of HbO and HbR differ substantially, as is well known in the visible range of the spectrum: The arteries appear red because they mainly transport oxygenated hemoglobin whereas veins are blue due to the high concentration of deoxygenated hemoglobin. For a sampled brain volume the relative concentration changes for HbO and HbR can be calculated from the light attenuation using a modified Beer-Lambert approach (Cope et al., 1989).

The contact based NIRS techniques mostly use optical fibres to guide the light from a source into the head and to transport the attenuated light from the head to an amplified detector. The source and detector probes (optodes) are placed with a distance of about 1.5 to 3.5 cm to ensure that the light samples the brain (sufficient depth penetration) and that a sufficient amount of light is collected at the amplifier. Because attenuation changes of at least two wavelengths are needed to infer changes in HbO and HbR in a sampled volume, the respective wavelengths are emitted either in temporal sequence or simultaneously by means of a frequency-encoded technique. The capability to measure simultaneously an increasing number of channels with sufficient acquisition rate allows optical topography of the whole brain. When using a sufficient number of probes with different distances between a source and several detectors even depth information can be obtained that allows high density optical tomography of a specific region of the cerebral cortex (Zeff et al., 2007; Koch et al., submitted).

6. Empirical studies

In this section the three studies the present dissertation is based on will be briefly summarized. The first two studies aimed to investigate the relationship between evoked potentials, alpha-rhythm and the hemodynamic response in a flicker-light paradigm. The goal of the third study was to investigate the relationship between gamma-band activity and the hemodynamic response for different contrast strength of a grating and to investigate the predictive value of fluctuations in the gamma-band on behavioural response latencies.

6.1. Study I: Synchronization between background activity and visually evoked potential is not mirrored by focal hyperoxygenation

Electrophysiological studies have shown that neurons in the visual cortex are entrained by external visual stimulation and respond phase-locked with the frequency of the stimulus (Herrmann, 2001). In both animals and humans such stimulus driven neuronal responses can be observed to stimulus frequencies up to 100 Hz (Gray et al., 1989; Gray et al., 1990; Herrmann, 2001). Another well documented fact is that applying frequencies which match internal oscillator frequencies, such as the alpha-rhythm, the response amplitude is enhanced compared to adjacent stimulus frequencies.
The goal of the first study was to investigate how this resonance phenomenon is reflected in the vascular response. With respect to the vascular response imaging methods as PET and fMRI observed a linear increase of the hemodynamic response with increasing stimulation frequency with a maximal response at about 8 Hz (Fox and Raichle, 1984; Kwong et al., 1992; Mentis et al., 1997; Thomas and Menon, 1998; Ozus et al., 2001) and a slight attenuation or a saturation of the response magnitude at higher frequencies (> 8 Hz). Because of the sluggish nature of the hemodynamic response, the experimental designs used for fMRI and PET covered a broad frequency range with only a few stimulation frequencies. Although 8 Hz is described as the lower tail of the alpha-frequency range no study so far addressed the impact of flicker frequencies in the alpha-range on the hemodynamic response. Study I therefore aimed to investigate whether the maximum response reported in the literature using vascular based imaging is functionally linked to the resonance phenomenon when flicker-light in the alpha-range is applied. To this end the study covered a broad range of stimulation frequencies with a 1 Hz step resolution in order to (a) validate previous electrophysiological reports on frequency specific resonance phenomena and (b) find out whether such frequency-specific local maxima are reflected in the hemodynamic response.

In a pre-study the homebuilt goggles for flicker-light and the necessary electronics were tested and several subjects underwent EEG to test the effect of various flicker frequencies. In the combined EEG-NIRS study, subjects (N = 11) were stimulated with flicker-light stimulation at frequencies of 1 Hz and 5 to 25 Hz in 1 Hz steps to cover (a) the peak frequencies reported from the hemodynamic literature, (b) the alpha-frequency range and (c) the 2nd harmonic of the alpha-rhythm. EEG was recorded from 21 electrodes according to the 10-20 system whereas optical probes (8 sources and 4 detectors, 14 sampling volumes) covered the occipital cortex. All frequencies were presented for 15 s in a pseudo-randomized order. To reduce the acquisition time, only a few baselines were inserted in order to (a) quantify power and frequency of the alpha-rhythm during rest and (b) allow the hemodynamic response to reach baseline values. Evoked potentials and steady state evoked potentials (for all frequencies > 5 Hz) were calculated from EEG-data. Frequency and power of the alpha-rhythm were calculated using Welch’s power spectral density approach (PSD). To reduce the influence of the evoked activity on alpha-power the averaged evoked potentials of each stimulation frequency were subtracted on a subject level from the raw-data before calculating the PSD. This procedure is of special relevance when stimulation frequencies are close to the alpha-frequency. The optical data were fed into a general linear model to estimate the concentration changes for HbO and HbR with respect to each stimulation frequency. Because of the overlapping hemodynamic responses a new algorithm based on linear time-invariant assumptions of the hemodynamic signal was developed and successfully applied in order to separate the individual responses for each flicker frequency. Based on the resting state alpha-frequency a normalization procedure was applied before averaging of the evoked activity, alpha-power and hemodynamic responses across

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2 Fox and Raichle (1984) used 8 frequencies to cover a range from 1 to 48 Hz
subjects to account for inter-subject variations of the alpha-frequency\(^3\): The individual response profiles (e.g. individual responses for neuronal and hemodynamic parameters across stimulation frequencies) were converted from stimulation-units in Hz to IAF-units, where 1 IAF denotes the individual alpha-frequency (IAF) and 2 IAF the second harmonic of the IAF. This procedure allowed comparing the hemodynamic response when the stimulation-frequency is close to the resting alpha-frequency.

Hypotheses
(a) The magnitudes of evoked potential, alpha-power and hemodynamic response vary in dependence of flicker frequency.
(b) The optical imaging technique allows to detect the hemodynamic response over a wide range of flicker frequencies in 1 Hz steps (22 frequencies).
(c) Critical flicker frequencies at 8 Hz and around a subject’s alpha-frequency lead to response maxima in the vascular and electrophysiological response respectively.
(d) The electrophysiological (evoked potential and/or alpha-power) and the hemodynamic signals reveal a positive relationship across frequencies (predictability of the hemodynamic response).

As expected, alpha-power and steady-state evoked responses revealed largest magnitudes at occipital electrodes. The evoked responses (measured as root mean square of the evoked response) declined with increasing stimulation frequency. Evoked responses were enhanced when the stimulation-frequency was close to the IAF (1 IAF, mean frequency: ~11 Hz) and it’s subharmonic (0.5 IAF, ~5 Hz), which might be regarded as further evidence that evoked response and background alpha-rhythm are not independent phenomena as stated by the additive model (Makeig et al., 2002; Hanslmayr et al., 2007). Evoked activity was strongest for frequencies below 15 Hz. Compared to the resting power the induced alpha-power without evoked fraction was reduced across stimulation frequencies. Alpha-power decreased with stimulus frequency to reach a minimum at 9 Hz. At the IAF, there was a clear increase in alpha-power, which was larger than the resting alpha-power but nearly identical to resting alpha-power after subtraction of the average VEP. For total (sum of induced and evoked alpha-power) and induced alpha-power a distinct local maximum was observed for frequencies that match the individual alpha-frequency. Beyond 11 Hz, alpha-power showed a tendency to decrease with stimulus frequency. Concerning the vascular response the decrease in HbR (which is inversely correlated with the BOLD-contrast, Kleinschmidt et al., 1996) increased in amplitude with increasing stimulation frequencies up to 7-8 Hz (~0.75 IAF) and yielded smaller responses for frequencies beyond 8 Hz. Hence, in line with previous vascular based imaging studies, the study revealed a maximized hemodynamic response

\(^3\) The stimulation frequencies were fixed for all subjects, whereas the individual alpha-frequency (IAF) varied between 8 Hz and 12 Hz. The normalization procedure was applied to sharpen the grand average response profiles of each parameter across frequencies.
for stimulation frequencies at about 8 Hz. However, the electrophysiological resonance at the IAF was not reflected in an enhanced vascular response. Compared to adjacent frequencies, stimulation with flicker frequencies close to the IAF elicited a hemodynamic response of about the same size as for neighbouring frequencies.

Based on the comparison between electrophysiological and hemodynamic parameters two main conclusions were drawn from this study. First, the resonance phenomenon at the IAF and the strongest hemodynamic response to flicker-light are two independent phenomena: A resonance phenomenon between evoked potentials and alpha-power yielding a maximal amplitude of the VEP at this frequency can be reliably found for all subjects, whereas the hemodynamic response is maximal at about 8 Hz. Here, the benefits of simultaneous EEG and NIRS and the normalization procedure allowed to verify whether the hemodynamic maximum at about 8 Hz is related to the alpha-rhythm. The second conclusion builds on this relative dissociation between the hemodynamic and the electrophysiological response modalities to stimulation in the IAF-range. Originally and in line with other sensory systems (see Arthurs et al., 2000) it was expected that the strongest electrophysiological response should be accompanied by a comparable maximization of the hemodynamic response amplitude. Although evoked responses and even alpha-activity were strongest for stimulation in the IAF-range, no comparable peak response was observed for the hemodynamic response. This phenomenon can be regarded as a trade-off between neuronal and hemodynamic signal. The magnitude of the evoked response can not explain the hemodynamic finding. It is known, however, that resting state fluctuation of occipital alpha-power is inversely linked to the hemodynamic signal (Goldman et al., 2002; Laufs et al., 2003a; Moosmann et al., 2003): Here a large alpha-power is related to a small hemodynamic change and vice versa. Thus, it was assumed that the insensitivity of the vascular response to the electrophysiological resonance is a sum of activation (evoked activity) and deactivation (alpha-power). This scenario will lead to a hemodynamic ‘zero’-effect because both evoked activity and alpha-power are enhanced for IAF-stimulation. Beyond that, another explanation of the findings was discussed. It was observed that the subtraction algorithm reduced alpha-power most dominantly if the stimulation frequency was close to the IAF. Therefore it was assumed that compared to other stimulation frequencies, stimulation with the IAF leads to a strong phase-alignment/phase-locking of ongoing alpha-activity which results in the observed peak magnitudes in the EEG without the necessity for a strong hemodynamic response. The hemodynamic response is small because the resonance peak observed in the EEG originates from an energy-efficient synchronization or resetting mechanism that rather strengthens the temporal precision of neurons instead of an additional recruitment of neurons.
6.2. Study II: Individual alpha-frequency correlates with amplitude of visual evoked potential and hemodynamic response

In summary Study I revealed that the coupling between neuronal and hemodynamic response is not simply a linear relation when comparing the stimulation induced changes in the alpha-rhythm, evoked potentials and the vascular response. This finding deviates from the straightforward coupling as reported based on the investigation of variations in stimulus intensities in the somatosensory system (Arthurs et al., 2000). In Study I neither evoked potentials nor alpha-power predicted the magnitude of the hemodynamic response. Even a superposition of both predictors failed to explain the observed hemodynamic response profiles (analysis not included in the original publication), although both evoked potentials and alpha-rhythm dominate the EEG signal and are known to originate from occipital sources. Nonetheless (inverse) correlations between alpha-power and vascular response have been reported (Goldman et al., 2002; Moosmann et al., 2003). To address this apparent discrepancy it was hypothesized that specific aspects of the EEG-signals might have a modulatory influence on the hemodynamic response. Since spontaneous fluctuations in alpha-power have been shown to inversely correlate with BOLD-contrast it was examined whether and which resting state parameters of the individual alpha-rhythm govern frequency and amplitude of an alpha-band and whether such an interindividual variance may be reflected in the magnitude of the hemodynamic response. The aim of the second study was therefore to shed light on the predictive values of amplitude and frequency of the resting state alpha-rhythm on evoked potentials, alpha-power and the hemodynamic response during stimulation. This hypothesis is linked to the observation that evoked potentials and background activity are no independent phenomena (Brandt and Jansen, 1991; Schurmann and Basar, 1994; Barry et al., 2000; Makeig et al., 2002; Shah et al., 2004). The resonance phenomenon (Study I), which occurs when an input frequency matches an endogenous oscillatory frequency can be regarded as example for the evidence of a functional link between an internal rhythm and the evoked potential (Pigeau and Frame, 1992; Herrmann, 2001). As opposed to the additive model which treats background activity as noise, the phase-resetting model presumes a phase-realignment of ongoing activity upon stimulus onset. This latter model highlights the possibility that components of the evoked potentials may partially originate from synchronization of background-rhythms (Kawabata, 1972; Sayers et al., 1974; Nogawa et al., 1976; Makeig et al., 2002). Indeed, a number of studies supply evidence for the evoked potential to be partially generated by phase-resetting of background activity including the alpha-rhythm (see also Makeig et al., 2002; Yeung et al., 2004; Hanslmayr et al., 2005; Hanslmayr et al., 2007; Sauseng et al., 2007; Yeung et al., 2007). Most of the studies investigated the trial-by-trial relationship between amplitude or phase of the baseline alpha-activity

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4 Besides of other frequency bands such as theta- and beta band, gamma-activity was analyzed in Study I. Higher frequencies however revealed no systematic variation with stimulation frequency and failed to predict the hemodynamic response profile.
and the response magnitude of early components of the evoked potential to single flash stimulation. The second study addressed the question whether interindividual differences between resting state alpha-frequency and -amplitude may explain intersubject response variability to visual stimulation. A pilot-study without visual stimulation revealed a stable inverse relationship between alpha-frequency and -amplitude across subjects (assessed during 20 resting blocks with open and closed eyes, 16 subjects). In Study II subjects were measured simultaneously with EEG-NIRS. The experimental paradigm slightly differed from Study I: instead of being exposed to the same fixed stimulation frequencies, the flicker frequencies in Study II corresponded to parts and multiples of the IAF (0.1 to 1.5 IAF) whereas 1 IAF denotes the subject’s alpha-frequency at rest. For each subject the stimulation protocol was adjusted to the IAF determined from a resting epoch.

Hypotheses
(a) Individual resting state alpha-frequency and alpha-amplitude reveal an inverse relationship.
(b) These resting state properties bias the magnitudes of electrophysiological and hemodynamic parameters during stimulation.
(c) During stimulation the electrophysiological and the hemodynamic signals reveal a relationship across subjects: The hemodynamic response positively correlates with the evoked potential amplitude but negatively with the alpha-power (predictability of the hemodynamic response).

As far as the resting state alpha-rhythm is concerned, Study II revealed an inverse relationship between frequency and amplitude across subjects: Subjects with low resting alpha-frequency showed a large alpha-amplitude, whereas subjects with a high frequency showed a small amplitude. This finding has been also reported by Lopes da Silva et al. (1976, see also Pfurtscheller and Lopes da Silva, 1999). A second relationship was found between resting IAF and electrophysiological and hemodynamic parameter during visual stimulation when subjects were pooled into a low and a high IAF group: Subjects with a low alpha-frequency and large amplitude during rest exhibited large neuronal and vascular responses during stimulation compared to subjects with high resting alpha-frequency and low amplitude. More specifically, during stimulation subjects with a low IAF showed (a) larger evoked potentials, (b) larger alpha-amplitudes and (c) stronger hemodynamic responses across stimulation frequencies as compared to subjects with a high IAF. Correlation analysis across subjects revealed a good relationship between resting state IAF and the magnitudes of evoked potentials, alpha-power and the hemodynamic response (HbR). Thus, an individual’s resting state alpha-frequency partially predicts the amplitude of neuronal and hemodynamic response during stimulation. Based on these findings and on Study I, a heuristic model was introduced to explain the relationship between IAF and alpha-amplitude and the predictive outcome for evoked potential, alpha-amplitude and hemodynamic response during stimulation accommodating the following features:
A neuronal network is engaged in the generation of spontaneous alpha-oscillations during rest.

During stimulation some neuronal elements of the network generic for the alpha-rhythm will be engaged in stimulus processing.

Some other neuronal elements, which are not engaged in the alpha-rhythm, will be additionally recruited to process the stimulus (participate in the evoked potential).

The model assumes that the two networks – generic for either VEP or resting alpha-rhythm are partially overlapping, which might explain the relationship between evoked potentials and alpha-activity (Brandt and Jansen, 1991; Barry et al., 2000; Makeig et al., 2002). It has been suggested that the network involved in the generation of the alpha-rhythm during rest can be roughly classified by the frequency and amplitude of the underlying oscillation (Elul, 1971; Singer, 1993a, 1993b; Pfurtscheller and Lopes da Silva, 1999). Therefore, it was hypothesized that in subjects with a low IAF and a high alpha-power a larger network participates in the alpha-oscillation compared to subjects with a high IAF and low amplitude. It was postulated that the magnitude of the VEP depends on the size of the overall alpha-network: Subjects with a large alpha-network (e.g. low frequency and high amplitude) show enhanced evoked potentials, because more neuronal elements are potentially available and will be recruited for stimulus processing. Furthermore, subjects with a low IAF show an enhanced decrease of alpha-activity (alpha-desynchronization) during stimulation (more neurons engaged in stimulus processing) and a stronger hemodynamic response compared to subjects with a high resting alpha-frequency. Thus, the magnitude of the hemodynamic response corresponds to the size of the underlying neuronal network that is involved in stimulus processing and can be estimated by the resting alpha-frequency. The merely descriptive model relies and extends a previous mathematical alpha-model from Lopes da Silva and colleagues (1976) and is suited to explain the findings of Study I and II but also accommodates previous reports on the relationship between resting alpha-rhythm and ERP components across subjects (Kooi and Bagchi, 1964; Rodin et al., 1965; Pigeau and Frame, 1992). It may also explain the trial-by-trial variability within the same subject (Makeig et al., 2002). Beyond other previously used techniques and methods (Brandt and Jansen, 1991; Arieli et al., 1996; Barry et al., 2000) Study II furthermore demonstrates an interesting avenue to study the relationship between ongoing activity and evoked signal by assessing neuronal and hemodynamic parameters simultaneously.
6.3. Study III: Stimulus-induced and state-dependent gamma-activity is tightly coupled to the hemodynamic response in humans

Although flicker-light stimulation, used in Study I and II generates robust stimulus-locked EEG signals, the stimulus bears some limitations and disadvantages such as (a) functional relevance and naturalness of flicker-light and (b) control of internal state variations. The limited analytical frequency-range is an additional disadvantage, because higher harmonics of flicker frequencies cover oscillatory activity in higher EEG-bands. Study III aimed to investigate a more natural stimulus, incorporating a task yielding in behaviour data and especially focused on higher oscillatory activity in the gamma-range and their potential contribution to the hemodynamic signal.

It has been shown that gamma-band activity plays an important role in visual perception (Hoogenboom et al., 2006) and top-down processing such as visual feature binding (Eckhorn et al., 1988; Gray et al., 1989; Gray and Singer, 1989; Tallon-Baudry et al., 1996; Mima et al., 2001), attention (Fries et al., 2001a) and visuomotor control (Rodriguez et al., 1999). The modulation of gamma-band activity upon parametric variation of stimulus properties (e.g. stimulus contrast, spatial frequency, motion strength, size and eccentricity) has been studied in animal and human (Tzelepi et al., 2000; Bodis-Wollner et al., 2001; Logothetis et al., 2001; Busch et al., 2004; Hall et al., 2005; Henrie and Shapley, 2005; Liu and Newsome, 2006; Hadjipapas et al., 2007; Schadow et al., 2007; Siegel et al., 2007). For example, Hall and colleagues (2005) used a paradigm described by Logothetis and group (2001) to investigate the coupling in humans. By means of MEG the group found a linear increase of occipital gamma-activity with stimulus contrast. The finding is strikingly similar to the LFP gamma-activity in macaques (Logothetis et al., 2001). This positive relationship between contrast strength and gamma-band activity was also observed in a recent EEG study (Schadow et al., 2007). Beyond the fast growing number of reports in the electrophysiological field only a few studies investigated the relationship between gamma-activity and the hemodynamic response. A close coupling between gamma-range LFP oscillations and the invasively measured hemodynamic response has been reported in the cat’s visual cortex (Niessing et al., 2005). The simultaneous study (implanted microelectrodes and optical imaging) used visual gratings of varying contrast strength. Niessing and colleagues observed enhanced gamma-activity accompanied by a stronger hemodynamic response with increasing contrast level. Although both signals fluctuated during constant stimulus properties the tight coupling between neuronal and vascular response was preserved. In the human visual cortex sustained gamma-activity has been reported in response to checkerboard pattern and gratings by means of MEG (Brookes et al., 2005; Hoogenboom et al., 2006). Both studies found a good co-localization between gamma-activity and BOLD response derived from a successively performed fMRI assessment. The authors also observed sustained alterations in other frequency bands (DC-signal change, alpha-desynchronization). Hence it may be concluded that several frequency bands contribute to the hemodynamic signal. Which neuronal signal (DC, alpha- or gamma-band) is the major source of the hemodynamic signal remains an open
question. In humans Mukamel et al. (2005) and Nir et al. (2007) combined invasive electrocorticography and fMRI in a simultaneous approach. They investigated patients who underwent presurgical evaluation due to intractable epilepsy in an elegant approach using a highly complex stimulus (movie). Both studies report a good relationship between gamma LFP and BOLD.

So far, only Niessing et al. (2005) investigated the influence of a parametric stimulus variation and the relationship between gamma-band activity and the hemodynamic response in animal. Study III hence addressed the question whether the findings of Niessing et al. (2005) are transferable to humans. In two EEG pre-studies the spatial frequency and velocity of a concentrically moving sine wave grating were varied at different contrast strengths in order to obtain basic stimulus parameters which elicit a strong gamma-band activity. In two other subjects (EEG and NIRS) a sustained gamma increase was observed for the entire stimulus duration of up to 70 s accompanied by an increase of HbO and a decrease of HbR for the same stimulus duration. In Study III EEG and NIRS were recorded simultaneously using a comparable setup as in Study I and II. The experimental paradigm consisted of an ongoing concentrically moving sine wave grating with five different contrast levels and an additional ‘zero’-contrast serving as a baseline. The behaviourally irrelevant contrast changed pseudo-randomly every 7 s, whereas subjects had to respond to a slight velocity alteration (2 levels) of the grating during constant contrast periods. The rationale of the task was to maintain a certain level of attention and to obtain a behavioural parameter as an additional source to explain fluctuations of the neuronal and hemodynamic signal. The latter is of relevance: A recent study in trained monkeys showed a clear augmentation of gamma-band LFP and spike-field coherence when attending a stimulus as compared to the unattended perception of the same stimulus (Womelsdorf et al., 2006). The data analysis consisted mainly of (a) a wavelet approach to analyze the contrast-related temporal spectral dynamics in the EEG data, (b) a general linear model to estimate the accompanied contrast-specific hemodynamic response and (c) a trial sorting technique for temporal-spectral EEG data and hemodynamic signals to account for the detection related behavioural response latencies within the same contrast.

Hypotheses
(a) An increase in contrast-strength leads to an increase in behavioural performance (faster reaction time (RT) and improved detection rate for the velocity change of the grating).
(b) A positive relationship exists between contrast-strength, occipital gamma-activity and the magnitude of the hemodynamic response (external modulation: predictability of the hemodynamic response).
(c) The behavioural response variability during the same contrast-strength is also evident in the magnitudes of gamma-activity and the hemodynamic response (internal fluctuation: predictability of the hemodynamic response).
Study III revealed a sustained gamma-activity enhanced over the entire 7 s stimulation period at occipital electrodes. Both gamma-activity and the hemodynamic response were driven by stimulus contrast and increased logarithmically with Michelson contrast. Lower EEG frequency bands varied but revealed no systematic modulation with contrast strength. Within the same contrast, reaction times to the velocity change varied substantially. To further examine this ‘spontaneous’ variability the neuronal and hemodynamic data during the strongest contrast were grouped based on the reaction time. Gamma-activity in the time window 200–100 ms prior onset of the velocity change revealed an inverse relationship with reaction time: Fast reaction times were heralded by stronger gamma-activity compared to medium and slow RTs. Moreover, fast reaction times also revealed stronger oxygenation changes compared to medium and slow RTs. Thus, gamma-power prior to the relevant stimulus predicted the speed of reaction. The latter finding closely matches the results from the invasive study on speed of change detection in monkeys (Womelsdorf et al., 2006). Taken together, Study III shows a close coupling between gamma-activity and the hemodynamic response in human, irrespective of whether the modulation is triggered by the stimulus level or originates from internal state variations such as shifts in the level of attention. Therefore, Study III supports the view that oscillatory activity in the gamma-range and the hemodynamic response are also tightly coupled in humans. Furthermore, this study provides further evidence for the pivotal role of gamma-oscillations in visual perception and attention (Gray and Singer, 1989; Fries et al., 2001a) and demonstrates the feasibility to use near-infrared spectroscopy as an indirect measure for higher oscillatory EEG-activity.

7. General discussion

Simultaneous assessment of the electrophysiological and vascular response is mandatory to better elucidate neurovascular coupling also in humans. While simultaneous EEG-fMRI approaches have been increasingly used over the past decade, until now this approach is challenging, particularly because EEG data are strongly contaminated with fMRI-related gradient and cardioballistic artefacts. Especially the recovering of high oscillatory EEG activity in the gamma-range bears technical and analytical challenges (Anami et al., 2003; Freyer et al., 2009).

The presented work aimed to investigate the relationship between electrophysiological and hemodynamic signals noninvasively in the human visual cortex focusing on rhythmic brain activity and their potential contribution to the hemodynamic response. Methodologically all studies build on the combination of EEG and optical imaging, which have been demonstrated to be interference-free. To study the neurovascular coupling in the human visual system two different types of stimuli were used. The stimuli evoke characteristic electrophysiological pattern known from animal and human and focus either on rhythmic activity in the alpha-range or in the gamma-range. Both alpha- and gamma-activity have been localized in visual areas and have been linked to
cortical activation and visual processing. Specifically, the present studies were conducted to address the question how the hemodynamic signal is linked to the elicited electrophysiological pattern during variation of a stimulus property such as flicker frequency and contrast-strength.

In the first study flicker-light over a broad range of frequencies was used. The study focused on the resonance phenomenon in the alpha-range known from electrophysiological studies and the repeatedly reported finding derived from hemodynamic based studies, a maximum hemodynamic response at around 8 Hz flicker-light. One question was whether the two findings, derived from electrophysiological or hemodynamic based techniques, refer to the same phenomenon. Beyond this, the question was addressed whether alpha-activity during stimulation provides additional useful information to predict the hemodynamic signal. As shown by others flicker-light yielded to stimulus-locked neuronal responses with the same frequency as the stimulus (Herrmann, 2001). Alpha-activity was attenuated during application of flicker-light, regardless which frequency was used, corroborating the link between perceptual processing and alpha-band desynchronization (Pfurtscheller, 2001). Compared to adjacent stimulation frequencies the EEG revealed a resonance-like enhancement in VEP-magnitude and augmented alpha-activity when the stimulation frequency matches the resting state alpha-frequency. This finding, also observed in animals and humans, indicates an interaction between stimulus frequency and an internal oscillator frequency (Herrmann, 2001). Although the electrophysiological activity was augmented during alpha-stimulation, the hemodynamic response revealed no systematic changes compared to adjacent stimulus-frequencies. Conversely, the strongest hemodynamic changes were observed at about 8 Hz, replicating fMRI and PET results (Fox and Raichle, 1984). At 8 Hz no electrophysiological change was found for VEP and alpha-activity. The finding of Study I is therefore twofold. First, the resonance phenomena observed in electrophysiological studies and the strongest response at 8 Hz derived from hemodynamic based studies refer to different phenomena. It has been suggested that the strongest hemodynamic response at 8 Hz refers to the resonance phenomena in the alpha-range. Based on the simultaneous EEG-NIRS assessment and a finer sampled flicker frequency range the study shows that both phenomena do not coincide. The 8 Hz maximum of the vascular response remains obscure. Although the Brücke-Bartley effect has been discussed by Fox and Raichle (1984), predicting that the largest population of cortical neurons respond to frequencies adjusted to the ‘activity–recovery cycle’ in the retina-cortex pathway (Grusser and Creutzfeldt, 1957; Bartley, 1968), the here used non-invasive methods cannot verify this assumption. Moreover, compared to adjacent frequencies stimulation at 8 Hz rather revealed a moderate VEP-amplitude and non-specific changes in the alpha-amplitudes. The resonance phenomenon on the other hand yielded no additional increase in the hemodynamic response. The study could demonstrate that compared to other stimulation frequencies the phase-locking of alpha-activity is enhanced when the flicker frequency is close to the alpha-frequency. Based on the ‘insensitivity’ of the hemodynamic response for the electrophysiological resonance at stimulation frequencies in the alpha-range it was
inferred that no additional metabolic demand is elicited. It was assumed that the resonance phenomenon rather origins from low energetic synchronization via a phase-resetting mechanism instead of enhanced synaptic activity for example via recruitment of additional neurons. Study I also revealed that neither evoked potential nor alpha-activity sufficiently predicts the hemodynamic response across flicker frequencies. For variation of temporal frequencies of a visual stimulus the results strongly contrasts findings in human somatosensory cortex where a linear relationship between evoked potential amplitude and fMRI-BOLD response was observed during increase of stimulus intensity (Arthurs et al., 2000).

Study II was conducted to extend the findings of Study I and to focus on the potential contribution of resting state parameters on electrophysiological and hemodynamic measures during flicker-light application. For resting state an inverse relationship between alpha-amplitude and frequency was observed across subjects, corroborating findings of Lopes da Silva (1976) and Pfurtscheller and Lopes da Silva (1999). When comparing resting state alpha-frequency and magnitudes of evoked EEG and NIRS parameters, Study II revealed that background activity and stimulus-induced neuronal and hemodynamic activity are no independent phenomena. The resting alpha-frequency showed an inverse relationship with the amplitudes of evoked potentials, alpha-power and the hemodynamic response across subjects during flicker-light. The finding might explain the large intersubject variability in EEG and fMRI studies observed during stimulation and also during resting states (Gonçalves et al., 2006). Therefore Study II provides further evidence that background activity and evoked potentials interact with each other (Brandt and Jansen, 1991; Schurmann and Basar, 1994; Barry et al., 2000; Pfurtscheller, 2001; Makeig et al., 2002; Shah et al., 2004). Furthermore, it can be shown that resting state parameters have predictive value for the hemodynamic response, highlighting the fact that both stimulus-related electrophysiological and hemodynamic changes behave similar across subjects.

Study III aimed to investigate the role of higher oscillatory activity in the gamma-range and its potential contribution to the hemodynamic response. A number of electrophysiological studies corroborate the assumption that gamma-activity is of central relevance for visual perception, visual feature binding and attention (Eckhorn et al., 1988; Gray et al., 1989; Gray and Singer, 1989; Tallon-Baudry et al., 1996; Fries et al., 2001a; Mima et al., 2001; Hoogenboom et al., 2006). In human, the relationship between higher oscillatory activity and hemodynamic response is still insufficiently resolved. Since gamma-modulations were not observed in Study I and II and also not reported for flicker-light in the literature (Sewards and Sewards, 1999), Study III used a moving grating in a detection task and tested the influence of a parametrical contrast-variation. Similar to MEG results (Brookes et al., 2005; Hoogenboom et al., 2006) Study III revealed a sustained response in the gamma-range over the entire stimulation period. The behavioural performance during the detection task strongly depended on the particular contrast. The contrast on the other hand modulated the gamma-power to that effect that an increase in contrast-strength revealed an
equal increase in gamma-band activity. This finding is in line with results in animal (Henrie and Shapley, 2005; Niessing et al., 2005) and shows the contrast-related modulation of sustained activity also in human. The result extends previous findings for contrast-related transient gamma-band modulation in humans (Schadow et al., 2007). In comparison, the lower frequency bands revealed no systematic variation with contrast-level. As for the gamma-band activity, the hemodynamic response increased parametrically with the contrast of the grating. A tight relationship between gamma-activity and the hemodynamic response has been reported in animal (Niessing et al., 2005). Study III is capable to reveal a similar relationship also in human: although gamma-activity and the hemodynamic response increased logarithmically with Michelson contrast, both signals revealed a tight linear relationship. Additionally it was found, that fluctuations of the behavioural response during a constant contrast were linked to the power in the gamma-range some hundred milliseconds prior onset of the behaviourally relevant task. Here, an inverse relationship was observed between reaction time and gamma-band activity. This observation closely matches the results from the invasive study on speed of change detection in monkeys (Womelsdorf et al., 2006). Furthermore, also the hemodynamic response varied systematically with response time. Thus fast reaction times were preceded by comparable strong gamma-activity and revealed strong oxygenation changes compared to medium and slow reaction times. Study III is therefore capable to replicate the findings in animal (Niessing et al., 2005) and even extends the results with respect to internal state variations in gamma-activity which have been found to appear behaviourally relevant (response time). For parametrical variation of contrast-strength but also state-dependent fluctuations during a constant contrast Study III is feasible to show that gamma-activity and the hemodynamic response are tightly linked.

The studies focus on oscillatory components of the EEG and their potential contribution to the hemodynamic signal. Rhythmic activity in the alpha- and gamma-range was investigated since both brain rhythms seem to have a multifunctional purpose and appear to be functionally related to the visual system. Although a number of attempts were made to elucidate the coupling behaviour in the last two decades, an explicit linkage between the two is still missing and the neuroimaging community faces two complementary but unrelated sets of functional descriptions of the human brain (Lachaux et al., 2007). The question which combinations of EEG patterns affect most strongly the hemodynamic signal is by far not resolved and needs to be answered in the future in order to overcome the interpreting limits of the hemodynamic signal with respect to the underlying neuronal activity.
8. Conclusion

The results of this dissertation contribute to the existing literature on neurovascular coupling by suggesting that:

- Simultaneous measurement of neuronal and hemodynamic signals by means of EEG and NIRS represents a powerful tool to study the relationship between direct and indirect neuronal signals in human.
- The resonance phenomenon, a local maximum that appears when the stimulation frequency matches the endogenous alpha-frequency, is not accompanied by an increase in vascular parameters. The scenario depicts a mismatch between the hemodynamic response and dominant electrophysiological signals such as evoked potentials and alpha-power.
- Electrophysiological resting state parameters can provide predictive information for the magnitude of evoked potential, alpha-power and the magnitude of the hemodynamic response upon stimulation.
- Evoked potential and ongoing oscillatory activity in the alpha-range represent no independent electrophysiological signals. The entangled relationship of both signals is manifested in the resonance phenomenon and the relationship of resting alpha-frequency and magnitude of evoked potentials.
- Oscillations in the gamma-range play a pivotal role in visual processing and behavioural performance. The parametrically varied visual stimulus yield to systematic changes in the gamma-band only. Faster response times were preceded by a phasic increase in gamma-band activity.
- Higher oscillatory activity represents a neuronal component with a major influence on the hemodynamic signal, irrespective whether the modulatory component origins from external stimulus variation or internal state modulation.

Taken together, the work summarized in this dissertation supports the view that evidence from complementary experiments in humans and animals, as well as the application of concurrent direct electrophysiological based and indirect vascular based methods, can help to elucidate the neurovascular coupling mechanisms. These are of relevance on basic scientific grounds and need to be understood in more detail to fully appreciate the results of neuroimaging techniques relying on the vascular rather than electrophysiological response.
References


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Appendix

Original articles, Study I-III
Publications and Presentations
Peer Reviews
Curriculum Vitae
Eigenständigkeitserklärung
Synchronization between Background Activity and Visually Evoked Potential Is Not Mirrored by Focal Hyperoxygenation: Implications for the Interpretation of Vascular Brain Imaging

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We performed an electroencephalography and optical topography study simultaneously exploring electrophysiological and vascular response magnitude as a function of stimulus frequency. To elicit a response in the visual cortex, subjects were exposed to flicker frequencies varying from 1 to 25 Hz (1 Hz steps, eyes closed). Extending the standard view to compare magnitudes of the evoked neuronal to the evoked vascular response, we additionally investigated modulations of $\alpha$-power, a marker of “background” EEG activity.

The results show two discrepancies between the electrophysiological and vascular response: (1) VEP and $\alpha$-power exhibit a discontinuous peak when stimulating at the individual $\alpha$-frequency (IAF) ($\sim 10 – 11$ Hz), indicating resonance between background oscillations and evoked response; this is not mirrored by the vascular response. (2) The vascular response, in contrast, steadily increases up to a maximum at 7 – 8 Hz and slightly decreases with higher frequencies. This continuous frequency dependence is partly reflected by the decrease in $\alpha$-power up to frequencies of 8 – 9 Hz and a slight increase in $\alpha$-power beyond the IAF resonance. Although indicating an inverse relationship between $\alpha$-power and vascular response, the frequency dependence of the evoked response does not show such a correlation.

Thus, electrophysiological resonance between an individual’s $\alpha$-frequency and isofrequent stimulation is not mirrored by the vascular response. Also, spontaneous background EEG activity is an important modulator of the vascular response magnitude. We discuss these deviations from a simple one-to-one translation between evoked potential and vascular response amplitude in the light of questions concerning synchronization, attenuation, and induction of background oscillations such as the $\alpha$-rhythm.

Key words: flicker; steady-state visual-evoked potential (ssVEP); $\alpha$ oscillatory activity; hemodynamic response; near-infrared spectroscopy (NIRS); neurovascular coupling; visual cortex

Introduction

Beyond doubt, changes in neuronal activity are tightly coupled to focal changes in cortical blood flow. This constitutes the option to map the hemodynamic response and infer principles of the cortical processing, even of complex tasks (Villringer and Dünagl, 1995). Nonetheless, recent research has highlighted the fact that the basis of such noninvasive brain imaging may be far more complex because of fundamental deviations from a straightforward translation of neuronal excitation and inhibition into respective increases or decreases in vascular response (Mathiesen et al., 1998; Logothetis et al., 2001; Hewson-Stoate et al., 2005).

Here we highlight a related issue, dealing with the fact that “at rest” the brain entertains an impressive electrophysiologically accessible activity, which is modulated and/or superimposed by an evoked response (Makeig et al., 2002). [For the complex relationship between intracortical and scalp-recorded electrophysiological potentials, see Mitzdorf (1985) and Lopes da Silva (2004)]. Because modulation of such “background” activity has been shown to elicit regional blood oxygenation level-dependent (BOLD) changes (Goldman et al., 2002; Laufs et al., 2003; Moosmann et al., 2003), one motivation for our study was to modulate both spontaneous oscillations and evoked potential amplitude in a parametric visual stimulation design, thus investigating their respective influence on regional cerebral blood flow (rCBF) changes. To this end, we examined a flicker-light stimulation over a wide frequency range, while subjects kept their eyes closed. Separating the amplitude of the visual-evoked potential (VEP) from $\alpha$-power modulation, two potential predictors of the simultaneously assessed vascular response can be analyzed.

Another goal of the study was to address a surprisingly neglected, although striking discrepancy between the frequency dependence of the vascular response and VEP amplitude. Vascular-based techniques report on a continuously differentiable increase in response up to $\sim 8$ Hz (Fox and Raichle, 1985; Kwong et al.,...
was started. Stimulation blocks. Montage of EEG and near-infrared spectroscopy experiment lasted 120 trials without stimulation and 18 trials without stimulation. All trials had a duration of 15 s. The interval between flashes, i.e., for the highest frequency (25 Hz), the 5 ms flashes were separated by 35 ms intervals, whereas at 1 Hz stimulation the interval between flashes, i.e., for the lowest frequency (1 Hz), the 5 ms flashes and an interflash interval of 45 ms. For a stimulation frequency of, e.g., 10 Hz, the duration of the flash (5 ms) remained constant, and the interflash interval was 95 ms (data not shown).

**Subjects**

From an initial EEG study (without coregistration of NIRS; data not reported here), we selected 11 of 21 subjects, who showed a well detectable α-rhythm. None of the thus selected subjects had to be excluded from additional analysis in the here reported combined EEG-NIRS study (2 male, 9 female; mean age, 25.4 years (range, 18–28 years)). All subjects had normal vision or mild hyper-/myopia, were neurologically and otherwise healthy with unremarkable medical history (especially no migraine or epileptic seizures). Correction of mild hyper-/myopia was not mandatory because full-field stimulation was applied while eyes were closed. All subjects gave informed consent and were financially rewarded for their participation. Beyond tiring, none of the subjects reported any major discomfort.

**Data acquisition**

EEG. To assess α-power and sVEP, the EEG (BrainAmp amplifier and Visor Recorder software; Brain Products, Munich, Germany) was recorded over 21 standard positions according to 10-20 system (Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, FC1, FC2, CP1, CP2, FC5, FC6, CP5, CP6, Pz, Cz, Pz). Three additional channels recorded the horizontal and vertical EOG (hEOG, vEOG; left horizontal, right horizontal, and vertical upper electro-oculogram, respectively). During recording, all electrodes were referenced to Fcz, and data were recorded at a 1000 Hz sampling frequency (0.3–70 Hz frequency range with 50 Hz notch and 3 s amplification).

**Optical imaging.** As an indicator of the vascular response, we assessed changes in hemoglobin oxygenation measured over the occipital region using a frequency-domain NIRS imaging system, based on a multichannel Omnirad Tissue Oxymeter (ISS, Champaign, IL). We here focus on the decrease in deoxy-hemoglobin, which can be only explained by a faster washout during an increase in regional cerebral blood flow. Also, the content of [deoxy-Hb] is the most relevant physiological parameter determining the BOLD contrast. A decrease in [deoxy-Hb] is correlated to an increase in BOLD contrast (Kleinschmidt et al., 1996). Details of the methodology and the underlying physiology were detailed previously (Obrig and Villringer, 2003). In brief, light in the near-infrared penetrates biological tissue rather well, thus allowing for optical spectroscopy in deeper tissue layers such as the cerebral cortex. Changes in oxygenation of hemoglobin elicit a change in absorption at different wavelengths, broadly corresponding to the well known color change of venous and arterial blood in the visible range. Using two wavelengths and applying a modified Beer-Lambert law, concentration changes in oxygenated and deoxygenated hemoglobin can be thus assessed (δ[oxy-Hb] and δ[deoxy-Hb]).
Figure 2. The flow chart illustrates the analysis of EEG and NIRS data. NIRS: After filtering and downsampling, a GLM was applied to determine the pixels (ROI) in whom the difference between rest and stimulation at any frequency was most significant. Next, an attenuation of global effects was performed (see Materials and Methods). For the time courses in the ROI (see data for the different frequencies, compare Fig. 4b, open vs filled circles).

EEG

- Evoked activity
- Alpha activity
- Oxygenation changes

Data analysis

Data were analyzed with programs written in Matlab in part using pre-programmed routines (version 6.5; The Mathworks, Natick, MA). For all analyses concerning frequency dependence of the stimulus-locked activity and α-activity, data were derived from electrode O2. Because the detailed description of the different steps of our analysis of EEG and NIRS data may be beyond some readers’ interest, we supply a simple flow chart of the data processing in Figure 2. In summary, we performed the analysis to yield frequency dependencies of (1) the evoked electrophysiological response (VEP/ssVEP); (2) an indicator of the ongoing electrophysiological background activity (α-power); and (3) the vascular response as indicated by the hemoglobin-oxygenation changes. Because the IAF differs between subjects, we normalized the frequency dependencies to the IAF.

6% (deoxy-Hb)) (Cope and Delpy, 1988). Our system uses laser diodes emitting light at 690 and 830 nm, respectively. The intensity of the incident light is modulated at 110 MHz. Guided to the subject’s head by fiber-optic bundles (diameter, 0.4 mm), some of the light will be reflected and collected by another optic probe (diameter, 3 mm) some centimeters apart, to guide the light to the detector (photon multiplier tube). The resulting changes in the intensity changes and in the phase shift of the modulation wave allow a description of changes in optical density in the illuminated tissue (the option of depth resolution (Kohlf-Barreis et al., 2002) was not applied here). Assuming constant scatter, these changes can be converted into changes in [oxy-Hb] and [deoxy-Hb] in the tissue illuminated. To provide a rough topographical image, the system uses seven emitter and four detector channels arranged in an array covering 50 cm² of the occiput. The center of the probe array was 2 cm above the inion. Thus, the array covers the area around electrodes O1 and O2. The position of the optical probes in relation to the electrodes is 2.7 cm). Within one subject, the interprobe distance was constant. Data were recorded at 10 Hz.

Optical imaging

The vascular response was assessed by the analysis of changes in [deoxy-Hb] and [oxy-Hb] as measured by near-infrared spectroscopy. Because changes in [deoxy-Hb] correspond to BOLD contrast changes as assessed by functional magnetic resonance imaging (fMRI) (Kleinschmidt et al., 1996), the forthcoming analysis focuses on [deoxy-Hb]. After calculation of the concentration changes in the concentration of hemoglobins, we attenuated the pulse-related signal changes by a lowpass filter (Butterworth, fifth order) at 0.5 Hz. Optical images were generated over a ~50 cm² area overlying the occipital cortex by interpolation of the 14 emitter–detector combinations measured (Fig. 1a).

The additional analysis comprised a number of successive steps. Selection of “activated pixels.” The first step was performed to determine the pixel with the most significant difference between rest periods and stimulation. Thus, all trials with stimulation (1 Hz and 5–25 Hz) were considered the on-condition, whereas resting periods (n = 18) served as off-condition. In analogy to statistical parametric mapping (e.g., Statistical Parametric Mapping software SPM99; Wellcome Department of Cognitive Neurology, London, UK), the predictor (boxcar of on-condition vs off-condition) was convoluted with a negatively accelerated exponential function corresponding to the average rise of the dipole direction stems from the fact that a decrease in [deoxy-Hb] corresponds to an increase in BOLD contrast. Statistics followed β-value estimates along the principles of the general linear model (GLM).
Attenuation of “global effects.” In a second step, global effects were attenuated across all measurement positions. The hypothesis is that such effects are likely to stem from movement artifacts or from hemodynamic changes unrelated to the stimulation (breathing, low frequency oscillations, slow changes in blood pressure, and residual heart beat). Because our prediction is that the global effects are not correlated with the stimulation, we chose those six positions with the smallest t values extracted from the first analysis. Those time series were then fed into a principal component analysis (PCA). Note that only six pixels where used, to prevent an attenuation of the stimulation-induced changes. The resulting orthogonal time courses were used as covariates in the subsequent analysis.

Frequency dependence. To compare [deoxy-Hb] changes for the different stimulation conditions (frequencies), the input response function for each frequency was convoluted with the hemodynamic response function (i.e., predictors for each stimulation frequency were determined). Finally, all predictors and the covariates (as obtained by the PCA) were integrated in the GLM analysis. For each subject, the location with the largest t value was selected to determine the dependence of [deoxy-Hb] changes from the stimulation frequencies.

Before averaging across subjects, we normalized the individual subject’s data with respect to frequency and amplitude.

Frequency normalization. Frequencies of the human EEG can be expressed as units relative to the individual central α-frequency. Because we here focus on stimulation-induced modulation of α-rhythm, we performed a frequency normalization. For example, in a subject with an IAF of 8 Hz, the 5 Hz stimulation corresponds to a stimulation at 0.63*IAF, whereas the stimulation at 25 Hz corresponds to 3.13*IAF. In a subject with an IAF of 12 Hz, correspondingly, frequencies from 0.42*IAF to 2.08*IAF were tested. This conversion is based on the underlying hypothesis that biological response will depend on the biological frequencies rather than on absolute numeric values.

Amplitude normalization. For each subject, the frequency-normalized data of all parameters were z-scored using the formula Zi = (Pi – mean (Pi))/std (Pi), where P means the data of the particular parameter for the subject i. Next the z-scored data were averaged across subjects. Finally, we retransformed data to absolute values conserving mean and variance of the original data. For an example of the normalization procedures, see supplemental material, available at www.jneurosci.org.

Results
The central issue of the present paper is the differentiation between evoked electrophysiological activity (ssVEP) and spontaneous background activity to investigate their respective influence on the vascular response. This section first presents the ssVEP results to then describe stimulation-frequency dependent changes in α-power. The latter is considered the most prominent feature of electrophysiological background activity over the occipital cortex. Finally, the results of the NIRS measurements are shown to yield a tentative hypothesis on the mutual dependence of the two electrophysiological predictors and the vascular response. It should be noted that evoked or induced γ-power changes are another intriguing oscillatory electrophysiological signal, which has been discussed with respect to the vascular response (Niessing et al., 2005). We did not find any reliable modulation in the γ-band, which may stem from the “stationary” nature of the stimulus (Sewards and Sewards, 1999) but may likewise result from a low signal-to-noise ratio. Therefore, we here focus on the α-band changes, which were clearly modulated in all subjects.

As described in Materials and Methods, we normalized the frequency dependence to the individual mean α-frequency derived from the resting periods. This procedure results in different scales for each subject, resulting from the interindividual difference in α-frequency. Also, because the study design only included integer steps of 1 Hz for the stimulation frequencies, a stimulation at the exact α-frequency was usually not available. Therefore, after averaging, we rescaled the stimulation frequency range to integer frequencies (in hertz) by multiplying with the mean α-frequency across all subjects (10.6 Hz). Thus, the presentation of the results gives data at frequencies that were actually tested. The mean α-frequency across subjects and its harmonic are indicated by broken bars in the plots. In the text we refer to frequencies in hertz, which can be converted into multiples of the IAF by division by 10.6.

Electrophysiological results

ssVEPs

VEP or ssVEP traces for all frequencies are shown in Figure 3a (the transition from a separable VEP to a steady-state oscillation is somewhat arbitrary. In a strict sense, only the result at 1 Hz can be considered a typical VEP, because at 5 Hz, components with a latency >200 ms will be aliased and cannot be differentiated). They are a grand average across data at electrode position O2 from all subjects and all stimulation blocks. After z-transformation (across stimulus frequencies) of the individual subjects, the averaged data were rescaled to microvolts by multiplication of the mean amplitude across subjects. For low stimulation frequencies (1, 5, and 6 Hz), two relatively distinct early components with latencies of ~80 and ~110 ms can be discriminated. These components broadly correspond to the N2 and P2, as defined in clinical VEP guidelines for flash-evoked VEP (Odom et al., 2004). From 7–9 Hz, the ssVEP shows a biphasic response, without clearly discernable components to yield roughly sinusoidal oscillations beyond 9 Hz. As a measure of mean electrophysiological activity, the root-mean square (RMS) of stimulus-evoked potentials was assessed over the 1 s data segments. In Figure 4a, this measure of mean amplitude and SEM across subjects is plotted against stimulus frequency. The stimulus-evoked response is largest in amplitude at 5 and 11 Hz, and a local minimum can be seen at 7 Hz. Beyond 11 Hz, evoked responses decrease in amplitude with increasing stimulation frequency. Small local maxima appear at 20 and 25 Hz.

α-Power modulation

As described in Materials and Methods, we performed two types of analysis to assess the dependence of α-power from the stimulus frequency applied. Results for either analysis are presented in Figure 4b. The open symbols show the frequency dependence of α-power, when applying the conventional method. There is a clear peak around the mean α-frequency. Necessarily, stimulus-evoked response and α-frequency spectrally coincide at this frequency. Thus, the result cannot be easily interpreted with respect to the underlying question of the mutual interdependence between electrophysiological background activity and vascular response. Therefore, for the second type of analysis, we subtracted mean ssVEP from the raw data before the analysis in the frequency domain. The results for this analysis are given by the black symbols. In addition to the attenuation of the peak at the mean α-frequency, there is another relevant difference between the results of the two analyses. At ~5 Hz, the type I analysis shows a "subharmonic" peak in α-power not seen after attenuation of the evoked response. Note that the peak at ~21–23 Hz is not altered by the stimulus attenuation procedure.

To sum up, α-power decreases with stimulus frequency to reach a minimum at 9 Hz. At the individual α-frequency, there is a clear increase in α-power, which is larger than the resting α-power but nearly identical to resting α-power after subtraction of mean ssVEP (Fig. 4b, filled symbols). Beyond 11 Hz, α-power...
Hemodynamic response

Hemodynamic response was assessed by measured changes in [deoxy-Hb] and [oxy-Hb]. Because changes in [deoxy-Hb] inversely correlate with BOLD contrast as assessed in fMRI, the analysis is focused on this parameter. After selection of the focal maximum of stimulation-related decreases in [deoxy-Hb] (see Materials and Methods), we analyzed the dependency of [deoxy-Hb] decreases from stimulation frequency. Note that a larger decrease in [deoxy-Hb] is interpreted as an indicator of an increase in cerebral blood flow (washout of [deoxy-Hb]). Larger decreases thus denote a larger vascular response. Error bars denote SEM. Note that in contrast to a, giving the ssVEPs, the whole stimulation epoch (15 s) is shown, because the vascular response is too sluggish to analyze individual stimulus response.

Figure 4. Frequency dependence of the electrophysiological parameters. a, The average magnitude of the evoked potentials, calculated as the root mean square over the 1 s segments (as shown, in part, in Fig. 3a). b, The frequency dependence of the α-power before (open circles) and after (filled circles) attenuation of the stimulus-induced changes (for the procedure, see Materials and Methods and Fig. 2). Error bars give the SEM across subjects. Note that all data were normalized to the IAF before averaging. The data were transformed back to actually tested frequencies by multiplying with the mean IAF across subjects of 10.6 Hz. The vertical bars give the mean α-frequency (10.6 Hz) and its first harmonic (21.2 Hz). The horizontal solid bar denotes resting state α-power with SEM (dashed lines).

Figure 3. Grand-average time courses for ssVEPs and [deoxy-Hb] changes. a, The visual-evoked potentials averaged across all subjects. Segments of 1 s were averaged for all frequencies tested. The stimulation frequency is given on the left in hertz; vertical bars denote the flashes, horizontal lines denote 0 µV for each frequency. Note that only the first 330 ms of each frequency are presented for reasons of graphical clarity. The resonance at ~11 Hz and more weakly at ~21 Hz can be seen. b, The time course of the [deoxy-Hb] changes in response to the 22 different frequencies tested. The gray vertical box indicates the stimulation epoch. The decrease in [deoxy-Hb] is an indicator of an increase in cerebral blood flow (washout of [deoxy-Hb]). Larger decreases thus denote a larger vascular response. Error bars denote SEM. Note that in contrast to a, giving the ssVEPs, the whole stimulation epoch (15 s) is shown, because the vascular response is too sluggish to analyze individual stimulus response.

shows a tendency to increase with stimulus frequency. A broader peak in the β-frequency range (21–23 Hz) is not altered by the attenuation of stimulus-induced response, whereas at the peak at the subharmonic of α, at ~5 Hz is eliminated by the procedure.
seen. The reasons for differences between changes in [oxy-Hb] and [deoxy-Hb] are complex but beyond the scope of the present paper. For a more detailed discussion on the flow volume relationship and its implications for NIRS–fMRI measurements, we refer to a publication to be issued shortly (Steinbrink et al., 2006).

In analogy to the mean ssVEPs, Figure 3b depicts the averaged time courses for [deoxy-Hb] for all frequencies in units of hertz for a timescale of 30 s. Because stimulation frequencies are not separated by resting periods, a reconstruction based on linear superposition instead of simple averaging was done. After onset of stimulation, [deoxy-Hb] decreases at all frequencies. As also seen in Figure 5a, [deoxy-Hb] reveals a maximal decrease at stimulation frequencies of 7 and 8 Hz.

Relationship between electrophysiological and vascular response

The comparison between the two aspects of the electrophysiological response (i.e., the ssVEP and the α-power) and, in contrast, the hemodynamic response, critically depends on the sampling of a comparable region of the cerebral cortex. A fine-tuned spatial correlation is beyond the spatial resolution of either method. EEG and NIRS can localize on a scale of centimeters rather than millimeters. Nonetheless, we considered it mandatory to provide arguments for the comparability of the two response modalities. Figure 6a shows the topological distribution of visual-evoked potentials for all stimulation frequencies tested. The topological maps indicate that the largest potentials are located in the occipital region. The response was maximal over the leads O1 and O2. These two electrodes are at the center of the area investigated by the NIRS array. For NIRS, Figure 6b, c and d, shows the t-maps of [deoxy-Hb] and [oxy-Hb] for the comparison resting periods versus all stimulation periods. This test was performed to select the channel in which the frequency dependence of the hemodynamic changes was analyzed. Figure 6d shows the optical probe arrangement. Figure 6e gives the location of the highest t value of [deoxy-Hb] and [oxy-Hb] for each subject. The results show that the decrease in [deoxy-Hb] showed highest t values (navy-blue

Figure 5. Frequency dependence of the hemodynamic parameters. Changes in [deoxy-Hb] (a) and [oxy-Hb] (b) are given in analogy to Figure 4. The same normalization procedure was applied (IAF normalization and back-transformation). For a more detailed discussion on the flow volume relationship and its implications for NIRS–fMRI measurements, we refer to a publication to be issued shortly (Steinbrink et al., 2006).

Figure 6. EEG and NIRS topography. a, Spherical spline maps of the RMS of averaged visual-evoked potentials on a scalp in a two-dimensional circular view. The topography is generated using interpolation on a fine Cartesian grid based on the measurement over the 21 standard 10–20 system positions. Maps for each flicker frequency tested are given. Pseudocolor scaling is in μV. b, Topographical pseudocolor t-maps of changes in [deoxy-Hb] and [oxy-Hb] (c). Note the generally higher t values for [deoxy-Hb]. d, The optical probe arrangement; e, the location of the highest t values for [deoxy-Hb] and [oxy-Hb] for each subject demonstrating the intersubject variability.
areas in the pseudo-color scaling) for the area overlying electrodes O1 and O2. We decided to analyze the data in one electrode (O2) to yield a comparable result with respect to the NIRS results based on a selected single probe pair. We applied a frequency normalization to sharpen the resonance phenomenon between \( \alpha \)-oscillation and ssVEP. To judge whether such an electrophysiological resonance would translate into a likewise augmented vascular response at this frequency, the same normalization was performed for the NIRS data. Un-normalized EEG data in fact showed the expected broadening of the resonance peak, whereas the vascular response parameters did not show any discontinuous increase around the central or normalized \( \alpha \)-frequency (data not shown). Correlation analysis was performed between [deoxy-Hb]/ssVEP and [deoxy-Hb]/\( \alpha \)-power, respectively. This correlation analysis across all stimulation frequencies revealed no significant results for the group average. Nonsignificant correlations were also found in all but three comparisons in the single subjects (supplemental Table 1, available at www.jneurosci.org as supplemental material).

To sum up, evoked potentials and stimulation-induced hemodynamic changes were maximal over the occipital region roughly localized between O1 and O2, when relying on the 10-20 system. The simplifying hypothesis is that changes in regions outside this area will not substantially influence the evoked response elicited by the stimulus. Anatomically, the region sampled includes striate and extrastriate visual areas. The question of how far subcortical neuronal activity is relevant to explain the different response behaviors for the electrophysiological and the vascular response is addressed in the discussion section. This issue will be discussed in the light of the differentiation between evoked potentials and spontaneous background activity in the EEG measurements.

Discussion

Coupling between stimulus-induced electrophysiological and cerebralvascular changes allows for high-resolution functional mapping down to columnar (Cheng et al., 2001) and layer-specific topography (Pfeuffer et al., 2004). However, response modalities exhibit fundamental discrepancies. Because of a much longer latency, brief stimuli elicit a sluggish vascular response peaking after stimulus cessation (Martindale et al., 2003); oxygen demand generated by neuronal activity is overcompensated by a disproportionately large vascular response (Fox and Raichle, 1986). To explain the mismatch between oxygen consumption and blood flow, three major theories have been proposed: (1) Glucose uptake and metabolism determines blood flow (Frahm et al., 1996; Magistretti and Pellerin, 1996). (2) Limited oxygen diffusion to tissue necessitates an overly large increase in blood flow (Buxton and Frank, 1997). (3) Finally, a dominant role of neurotransmitter or interneuron links between the “functional unit” and the vessels has been highlighted (Attwell and Iadecola, 2002). Not mutually exclusive, theories are still controversial. The disproportionally large oxygen supply, however, constitutes the most powerful imaging signal, i.e., BOLD contrast reflecting decreases in [deoxy-Hb]. Temporal latency is efficiently dealt with by models of the “hemodynamic response function” (Friston et al., 1995).

Here, we highlight another discrepancy as yet not fully addressed. If neuronal networks encode information by synchronization of spontaneous oscillations (Gray et al., 1989; Fries et al., 2001), such neuronal processing may require neither more energy nor additional synaptic signaling. To test for hence predicted dissociations between evoked potential and vascular response, we investigated (1) ssVEP, (2) variation of \( \alpha \)-power, and (3) the hemodynamic responses to parametrically varied frequency of a flicker-light stimulation. We find a discontinuous maximum of the ssVEP and \( \alpha \)-power when stimulating at the individual \( \alpha \)-frequency. The oxygenation response does not show such a peak but steadily decreases in amplitude with stimulation frequencies >8 Hz. This indicates that the evoked potential is composed of several electrophysiological processes, including the synchronization of spontaneous oscillations. A comparison between evoked potentials and a vascular response must therefore respect both the summation of evoked neuronal signaling and the synchronization of spontaneous rhythmic activity, the latter potentially not reflected by a larger vascular response. In addition to this discontinuity of frequency dependence in the electrophysiological parameters assessed, the ssVEP steadily decreases with increasing stimulus rate. \( \alpha \)-Power decreases up to the IAF, showing a small increase for higher frequencies. Thus, neither of the frequency dependencies easily predicts the vascular response, which was maximal at a 7–8 Hz stimulation frequency. Lower stimulation rates elicit a smaller [deoxy-Hb]-response, whereas higher frequencies slightly attenuate the vascular response. This corresponds to reports on frequency dependence for visual stimulation with PET and fMRI (Fox and Raichle, 1985; Kwong et al., 1992; Thomas and Menon, 1998; Ozus et al., 2001; Hagenbeek et al., 2002). Although the experimental designs differed in stimulus conditions, stimulus intensity, and the frequency range tested, response saturation mostly at 8 Hz is agreed on. Higher frequencies yield a small decrease or no further amplitude modulation (Ozus et al., 2001). The reason for a peak response at 8 Hz remains unclear. Fox and Raichle (1985) discussed the “Brücke-Bartley effect,” predicting the largest population of cortical neurons to respond to frequency adjusted to the “activity–recovery cycle” in the retina–cortex pathway (Grüsser and Creutzfeldt, 1957; Bartley, 1968). Although ssVEP is a coarse measure of integrated electrophysiological activity, the expected maximal neuronal recruitment cannot explain a local minimum of the evoked vascular response. Figure 4a, however, demonstrates a local minimum of ssVEP at 7 Hz. Thus, we cannot confirm the maximal hemodynamic response at 8 Hz to simply reflect the Brücke-Bartley effect.

Alternatively, our results suggest that the hemodynamic response will also depend on the modulation of background activity (Brookes et al., 2005). Electrophysiologically, \( \alpha \)-rhythm has long been considered to indicate an “idling state” desynchronized by incoming visual stimuli (Berger, 1929; Fries et al., 2001). Because photic driving does not completely abolish \( \alpha \)-activity (Adrian and Matthews, 1934), we also assessed the modulation of \( \alpha \)-power at all stimulation frequencies. Assuming an inverse relationship between \( \alpha \)-power and BOLD contrast in the visual cortex (Singh et al., 2002; Laufs et al., 2003), we expected a correlation between the amplitude of the [deoxy-Hb] response and \( \alpha \)-power (Moosmann et al., 2003). Indeed, we find that \( \alpha \)-power and [deoxy-Hb] decrease with increasing stimulus frequency up to 7–8 Hz. There is a discontinuity of the \( \alpha \)-power amplitude around the IAF to slightly increase with flicker frequencies beyond 12 Hz. Thus, our data provide evidence for the view that \( \alpha \)-power modulation will influence the vascular response also during ongoing stimulation (Brookes et al., 2005).

The resonance between ssVEP and \( \alpha \)-power at the IAF deserves additional consideration. Typical VEP assessment assumes that background activity is independent of the EPs. Event-locked averaging recovers the VEP from a background, which is dominated by oscillations in the \( \alpha \)-range over the occipital lobe. How-
ever, evidence has been provided that ongoing activity is altered in amplitude and/or phase by incoming stimuli. Stimulus-induced phase-locking of the α-rhythm has been shown to substantially contribute to the generation of VEPs (Makeig et al., 2002). Also, stimulus-driven modulation of locked and unlocked α-, θ- and γ-bands have been reported (Schurmann and Basar, 1994; Basar et al., 1998; Pfurtscheller, 2001; Woertz et al., 2004). Thus, beyond clinical practice, it is appropriate to consider ongoing activity and evoked potentials, sharing a common corticothalamic pathway, mutually dependent (Lopes da Silva et al., 1980; Chatila et al., 1993). In our study, we find a resonance between the α-power and evoked potentials at the IAF. Frequency domain analysis of α-power introduces the problem that stimulus-locked evoked potentials and α-power will both peak at 10–11 Hz. Therefore, we attenuated the stimulus-evoked contribution by subtraction of the mean evoked potential before the spectral analysis. Such an additive model of the “reproducible response,” estimated by trial averaging and the “ongoing activity” to predict the measured activity was also proposed by Arieli et al. (1996). The persisting resonance of both VEP and α-power at the IAF indicates phase locking and augmentation of the background α-band activity. In line with previous publications (Herrmann, 2001), there is additional evidence for phase locking because the simultaneously assessed hemodynamic response does not show a local maximum at the IAF. The reasoning is as follows: although mechanisms of coupling are still controversial, there is ample evidence that integrated afferent signaling may best predict the hemodynamic response (Lauritzen, 2001). Even inhibitory synchronous activity evokes increases in rCBF (Mathiesen et al., 1998); hence, any increase in cortical processing should evoke an increased vascular response.

Our result allows for two explanations: (1) phase-locking between α-rhythm and equifrequent stimulation will not require a substantial recruitment of cortical processing; (2) inhibitory corticocortical connections are blocked when stimulating at the IAF.

Although we favor the first explanation, it is impossible to disprove the second potential interpretation. Metabolic demand and ensuing vascular response caused by inhibition in a region are highly controversial (Tagamets and Horwitz, 2001). Metabolically, GABA cycling has been shown to not increase glucose uptake in astrocytes (Chatton et al., 2003), and BOLD contrast has been considered insensitive to inhibition (Waldvogel et al., 2000). However, rCBF increases have been demonstrated in response to inhibition (Mathiesen et al., 1998). Theoretically, blocking GABAergic neurons might decrease rCBF, which might compensate for the increase in rCBF attributable to excitatory neuronal activity. This could explain the insensitivity of the vascular response to the electrophysiological resonance. Because inhibition is more efficient as a result of the strategic positioning of inhibitory neurons (Koos and Tepper, 1999), our prediction is that even complete blocking of GABAergic neurons will cause a net increase in metabolic demand and rCBF, leading to a larger washout of [deoxy-Hb]. However, noninvasive approaches such as those used here will not be able to prove or disprove such a prediction. In brief, by comparing the amplitude of an evoked potential with the corresponding vascular response, we find clear deviations from a more or less linear dependency for a parametrically varied stimulus. This conflicts with a notion of a simple translation of the evoked potential into changes in hemodynamics (Ngi et al., 1999; Arthurs et al., 2000).

In the face of the accumulating evidence for substantial non-linearities of neurovascular coupling, it may seem advisory to rather refrain from any inference as to neuronal computations based on vascular response mapping. One might indulge in a reappraisal of electrophysiology, providing direct precisely timed information on the most relevant structure, the neuron. However, beyond the “inverse problem” in scalp recordings, even invasive studies have not solved the question of whether a stimulus will be represented by a specific excitation/inhibition pattern or whether synchronization of spontaneous oscillations are the correlate of a stimulus-evoked percept or whether both holds true (Stryker, 1989; Paretì and De Palma, 2004). Our study demonstrates that the comprehensive view of either side of the coin can provide answers to these very questions.

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Individual alpha-frequency correlates with amplitude of visual evoked potential and hemodynamic response

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In a simultaneous electroencephalography (EEG) and near-infrared spectroscopy (NIRS) study, the predictive value of the individual alpha-frequency at rest (IAF) for the amplitude of neuronal and vascular responses to visual stimulation was investigated. Across subjects, we find (i) an inverse relationship between IAF and the amplitude of the alpha-rhythm at rest. The IAF also predicts (ii) the amplitude of the visual evoked potential (VEP), as well as (iii) the amplitude of the alpha-rhythm during stimulation. Most importantly, (iv) IAF correlates with the oxygenation response to visual stimulation: A high IAF predicts a low alpha-amplitude at rest, a small VEP amplitude and a small oxygenation response. Conversely, a low IAF predicts high alpha-amplitude and larger electrophysiological and vascular responses to stimulation. Based on these findings, we assume that the relationship between IAF and neuronal and vascular response stems from the size of the network recruited for visual processing. The relation between IAF, alpha-amplitude, evoked potential and vascular response is discussed in the framework of a simple heuristic model. The results may partly explain the large intersubject variability observed in recently published concurrent EEG-fMRI studies.

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Introduction

Ongoing activity in the human electroencephalogram is sometimes termed ‘background activity’ but plays an important role to assess cortical neuronal networks during wakeful resting state and functional stimulation (Berger, 1929; Singer, 1993; Basar et al., 2001). Besides augmentation and attenuation of different frequency bands (event-related synchronization/desynchronization; ERS/ERD; Schurmann and Basar, 1994; Pfurtscheller, 2001; Woertz et al., 2004; Brookes et al., 2005), the lower frequencies (α- and θ-bands) may contribute to evoked potentials, thus blurring the clear distinction between an evoked response and the modulation of ‘background activity’ (Makeig et al., 2002; Yeung et al., 2004). Models proposed to explain the relationship depend on the analysis strategy. As an example, the classical visually evoked potential is recovered from the much larger ‘background’ by averaging time-locked to a flash or a checkerboard-inversion whereas the analysis of oscillations (Tallon-Baudry and Bertrand, 1999) is estimated in the frequency domain temporally related but not necessarily phase-locked to the stimulus.

The additive model is based on the assumption that the ongoing activity such as the alpha-rhythm has no functional significance for evoked potential generation, hence ongoing activity is treated as background noise. On the other hand, the phase-resetting model presumes a phase-realignment of ongoing activity. This highlights the possibility that components of the evoked potentials may partially originate from synchronization of background-rhythms (0- and α-bands; Kawabata, 1972; Sayers et al., 1974; Nogawa et al., 1976; Makeig et al., 2002). The two models are not mutually exclusive and it seems reasonable to assume that either model only partly explains the recorded potential changes: beyond phase-resetting of background-rhythms the evoked potential partially reflects additional neuronal recruitment to process the stimulus. In line with such a notion, current analytical models do not allow a clear favoring of either of the models (Yeung et al., 2004).

The attempt to disentangle the recruitment of additional neuronal units from a modulation by synchronization of ongoing oscillations is limited when relying on electrophysiological recordings alone. Here we also propose considering the vascular response, which – at first sight – seems to even complicate the matter. However, this independent response modality can help to make inferences as to whether a stimulus will recruit more neurons and elicit more synaptic activity or whether the electrophysiological response is a consequence of phase resetting. Since the latter mechanism requires no substantial additional recruitment but instead, synchronization of neuronal spiking and synaptic transmission, it will be reflected by a negligible increase in the vascular response despite a clear increase in electrophysiological response amplitude.

In a previous study, we have shown that over a wide range of stimulation frequencies the visually evoked electrophysiological and vascular response may dissociate (Koch et al., 2006). Larger VEP amplitudes do not necessarily predict a larger vascular
response. Here we expand this finding by investigating differences between individuals. It is well known that subjects exhibit interindividual variations in response magnitude (VEP). Also, the occipital alpha-rhythm (~10 Hz), the most dominant background oscillation in the EEG under relaxed wakefulness with closed eyes, varies across subjects (8–13 Hz) but remains remarkably stable within subjects (Kondacs and Szabo, 1999). The question raised in the present paper is whether the resting state individual alpha-frequency is functionally linked to interindividual response variability to stimulation. Therefore, we investigated the predictive value of the IAF for (i) the magnitude of visual evoked potentials, (ii) the alpha-power during stimulation and most importantly and (iii) the vascular response to flicker-light stimulation. The motivation to report on our findings is two-fold: First, the predictive value of a resting state parameter as to the neuronal and vascular or behavioral response appears important for a better understanding of interindividual variability in imaging studies. The individual alpha-frequency at rest may partially explain why large intersubject variations are seen in VEP magnitude and in vascular response in various experimental designs. Secondly, in the context of the current debate, a functional relationship between IAF at rest and the evoked potential clearly speaks against the hypothesis that evoked activity is largely independent from background activity as would be predicted by a simple additive model.

**Methods**

Twenty-one female subjects were seated in an EEG chair, in a silent, dark room. In a 4 min resting epoch (eyes closed), the IAF of each subject was assessed from the average of the occipital

![Fig. 1. Experimental design. (a) For each subject the IAF was determined from a 4 min resting epoch. The IAF (e.g. subject 1: 8.5 Hz; subject 2: 11.5 Hz) was used to individually tailor the stimulation-frequencies. Thus, frequencies corresponded to parts and multiples of the IAF ranging from 0.1 * IAF to 1.5 * IAF. Step-size was 0.1 * IAF, with finer-grained steps of 0.05 * IAF around the alpha-peak-frequency. For example, subject 1 (IAF: 8.5 Hz) was stimulated with flicker-frequencies from 0.85 to 12.75 Hz in 0.85 Hz steps, with additional frequencies tested between 6.8 and 10.2 Hz (0.425 Hz steps). (b) Probe distribution over the occipital cortex. An area of 10 cm by 5 cm was covered by NIRS and EEG. For the oxygenation response (NIRS), 22 sampling volumes (ellipses) are defined by the optical probes (emitters: black circles; detectors: white circles). EEG response in this area is measured at PO3/4, PO7/8, O1/2 and O9/10. The use of ring electrodes allows for the fixation of electrodes and optical probes in the same site. Note that EEG was monitored at additional 10–20 positions over the entire scalp.](image-url)
electrodes O1 and O2. Three subjects were excluded from further experimental procedures because a clear alpha-peak could not be detected in the power-spectral analysis. Altogether, 18 subjects participated in the subsequent stimulation-protocol (mean age 25.1 years; range, 21–28 years).

After assessment of the IAF, subjects kept their eyes closed and were exposed to periodic flicker-light by goggles equipped with two light emitting diodes (9000 mcd at 600 nm Conrad Electronic, Berlin, Germany). The flicker-frequencies corresponded to parts and multiples of the IAF so that each subject was stimulated with frequencies ranging from 0.1 to 1.5× IAF incremented in 0.1 IAF steps. For the frequencies around the IAF (0.8–1.2 IAF), steps of 0.05 IAF were tested. For a subject with an IAF of e.g. 8.5 Hz, the applied frequencies ranged from 0.85 to 12.75 Hz in 0.85 Hz steps, with additional frequencies tested between 6.8 and 10.2 Hz using 0.425 Hz steps (Fig. 1a). The resulting 19 different frequencies were applied in trials of 15 s in duration. Each stimulation frequency was tested 6 times. Additionally, 24 resting trials of 15 s in duration were included in the stimulation protocol. To prevent fatigue, the subject was allowed 5 breaks of variable length during the experiment. The 19 different frequencies and the rest condition were presented in a pseudo-random order. The stimulation frequency was varied by changing the interval between successive light pulses, while the duration of the light pulse was kept constant (5 ms). For a precise timing, we used a pulse generator (BNC 555e, Berkeley Nucleonics, CA, USA). The generator was controlled by the presentation software Cogent 2000 (Wellcome Department of Neuroscience, London, UK), which operates in the Matlab (v6.5, The Mathworks, Natick, MA, USA) environment. Subjects were healthy, specifically there was no history of neurological or psychiatric illness. They gave informed consent and were financially rewarded for their participation. Subjects were instructed to relax but remain awake during the experiment. None of the subjects reported any discomfort.

**Data acquisition**

The EEG was recorded from 29 standard positions according to the 10–20 system (Fp1/2, F3/4, Fc5/6, Fc1/2, C3/4, Cp5/6, Cpl/2, P3/4, Po3/4, Po7/8, O9/10, O1/2, Fp2, Fz, F7, Cz, Pz, Oz) referenced against FCz (BrainAmp amplifier and Vision Recorder software, Brain products Inc., Munich, Germany). Additionally, the horizontal and vertical oculogram was recorded. Impedances were kept below 5 kΩ. Data were recorded at 1000 Hz sampling frequency with a low cut off at 0.016 Hz and a 50 Hz notch.

Changes in cortical hemoglobin oxygenation were measured over the occipital region using a homemade optical NIRS topograph with a sampling frequency of 3 Hz. The methodology and underlying physiology are detailed elsewhere (Obrig and Villringer, 2003). Since light in the near-infrared penetrates biological tissue rather well, optical spectroscopy with a depth reaching to the cerebral cortex is feasible when applied to the adult human head. Applying a modified Beer–Lambert law to changes in optical densities at two different wavelengths, concentration changes in oxygenated (δ[oxy-Hb]) and deoxygenated hemoglobin (δ[deoxy-Hb]) can be assessed (Cope and Delpy, 1988). The optical topograph used here emits light at 760 nm and 830 nm, which is guided to the head by fibre optic bundles (diameter: 0.4 mm). The reflected light is collected by another set of optic probes (diameter: 3 mm) at a distance of 2.5 cm to be amplified by a photon multiplier. Under the assumption of a high but constant scattering coefficient (μs), the intensity changes measured can be ascribed to changes in absorption (μa) caused by concentration changes in the dynamic absorbers oxy-hb and deoxy-hb. Our system has 8 sources and 7 detectors arranged in a rectangular array thus supplying a rough topographical image of the occipital cortex. The probe array was centered 2 cm above the inion, covering the co-registered EEG-electrodes PO3/4, PO7/8, O1/2, O9/10 and Oz (Fig. 1b).

**Data analysis**

Data were analyzed with Matlab. Electrophysiologic data analysis was based on averaged data of all electrodes in the region of the optical pad (PO3/4, PO7/8, O1/2, O9/10).

**Electrophysiology (EEG)**

The IAF was estimated as the peak between 8 and 13 Hz from the 4 min resting epoch using the Power Spectral Density (PSD) routines implemented in Matlab (nfft of 4000 data points, Hanning window, overlap of nfft/2, linear detrend). This estimate was performed following the 4 min EEG recording at the beginning of the experiment to tailor the individually applied flicker frequencies to the respective subject (Fig. 1a). After the full experiment was completed, all EEG-data were downsampled to 250 Hz using Vision Analyzer software (Brain Products Inc., Munich, Germany; 3rd order polynomial). First, VEPs were calculated for each stimulation frequency at all channels starting with the 3rd flash of a trial. For each frequency and channel, the resulting VEP was averaged across the six trials of identical stimulation frequency. The root mean square (RMS) of the VEP-trace over 500 ms served as a measure of amplitude for each frequency, because individual components cannot be compared between low and high stimulation frequencies. Beyond the ‘classical’ VEP, our focus is on stimulation-induced modulations of the alpha-power. However, the frequency analysis fails to segregate alpha-activity from evoked activity for stimulation frequencies close to the IAF. Therefore, prior to the assessment of alpha-power modulation in response to different stimulation frequencies, the evoked response was attenuated in each stimulation period. This procedure has been described in our previous study (Koch et al., 2006). In brief, for each subject and each EEG-channel, the corresponding evoked response is subtracted from the raw data, which results in an attenuation of the time-invariant and phase-
locked response. The power spectra of all stimulation trials were estimated in the frequency domain using the PSD (nfft of 1000 data points, Hanning window, overlap of nfft/2, linear detrend). Power spectra were averaged across all 6 trials of the same stimulation frequency. To obtain a measure for the alpha-amplitude during the resting epoch and each stimulation frequency, the PSD-power for the frequencies at the IAF ± 0.75 Hz was integrated.

Optical imaging (NIRS)

Since changes in deoxy-Hb have been shown to better correlate with the BOLD-contrast in fMRI, we focus on deoxy-Hb changes (Kleinschmidt et al., 1996). Cortical oxygenation changes of [deoxy-Hb] and [oxy-Hb] were assessed with NIRS. After calculation of the hemoglobin concentration changes, pulse-related signal changes were attenuated by low-pass filtering (Butterworth, 5th order, lower cutoff 0.45 Hz). The further analysis comprised two successive steps. (i) Selection of ‘activated pixels’: To determine pixels of statistically significant response to visual stimulation, all trials with stimulation were considered as being the ON-condition whereas resting periods served as OFF-condition. A general linear model (GLM) was calculated using a boxcar predictor convoluted with a gamma function. (ii) Frequency dependence: In order to compare changes in [deoxy-Hb] and [oxy-Hb] for different stimulation frequencies, a boxcar for each frequency was convoluted with the hemodynamic response function (i.e. predictors for each stimulation frequency were determined). Finally, all predictors were fed into a GLM analysis. For each subject, the location with the largest t-value in [deoxy-Hb] was used to determine the dependence of [deoxy-Hb] and [oxy-Hb] from the stimulation frequencies.

Results

In the present study, we address the question of whether the resting state alpha-frequency (IAF) correlates with the amplitude of both the electrophysiological and vascular response across subjects. This is motivated by reports on an inverse relationship between frequency and amplitude between individuals and putative to the result of larger or smaller neuronal ensembles generating the alpha-rhythm and partly the VEP. The presentation of the results therefore begins with the analysis of the resting state alpha-oscillations. The results of the IAF-adapted stimulation protocol are then described. We report on three response parameters: (i) evoked potential amplitude, (ii) alpha-power modulation during stimulation and (iii) oxygenation response as assessed by NIRS, to eventually compare their magnitude across subjects.

Relationship between amplitude and frequency of alpha-rhythm during rest

Based on the power spectral analysis over the 4 min resting epoch with closed eyes at the beginning of the experiment, the amplitude and frequency of the alpha-rhythm were assessed for each subject at the averaged leads O1/O2. Fig. 2 depicts the relationship between frequency and amplitude across the subjects.
In line with our expectation, we find an inverse relation between the two parameters of the alpha-rhythm: with increasing frequency the amplitude decreases. In other words, subjects with low resting alpha-frequencies show large amplitudes whereas subjects with high frequencies show low amplitudes of the resting state alpha-rhythm. The correlation coefficient across 18 subjects was $r = -0.550$ ($p = 0.02$). A possible confounding effect of the EEG impedance was examined. Across subjects, no systematic relation between alpha-frequency/amplitude and electrode impedance was found. Thus, we can confirm the inverse frequency-amplitude relation as previously reported (Lopes da Silva et al., 1976; Pfurtscheller and Lopes da Silva, 1999).

Dependency of response amplitude on IAF

After focussing on the group effect, we then performed a correlation analysis based on all individual subjects to challenge our hypothesis that the resting state frequency predicts the amplitude of all three dependent variables under stimulation. Each dot in Fig. 5 represents the stimulation effect in the parameter measured in a specific subject. The black line denotes the linear regression. The correlation analysis (Pearson’s product-moment

1 Irrespective of the complex dynamics of the neurovascular signal cascade upon functional activation (Buxton et al., 2004), it is generally agreed that predominantly synaptic activity rather than spike activity (Logothetis et al., 2001; Attwell and Iadecola, 2002) causes an increase in cerebral blood flow and blood volume to meet the increased demand for glucose and oxygen. A mismatch between an overcompensatory CBF increase and the neuronal oxidative metabolism results in a focal hyperoxygenation (Fox and Raichle, 1986; Buxton and Frank, 1997). The latter is reflected in an increase in oxygenated hemoglobin accompanied by a decrease in deoxygenated hemoglobin. The reduced concentration of paramagnetic deoxy-Hb in small local blood vessels (washout) also forms the basis of BOLD-fMRI (Kwong et al., 1992; Ogawa et al., 1992). Regarding the NIRS signal, several simultaneous fMRI-NIRS studies found evidence for a good relationship between oxy-Hb/deoxy-Hb and the BOLD-signal (Kleinschmidt et al., 1996; Toronov et al., 2001; Strangman et al., 2002; Yamamoto and Kato, 2002; Huppert et al., 2006) and blood flow (Hoshi et al., 2001; Sheth et al., 2004; Huppert et al., 2006).
parameters: (i) evoked response (vascular response during stimulation. A negative relation was between resting IAF and the respective electrophysiological and vascular response during stimulation. A negative relation was found for the relation between IAF and all four response parameters: (i) evoked response ($r = -0.489; p = 0.04$), (ii) alpha-power ($r = -0.654; p < 0.00$), (iii) [deoxy-Hb] response ($r = -0.504; p = 0.03$) and (iv) [oxy-Hb] response ($r = -0.44; p = 0.07$). Oxy-Hb data (4th panel) failed to reach significance. Note that the [deoxy-Hb] response is inverted because a larger decrease in [deoxy-Hb] denotes a larger vascular response.

Discussion

The interindividual variability is often hidden in group analysis in both vascular based imaging and electrophysiological studies. Therefore, important information is lost especially when the different response modalities are simultaneously assessed and the relation between the neuronal and vascular response is of interest. Although this relation is considered strong in most instances, deviations from a straightforward neuro-vascular coupling have gained increasing attention. In the present study, we address the question of how far a resting state electrophysiological parameter, the individual alpha-frequency, qualifies as a predictor for the interindividual response variability to visual stimulation. In a combined EEG and optical topography approach, we demonstrate that neuronal and vascular responses during stimulation are not statistically independent from the resting state IAF. While we supply further evidence for the previously reported inverse relationship between frequency and amplitude of the alpha-rhythm across subjects, this study is the first to demonstrate that these resting state parameters partially predict both electrophysiological and vascular response magnitude when compared between subjects. The interpretation of the results relies on the following considerations:

- The central resting state alpha-frequency is variable between but stable within subjects (Kondacs and Szabo, 1999).
- Amplitude and frequency of brain oscillations exhibit an inverse correlation, both within a frequency band as well as between different frequency bands (Singer, 1993; Pfurtscheller and Lopes da Silva, 1999).
- The amplitude of oscillations is proportional to the number of synchronized neural elements (Elul, 1971).
- Slower oscillations indicate a larger participating network or an extended recruitment of neuronal elements, while faster oscillations indicate a smaller participating network (Singer, 1993).

Since we use noninvasive techniques, a comprehensive model to explain this predictive power of the IAF is beyond the scope of this paper. Nonetheless, here we introduce a heuristic model to explain the findings of the present study and accommodate related findings of our previous work (Koch et al., 2006). The purely descriptive model is based on the relation between simultaneously assessed changes in (i) evoked potential amplitude, (ii) the modulation in amplitude of the alpha-rhythm and (iii) the oxygenation response, the latter an indicator of the vascular response (Obrig et al., 2002; Moosmann et al., 2003).

The model (Fig. 6) differentiates between 4 different cell populations in the visual cortex. Firstly, during rest, a neuronal network engaged in the generation of spontaneous oscillations in the alpha-range (light orange elements) is differentiated from neuronal elements which do not participate in the alpha-rhythm (grey elements). Upon stimulation, we introduce a second distinction: Some neural elements will be additionally recruited to participate in the neuronal activity time-locked to the stimulus, generic for the VEP. These elements (filled dark orange) are recruited from the neuronal network which did not participate in the generation of the alpha-rhythm at rest (i.e. grey elements during rest are additionally recruited to process the stimulus). Another neuronal population (hatched light/dark orange) is synchronized to the stimulus frequency and will thus contribute to the generation of the VEP. However, this population is part of the network generic for the alpha-rhythm at rest (light orange elements during rest). We assume that the two networks – generic for either VEP or resting alpha-rhythm – are partially overlapping. Our finding that during rest the individual alpha-frequency inversely correlates with the evoked potential magnitude supports this assumption. The assumption is also supported by a number of previous studies showing that evoked potentials are not independent from background activity (Brandt and Jansen, 1991; Barry et al., 2000; Makeig et al., 2002; Shah et al., 2004). Based on the grossly simplified definition of four different neuronal elements in the visual cortex, our findings in the present paper can be explained and can be linked to findings of our previous work (Koch et al., 2006). Note that the present study compares the neuronal and vascular responses between different subjects (Fig. 6a), whereas our previous study addressed the question of neurovascular coupling within a subject (Fig. 6b) across a wide range of stimulation frequencies.

Comparison between subjects (Fig. 6a)

The size of a network involved in the generation of the alpha-rhythm during rest has been proposed to be roughly classified by means of frequency and amplitude of the underlying oscillation (Elul, 1971; Singer, 1993; Pfurtscheller and Lopes da Silva, 1999). Consequently, in a subject with a low alpha-frequency ($S^{LFG}$, upper row, Fig. 6a) and a high alpha-power at rest, a large network participates in the alpha-oscillation (light orange elements), whereas in a subject with a relatively high IAF ($S^{HFG}$) and a low amplitude, the alpha-network is smaller (lower row, Fig. 6a). This inverse relation between frequency and amplitude is most likely due to different running times for synchronization within the recruited alpha-network. The more neurons participate in the resting state alpha-network, the larger the running time for synchronization of the involved neurons, hence the slower the frequency. Upon stimulation, some elements of the alpha-network will be recruited for the processing of the stimulus (hatched light/dark orange elements). This results in a partial decrease of the alpha-amplitude (light peak in the PSD-sketch) because these elements are now synchronized to the stimulation frequency (dark orange peak in the PSD-sketch). The magnitude of the VEP depends on the size of the overall network recruited (network is delineated by dashed line). Therefore, in $S^{LFG}$ the larger VEP amplitude is explained by the greater number of cumulatively recruited network elements (compare dashed areas between upper and lower row in Fig. 6a). Since the alpha-network in $S^{HFG}$ is small, the number of neuronal elements potentially recruited for the VEP is smaller (dashed area). The smaller overall network also explains why the peaks in the frequency domain analysis (PSD-sketch) are smaller in a subject with a high IAF ($S^{HFG}$) when compared to a subject with a low IAF ($S^{LFG}$). With respect to the ensuing vascular response, the size of the network also predicts the
amplitude of the vascular response. This can be explained by the fact that both the recruitment of additional neural elements (dark orange) and the recruitment of neurons which are part of the alpha-network generate a metabolic demand. Owing to its purely descriptive nature, the model does not make any specific assumptions as to whether cortico-cortical or thalamo-cortical connections cause synchronization and in which way inhibitory and excitatory synapses contribute to the overall resulting effect. Also a quantitative prediction cannot be reached by the model. However, this simple neuronal mass model explains the larger vascular response in LFG subjects during stimulation due to a larger number of engaged neurons compared to HFG subjects. In other words, the generation of the VEP by a smaller network requires less synaptic signaling and thus elicits a smaller vascular response compared to a larger network.

In summary, the different sizes of the alpha-network in a between subject comparison can be approximated by the IAF. However, the model also accommodates the findings of our previous study (Koch et al., 2006) highlighting a dissociation between the vascular and the electrophysiological response. In Fig. 6b, the respective scenario within a single subject is illustrated following the model assumptions. In this case, the size of the network is constant. Upon stimulation, the VEP is much larger in response to stimulation at the IAF \((f_{stim} \approx \text{IAF})\) compared to adjacent stimulation frequencies \((f_{stim} \neq \text{IAF})\).
stimulation at the IAF synchronizes a major part of the alpha-network to the stimulation frequency (hatched light/dark orange elements). This results in an overly large VEP (resonance phenomenon). When stimulating at adjacent frequencies, the alpha-network is divided into two frequency components: the IAF (light orange elements) and the stimulation frequency ($\text{stim}$, hatched elements). The finding that the vascular response does not substantially differ between these two scenarios in the same subject (Fig. 6b) suggests that the recruitment of the alpha-network for the stimulus processing generates a smaller metabolic demand. We infer that when stimulated with frequencies close to the IAF, enhanced phase-resetting rather than the recruitment of additional synaptic signaling explains the dramatic increase in the VEP amplitude. This mismatch between evoked potential and vascular response magnitude across stimulation frequencies highlights the fact that larger VEP amplitudes can also indicate more efficient phase resetting as opposed to the recruitment of a larger network. In support of our model, it has been argued that synchronization reflects neuronal processes of comparably low energy demand compared to additional excitatory recruitment (Buzsaki and Draguhn, 2004).

To sum up, the model describes why the magnitude of the evoked vascular response can be predicted by the resting state alpha-power across subjects, while it also accommodates our previously reported findings – replicated here – of a dissociation between vascular and electrophysiological response when comparing different stimulation frequencies within subjects. Below we will discuss the results concerning the difference between HFG when compared to LFG subjects with respect to previously published work by other groups.

Inverse relationship between resting state alpha-frequency and alpha-amplitude

For the resting state, we find an inverse frequency-amplitude dependence as has been previously reported (Lopes da Silva et al., 1976; Pfurtscheller and Lopes da Silva, 1999): the lower an IAF the larger the amplitude and vice versa. Within the simple neuronal mass model proposed here, this can be conceptualized by the assumption that an oscillation's amplitude reflects the number of synchronized neural elements (Elul, 1971), and hence predicts the size of a subject's potentially recruited network based on the baseline assessment of resting state alpha-frequency. Though the origin of the alpha-rhythm is not fully understood, cortico-thalamic and cortico-cortical excitation–inhibition cycles are generally considered generic. In a theoretical approach, Lopes da Silva et al. (1976) proposed a model consisting of an interplay between excitatory corticothalamic relay neurons (TCR) and inhibitory interneurons (IN). Depending on the power of inhibitory influence on the TCR, the alpha-frequency, i.e. the model output, varies in frequency and amplitude. Compared to a reference model (e.g. one IN inhibits 12 TCR), a decrease in inhibitory effectiveness (e.g. one IN inhibits 4 TCR) results in an increase in frequency but a decrease in amplitude. The augmentation of inhibitory effectiveness (e.g. one IN inhibits 21 TCR) yields the complementary result. Conceptually, this means that with an increase in size of a neuronal network generating an oscillation, not only the summed amplitude will increase but also the regional running time for phase tuning will be longer. The longer running time in this larger network can be expected to decrease the frequency of the macroscopically detectable oscillation (G. Curio, personal communication). Lopes da Silva's model makes assumptions on their respective kinetics and efficiency (Pfurtscheller and Lopes da Silva, 1999; Wendling et al., 2002). Our noninvasive approach does not allow for measuring specific inhibitory and excitatory neuronal populations and cannot directly assess the thalamic influence. Inherently, our descriptive model (Fig. 6a) sums the net synchronized electrophysiological activity in the cortical area monitored and compares it to the resulting vascular response.

Relation between IAF and evoked potential amplitude

The model proposed here predicts that lower resting state alpha-frequencies indicate a larger alpha-network. If this network is also involved in the generation of evoked potentials, it is expected that LFG subjects show larger VEP amplitudes compared to HFG-subjects. To our knowledge, this prediction has not yet been explicitly tested, but previous studies (Kooi and Bagchi, 1964; Rodin et al., 1965; Pigeau and Frame, 1992) found larger visual evoked responses in subjects with a high resting alpha-amplitude compared to subjects with low alpha-amplitude. If the inverse relation between alpha-frequency and amplitude holds true, these reports are in line with the current results and the model. Assuming that similar mechanisms determine the trial-by-trial variability within the same subject, the model is also in line with the previously reported positive relationship between fluctuations of alpha-power and magnitude of evoked potential for both visual and auditory stimuli (Brandt and Jansen, 1991; Brandt et al., 1991; Barry et al., 2000; Makeig et al., 2002). This is controversial because other studies addressing the issue reported a negative relationship between pre-stimulus alpha-power and ERP components (Rahn and Basar, 1993a; Rahn and Basar, 1993b). It has been argued that different experimental designs and different influences of background activity (alpha and theta) on specific components of the evoked response might explain the results found (Klimesch et al., 2004). However, we propose that variations within the same subject can be explained by the size of the recruitable network and the degree of functional connectivity. While the physiologically recruitable network is limited by anatomically defined populations, functional connectivity and hence the number of oscillatory neurons during rest and during stimulation can vary within a subject depending for example on the level of arousal, circadian rhythm, mental activity, stimulus strength and duration. In summary, we find that the IAF inversely predicts the magnitude of a subject's evoked potential. This is an extension of the view that the 'resting' state of the brain, reflected by the 'background' EEG-variables, determines the response to a stimulus (Barry et al., 2003).

Relation between IAF and deoxy-Hb response

Concerning the vascular response, we found an inverse relation between the resting alpha-frequency and the amplitude of the vascular response as derived from the changes in deoxygenated hemoglobin. Across flicker-frequencies, subjects with a low resting alpha-frequency show a larger deoxy-Hb response compared to subjects with a high IAF. Upon stimulation, the vascular response is largest in subjects with a low IAF. The very same subjects also exhibit the largest evoked potentials, whereas subjects with a higher IAF exhibit both smaller evoked potentials and smaller vascular responses. This supports the notion that across subjects

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2 A decrease in [deoxygen-Hb] is expected as an indicator of an increase in rCBF and corresponds to an increase in BOLD-contrast when compared to the results reported with functional MRI (KleinSchmidt et al., 1996; Huppert et al., 2006). To ease this comparison [deoxygen-Hb] is inverted in Figs. 3–5.
the magnitude of the vascular response corresponds to the size of the underlying neuronal network which participates in the processing of the stimulus. Since both the EEG and the vascular response reflect events associated with the depolarization of postsynaptic membranes (for a discussion, see Heeger et al., 2000; Rees et al., 2000; Lauritzen, 2001; Logothetis et al., 2001; Attwell and Iadecola, 2002), it seems reasonable to assume that during stimulation subjects with a low IAF expend more synaptic activity associated with larger evoked potentials and stronger vascular responses when compared to subjects with a high IAF. The enhanced synaptic activity in subjects with a low IAF probably results from a larger network involving more neurons in the resting state alpha-rhythm and in the generation of the evoked potential. Along this line of reasoning, the mutual interdependence between the alpha-rhythm and the evoked potential is caused by the recruitment of a partly overlapping network.

The inverse relation between IAF and the amplitude of evoked potentials speaks against a purely additive model: the resting state alpha-frequency is functionally linked to the magnitude of the evoked signal upon stimulation. The positive relation between resting alpha-amplitude and magnitude of the evoked potential supports the hypothesis that alpha-activity and evoked potentials are not independent phenomena. The observed linear dependency between IAF and vascular response can be functionally linked to the number of engaged neurons and the net synaptic activity for stimulus processing. Therefore, we supply further evidence for the observation that the response magnitude depends on the nature and size of ongoing network dynamics at stimulus onset (Brandt and Jansen, 1991; Arieli et al., 1996; Barry et al., 2000; Makeig et al., 2002) by demonstrating that the individual response magnitude is predictable on both a neuronal and vascular level by the resting alpha-frequency estimated prior to stimulation.

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References


Behavioral/Systems/Cognitive

Stimulus-Induced and State-Dependent Sustained Gamma Activity Is Tightly Coupled to the Hemodynamic Response in Humans

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A prompt behavioral response to a stimulus depends both on the salience of the stimulus as well as the subject’s preparedness. Thus, both stimulus properties and cognitive factors, such as attention, may determine the strength of neuronal synchronization in the gamma range. For a comprehensive investigation of stimulus–response processing through noninvasive imaging, it is, however, a crucial issue whether both kinds of gamma modulation elicit a hemodynamic response. Here, we show that, in the human visual cortex, stimulus strength and internal state modulate sustained gamma activity and hemodynamic response in close correspondence. When participants reported velocity changes of gratings varying in contrast, gamma activity (35–70 Hz) increased systematically with contrast. For stimuli of constant contrast, the amplitude of gamma activity before the behaviorally relevant velocity change was inversely correlated to the behavioral response latency. This indicates that gamma activity also reflects an overall attentive state. For both sources of variance, gamma activity was tightly coupled to the hemodynamic response measured through optical topography. Because of the close relationship between high-frequency neuronal activity and the hemodynamic signal, we conclude that both stimulus-induced and state-dependent gamma activity trigger a metabolic demand and are amenable to vascular-based imaging.

Introduction

Local field potentials (LFPs) in the gamma range are an auspicious electrophysiological candidate measure of stimulus-related and top–down processing (Eckhorn et al., 1988; Gray and Singer, 1989; Gray et al., 1989). Noninvasive neuroimaging, however, primarily relies on the vascular response. Thus, the question arises of how neuronal synchronization evokes a vascular response. Recently, a close coupling between gamma oscillations and the hemodynamic response was reported in the cat’s visual cortex (Niessing et al., 2005). Increases in visual contrast yielded stronger gamma synchronization and a larger vascular response. Thus, in the animal, gamma band and hemodynamics likewise indicate externally driven brain responses. Interestingly, also for a given contrast, both signals fluctuated while the tight coupling between neuronal and vascular response was preserved. This indicates that both responses are modulated by state-dependent variables. Highlighting this state dependence, trained monkeys show a clear augmentation of gamma-band LFP when attending to a stimulus compared with the unattended perception of the same stimulus (Womelsdorf et al., 2006).

Although variation in internal states such as attention cannot be easily monitored in the anesthetized animal, reaction time (RT), a measure concatenating both externally and internally generated response determinants, allows for a differentiation between stimulus-related and state-dependent variables in humans. Here, we combine noninvasive EEG and optical topography to simultaneously assess gamma-band and cortical oxygenation changes. Optical topography is a noninvasive extension of intrinsic optical signals as used in the study in the cat’s visual cortex (Niessing et al., 2005). Since focal decreases in the concentration of deoxygenated hemoglobin are the source of blood oxygen level-dependent (BOLD) contrast increases, results using optical topography are applicable to the large body of functional magnetic resonance imaging studies in humans (Kleinschmidt et al., 1996; Huppert et al., 2006).

In the present study, subjects had to report unpredictable changes in velocity of a moving grating whose contrast changed parametrically (see Fig. 1A) but was irrelevant for the task. Thus, salience of the stimulus was altered by external modulation (contrast) independent of the cue for behavioral response (velocity). We hypothesized that increasing salience would decrease reaction time. The variability in reaction time for a fixed contrast (velocity) independent of the cue for behavioral response (velocity).

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fluctuations of stimulus processing as reflected in the gamma-band response (GBR). Additionally, we investigated (3) the relationship between both types of GBR and the simultaneously assessed hemodynamic response.

The need to experimentally demonstrate that both state-dependent and stimulus-driven GBR changes are reflected in the vascular response results from increasing evidence for substantial deviations from a straightforward neurovascular coupling (Caesar et al., 2003; Sirotin and Das, 2009). This also applies to macroneuroscopically derived coupling between slow oscillatory activity and the vascular response (Goldman et al., 2002; Moosmann et al., 2003; Koch et al., 2006). Hence it cannot be easily assumed that stimulus-driven GBR changes will create the same metabolic demand as changes in gamma synchronization related to top–down processes (Fries et al., 1997).

On a conceptual level, it has been proposed that attention enhances perceptual sensitivity. In animals, V4 and MT neurons respond similarly to an increase in “physical” salience and the vascular response (Goldman et al., 2002; Moosmann et al., 2003; Koch et al., 2006). Hence it cannot be easily assumed that stimulus-driven GBR changes will create the same metabolic demand as changes in gamma synchronization related to top–down processes (Fries et al., 1997).

Data acquisition

EEG

EEG was recorded from 32 channels placed according to the 10–20 system (BrainAmp; Brain Products; Easycap; Falk Minow Services). Electrode impedances were kept at <5 kΩ. FCz served as a reference. Data were filtered between 0.01 and 250 Hz and sampled at 1000 Hz. Horizontal and vertical electro-oculograms (EOGs) were registered with four additional electrodes.

Optical topography

Changes in cortical hemoglobin oxygenation were measured concurrently with EEG over the parieto-occipital region with an optical topography system (ISS, Imaget; 10 Hz sampling frequency). Because light in the near-infrared range penetrates biological tissue rather well, optical spectroscopy of the cerebral cortex is feasible when used on the adult human head (Obrig and Villringer, 2003). The methodology renders concentration changes in oxygenated (HbO) and deoxygenated hemoglobin (HbR), which can be quantified under the assumption of specific and constant optical background properties. The optical topograph used here emits light at 760 and 830 nm, which is guided to the head by fiber optic bundles (diameter, 0.4 mm). The reflected light is collected by another set of optic probes (diameter, 3 mm) at a distance of 2.5 cm and amplified by photon multipliers. The system uses four sources and eight detectors arranged in a rectangular array, thus supplying a rough topographical image from 14 measuring volumes over the parieto-occipital cortex. The optical probes were mounted into electrode rings, covering the coregistered EEG electrodes P1/2, Pz, PO3/4 PO7/8, O1/2, and Oz (Fig. 1C).
Data analysis

Effect of the parametrically varied Michelson contrast

EEG. Off-line analysis of the EEG and near-infrared spectroscopy (NIRS) data was performed with Matlab. EEG data were bandpass filtered (0.3–120 Hz) and downsampled to 250 Hz. Data were segmented for additional analysis. For each contrast trial, the segment started 1 s before and ended 8 s after each contrast change. Baseline trials were segmented into four epochs of 4 s starting 1 s after the presentation of the gray screen. Data epochs contaminated by eye movements, muscle activity, or line artifacts were discarded using semiautomatic artifact rejection routines. All epochs were visually reinspected for artifacts. Time–frequency representations (TFRs) were calculated using wavelet analysis (Morlet; nine cycles; frequency range, 4–100 Hz; resolution, 1 Hz) to estimate the spectral activity for baseline and each contrast condition. The procedure provides a continuous measure of the amplitude of frequency components (Tallon-Baudry and Bertrand, 1999). Wavelet analysis was calculated for each trial individually. For baseline and each contrast condition, the resulting TFRs were averaged across trials. TFRs were expressed as changes relative to the baseline (Henrie and Shapley, 2005; Hoogenboom et al., 2006) (i.e., for each contrast condition, electrode, and subject, the activity of each frequency bin of the respective TFR was divided by the mean baseline activity of this frequency bin). To obtain a measure for each frequency band, relative changes were averaged in time (from 0.5 to 6.5 s after onset) and frequency (theta, 4–7 Hz; alpha, 8–13 Hz; beta, 15–25 Hz; gamma, 35–70 Hz). These values were pooled for the occipital electrodes (O1 and O2).

Optical topography. Concentration changes of HbO and HbR were calculated using the modified Beer–Lambert law. To attenuate pulse-related changes, data were low-pass filtered (Butterworth; third order; cutoff, 0.3 Hz). Channels were visually inspected for artifacts. Brief movement artifacts were corrected by a linear interpolation of the first and the last data points surrounding the artifacts. To obtain the hemodynamic response to each Michelson contrast, a design matrix consisting of five 0–1 boxcar predictors was convoluted with a hemodynamic response function with a peak at 5 s (Boynton et al., 1996). This model and the data were fed into a general linear model (GLM). To improve the model, the predictor matrix of the GLM comprised an additional cosine filter matrix to deal with drifts and slow oscillatory changes (low cutoff at 0.033 Hz). Because the experimental design included a baseline trial only after every fifth trial, and because of the sluggishness of the hemodynamic response, the responses to individual trials partly overlapped. As long as the interstimulus interval does not fall below several seconds, the hemodynamic responses to temporally adjacent events can be described as a linear time-invariant system (Boynton et al., 1996). To extract the time courses of the response to each contrast (see Fig. 4), a pseudodeconvolution technique was applied (Koch et al., 2006): for each contrast level, the GLM-derived β value of the respective condition was set to zero while all other β values remained unchanged. Afterward, the β values were multiplied with the predictor matrix and subtracted from the real data. This analytical step results in a time course with the length of the experimental session that approximates the effect of the respective contrast alone, minimizing the hemodynamic effects of all other contrasts. To obtain time courses for HbO and HbR, the hemodynamic response from 0 to 20 s after stimulus onset was averaged for each contrast and channel (see Fig. 4CD). Baseline correction was applied before averaging using the first 0.5 s after stimulus onset as a reference. NIRS data of the two occipital channels corresponding to the EEG electrodes O1 and O2 were pooled. Repeated-measure ANOVAs were performed for EEG bands and hemodynamic responses separately. Post hoc t tests (Bonferroni corrected) were applied if an ANOVA revealed a significant effect.

Internal state-dependent effect

EEG. Single-trial analysis was performed for the highest contrast (c5, 91% Michelson contrast). EEG data of c5 were segmented from 0.8 s before to 0.8 s after the onset of each velocity change. Of the resulting 210 trials, only trials with a behavioral response within 250–700 ms were considered as valid trials. TFRs were calculated using the same approach as for the contrast effect and expressed as relative change compared with baseline. For each subject, TFRs were sorted according to the response time and grouped with equal size (one-third of the trials) into fast, medium, and slow response classes. For each response group, TFRs were averaged in the time window 200–100 ms before the onset of the velocity change and across the frequency dimension corresponding to gamma band activity (35–70 Hz). These values were pooled for the occipital electrodes (O1 and O2).

Optical topography. The analysis of the hemodynamic data followed the steps described above except that single-trial time courses for the highest contrast were not averaged. The extraction of single-trial hemodynamic responses for events following in rapid succession is critical especially if the exact individual hemodynamic response function is not known. In this case, the hemodynamic response of an event can be seriously confounded by the influence of adjacent events. Therefore, another approach was chosen: the same hemodynamic response was ascribed to the three respective behavioral responses within one trial of contrast c5. Next, the time courses were sorted according to the behavioral responses. The rationale of the approach is that the hemodynamic responses should be distributed uniformly across the RT dimension if no underlying relationship exists between reaction time and hemodynamic response. Time courses that did not match the criteria for a correct response (see above) were skipped. Again, NIRS time courses were grouped into fast, medium, and slow RT classes (one-third of the total trial number, equally large in size) and averaged. EEG data were averaged across O1 and O2. In correspondence to EEG, the two channels overlaying O1 and O2 were averaged. Differences between fast and slow responses were tested with paired t tests.

Results

Externally driven response variance by stimulus contrast

Subjects were instructed to concentrate on a concentrically moving grating of different contrast levels and press a button after a small velocity change of the grating. As expected, across subjects, mean reaction time and error rate decreased with increasing contrast (Fig. 2A, B). With respect to the EEG frequency bands, we found that occipital gamma power (pooled electrodes O1 and O2) parametrically increased with stimulus contrast (Fig. 2F). None of the other frequency bands (theta, alpha, beta range) varied systematically with contrast (Fig. 2C–E; supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

One-way repeated measures ANOVAs confirmed the finding of a significant contrast effect exclusively for the gamma band (gamma: F(4,44) = 12.962, p < 0.001; theta: F(4,44) = 1.869, p = 0.133; alpha: F(4,44) = 1.382, p = 0.256; beta: F(4,44) = 1.407, p = 0.248). Post hoc t tests revealed significant differences between contrast conditions for gamma power, and tests of within-subjects contrasts indicate a positive linear relationship (F(1,11) = 18.773; p = 0.001) between contrast level and gamma power (supplemental Table 1, available at www.jneurosci.org as supplemental material). No systematic increase of gamma-band activity with contrast strength was found for the channels monitoring ocular movements (EOG) (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). Gamma activity was tonically enhanced over the entire 7 s stimulation period for each contrast (example single subject data depicted in Fig. 3A). Thus, the measured activity in the gamma range is not attributable to a phasic response to the onset of the contrast change or fluctuations of attention but reflects an externally driven tonic response to the ongoing stimulus.

Coupling between gamma activity and hemodynamic response

As suggested by a report on an invasive approach (Niessing et al., 2005), we next addressed the question whether in humans the hemodynamic response would also be linked to the observed tonic changes in externally driven gamma changes. Data from pooled occipital probes (colocated to electrodes O1 and O2)
Electrophysiological and oxygenation response to contrast changes. Figure 3.

Figure 2. Behavioral and neuronal response to contrast variation across subjects. A, B, Mean reaction time (in milliseconds) (A) and error rate (in percentage) (B) decrease with increasing contrast strength. C–F, Changes of oscillatory power in different frequency bands: theta (4–7 Hz) (C), alpha (8–13 Hz) (D), beta (15–25 Hz) (E), and gamma (35–70 Hz) (F) band. Oscillatory power was averaged from 0.5 to 6.5 s after trial onset from pooled occipital electrodes O1 and O2. The data are depicted as relative power changes for each frequency band with respect to the baseline (value of 1). Values <1 correspond to a decrease in power, whereas values >1 represent an increase in power; error bars represent SEM. Compared with the baseline, alpha and beta activity are desynchronized, whereas theta and gamma activity are synchronized during stimulation. Only gamma band activity shows a systematic increase in power with increasing contrast. The vertical bars denote SEM across subjects.

Figure 3. Electrophysiological and oxygenation response to contrast changes. A, Time–frequency representations averaged over all 7 s segments of each contrast from a single subject (pooled electrodes O1 and O2) showing a response in the upper gamma frequency range (~75 Hz). GBR is strongest for the highest contrast and decreases with lower contrasts. Note that gamma activity is sustained over the full length of stimulation. B, Fluctuations of occipital gamma activity (black; pooled electrodes O1 and O2) and HbR (red; pooled from colocated occipital probes) from a single subject for a period of ~16 min. The background shading (yellow–green) depicts the changes in contrast strength. The close coupling between gamma and HbR changes is clearly seen in the unaveraged data; both parameters follow the contrast changes showing highest values for the largest contrast (yellow shading) and lowest values for the 0% contrast baseline (dark green shading). Note that contrast changes and gamma power are convolved with a HRF to allow for a comparison with the hemodynamic response.

revealed that oxygenation increased with gamma power when the latter is convolved with a hemodynamic response function (HRF). As an example, Figure 3B depicts the fluctuation of gamma power and oxygenation changes in a single subject over the duration of ~16 min. It can be seen that electrophysiological activity in the gamma range and the hemodynamic response are both driven by stimulus contrast. The grand average across all subjects from occipital probes (colocated to electrodes O1 and O2) confirmed the finding, yielding a steady increase in the magnitude of the oxygenation response with contrast (Fig. 4A for the increase in HBO; Fig. 4B for HbR; note a decrease in HbR signals an increase in oxygenation) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). One-way repeated-measures ANOVAs revealed a significant effect of contrast modulation for HBO ($F_{(4,44)} = 6.268; p < 0.001$) and HbR ($F_{(4,44)} = 11.942; p < 0.001$) (for post hoc t tests of HbR, see supplemental Table 1, available at www.jneurosci.org as supplemental material). Tests of within-subjects contrasts indicated significant linear trends for HBO ($F_{(4,44)} = 6.268; p < 0.001$) and HbR ($F_{(1,11)} = 8.917; p = 0.012$). Furthermore, the averaged time courses of HbR and HBO represent a typical hemodynamic response and validate the gradual effect of the contrast strength (Fig. 4C,D).

To test whether electrophysiological and vascular responses were driven by stimulus contrast, we additionally analyzed the correlation between the five Michelson contrasts and the dependent neuronal and hemodynamic signals. As hypothesized, occipital gamma power and HbR correlated well with Michelson contrast (Fig. 5A). The best prediction for this correlation was obtained when applying a logarithmic model (gamma: $F_{(1,3)} = 46.6125$, $R^2 = 0.940$, $p = 0.006$; with model coefficients $b_0 = -0.72$ and $b_1 = 0.397$; HbR: $F_{(1,3)} = 67.666$, $R^2 = 0.958$, $p = 0.004$; with model coefficients $b_0 = -0.462$ and $b_1 = 0.336$). Thus, gamma activity and HbR logarithmically increased with Michelson contrast, whereas the relationship between gamma activity and hemodynamic response is best explained by a linear model ($F_{(1,3)} = 22.788$, $R^2 = 0.884$, $p = 0.017$; with model coefficients $b_0 = 0.001$ and $b_1 = -0.002$).

In sum, as opposed to the lower EEG frequency bands, occipital gamma band activity varies parametrically with stimulus contrast. Furthermore, this systematic variation in gamma power is tightly cou-
plied to the collocated hemodynamic response. We find a logarithmic increase in both parameters with Michelson contrast, resulting in a linear correlation between neuronal and vascular response. Additionally, we analyzed the correlation between the transient response [i.e., the visual evoked potential (VEP) elicited by the onset of each contrast]. The P100 component of the grand average VEP (pooled electrodes O1 and O2) also increased with stimulus contrast and revealed a strong correlation with both the GBR and the vascular response (supplemental Fig. 3, available at www.jneurosci.org as supplemental material).

Internal state-dependent response variance

So far, we have shown that gamma activity and vascular responses are coupled when they are driven by external parameters (i.e., the salience of the stimulus). However, invasive experiments in the monkey have shown that gamma power is also strongly modulated by attention (Womelsdorf et al., 2006). Hence, we next analyzed neuronal response variability originating from internal state-dependent parameters. To this end, all behavioral responses to the velocity change were grouped with respect to reaction time (mean RTs: fast, 395.9 ms; medium, 492.4 ms; slow, 611.1 ms). This analysis was performed for the highest Michelson contrast (c5; 91%) to minimize confound by error rate. The approach rests on the fact that behavioral response variability stems not only from differences in stimulus and task properties, but also depends on the instantaneous, internal state of the brain, which has been shown to fluctuate substantially (Arieli et al., 1996). Indeed, when the contrast was kept constant, occipital gamma power (pooled O1 and O2 electrodes) before the velocity change differed substantially for fast, medium, and slow reaction times across subjects (Fig. 6A). Both occipital time–frequency representations across the full frequency range (top panel) and the time course of averaged gamma power (35–70 Hz; bottom panel) demonstrated that mean reaction time is inversely related to the grand average gamma power before the velocity change. When collapsed over a time window from 200 to 100 ms before the onset of the velocity change (Fig. 6B), fast reaction times were preceded by stronger gamma activity compared with medium and slow RTs. Paired t tests confirmed significant differences in gamma-band activity between the fast and slow RT trials (t(11) = 2.871; p = 0.015). In comparison, for the same temporal window using the same band windows as defined for the analysis of contrast strength, the lower frequency bands revealed no significant power differences between fast and slow RT trials at the occipital region of interest (ROI) (theta: t(11) = 0.409, p = 0.691; alpha: t(11) = 0.204, p = 0.842; beta: t(11) = 1.736, p = 0.11) (supplemental Fig. 4, available at www.jneurosci.org as supplemental material).

The differences in gamma power were closely linked to the hemodynamic response (pooled from occipital probes colocated to O1 and O2 electrodes) (Fig. 6C). Larger oxygenation changes were found for fast RT compared with slow RT (Fig. 6D, E) (HbO, t(11) = 2.712, p = 0.02; HbR, t(11) = −2.935, p = 0.01). The topography of gamma-band changes shows that the electrophysiological response extends pari- etally beyond the area sampled by the optical imaging array (Fig. 6F–H). In analogy to the VEP elicited by the contrast change (supplemental Fig. 3, available at www.jneurosci.org as supplemental material), we averaged the evoked response to velocity changes. The most
pronounced component of the evoked potential peaking ~250 ms after the velocity change did not systematically vary with reaction time (data not shown).

In sum, the results supply evidence in the human that spontaneous fluctuations in gamma power before the behaviorally relevant stimulus change partly predict reaction time. Moreover, the spontaneous variations in gamma power correlate with the vascular response to the stimulus. It seems noteworthy that the finding with respect to gamma activity closely matches the results from the invasive study on speed of change detection in monkeys [compare Fig. 6A with Womelsdorf et al. (2006), their Fig. 2].

Discussion
Synchronization of neuronal signaling in the gamma frequency range is a putative neurophysiological mechanism to process stimuli and efficiently integrate a multitude of features (Engel et al., 2001). Therefore, gamma synchronization is suggested to play a key role in the human brain’s exceptional capacity to generate and integrate percepts in rapid succession, which is mandatory for most higher-order cognitive function. In the human, we here supply evidence to support two important hypotheses highlighting the central role of gamma-band oscillations for the imaging of cognitive function noninvasively. (1) Gamma band response is augmented with parametric increases in stimulus contrast in the human visual cortex (Siegel et al., 2007). Apart from this “externally” driven modulation, we show that gamma activity before a task-relevant cue (velocity change) determines reaction time, which illustrates internal state dependence of GBR. Thus, GBR can be considered as a hinge between external and internal determinants of the variability in response velocity. (2) Evidence is supplied that GBR is closely related to the vascular response in the human brain. Both GBR attributable to changes in stimulus salience and state-dependent GBR predicting reaction time within a given stimulus contrast are reflected by concomitant hemodynamic changes. Linking GBR to behavior and the vascular response is highly relevant to the neuroimaging of human cognition.

Stimulus-induced GBR modulation
With respect to the externally modulated GBR, we find a logarithmic relationship between Michelson contrast and gamma activity. Notably, GBR was sustained over the full duration (7 s) of the trial (Fig. 3A), extending the results of a recent magnetoencephalography (MEG) study (Hoogenboom et al., 2006) using a single contrast over 3 s. The current findings cannot be explained merely by top–down modulation. This is because lower contrasts cause relatively small GBR but necessitate more attentional resources, as indexed by slower reaction time; whereas stronger contrasts cause larger GBR and faster responses. Hence the positive relationship between contrast and GBR indicates a stimulus-driven signal. Comparing human MEG data to monkey data (Logothetis et al., 2001), a previous study reports a linear, as opposed to the logarithmic increase in GBR with contrast that we found (Hall et al., 2005). The difference between the human data reported by Hall et al. and our data may relate to the difference in stimulus presentation (stationary vs moving) and the choice of contrast levels. For the evoked potential on the changes in contrast, which selectively samples the transient response, we also find a logarithmic increase with contrast in the present study (supplemental Fig. 3, available at www.jneurosci.org as suppl-
mental material). In line with our finding, a recent study on transient gamma response shows a nonlinear but steady increase across contrasts in a forced-choice discrimination task (Schadow et al., 2007), whereas in cats, a linear dependence was observed at low and medium contrasts, which became logarithmic with stronger contrast (Tolhurst et al., 1981).

**Relationship between contrast and other frequency bands**

Beyond GBR, theta activity also increased during stimulation, whereas alpha and beta power were attenuated. The response in lower bands did not reveal a systematic variation with contrast. This finding is consistent with a number of invasive studies supporting the view that gamma activity rather than lower frequency changes reflect stimulus configurations and task sets (Frien and Eckhorn, 2000; Frien et al., 2000), in particular for visual contrast modulation as shown in the monkey (Henrie and Shapley, 2005). In humans, evidence for sustained GBR in response to parametrically varied stimulus strengths is scarce. Our data suggest that sustained GBR reflects processing and encoding of task relevant information, whereas lower frequency bands are probably less stimulus related.

**How is the GBR reflected in the vascular response?**

Theoretically, the augmentation of a specific frequency band may result from either the recruitment of a larger network or phase-locking of preexisting oscillators. Oscillatory activity at higher frequencies involves enhanced activity of GABAergic interneurons (Traub et al., 1996; Hasenstaub et al., 2005) and is thus likely to cause an enhanced metabolic demand (Niessing et al., 2005). In the cat’s visual cortex, synchronization rather than neuronal recruitment is altered on midbrain stimulation (Munk et al., 1996). Also, strabismic cats show no difference in cellular discharge on binocular rivalry stimulation, whereas synchrony in the gamma range was exclusive for the perceived, as opposed to the suppressed, monocular stimulus (Fries et al., 1997, 2002). Our results are compatible with the results in cats (Niessing et al., 2005) that a GBR increase is reflected by a hemodynamic increase, following the assumption of enhanced inhibitory activity without reduction of a global excitatory drive. In line with the prediction in animals using BOLD contrast (Logothetis et al., 2001) or optical imaging (Niessing et al., 2005), we find support in humans for a tight relationship between GBR and its hemodynamic counterpart. Recent approaches in humans using complex stimuli relied on a comparison between invasive recordings in neurosurgical patients and the BOLD response in healthy volunteers (Mukamel et al., 2005; Nir et al., 2007). Although limited by interindividual comparison, these approaches also demonstrate a close coupling between both parameters.

**State-dependent GBR changes**

GBR also varied within the same contrast, which presumably reflects endogenous fluctuations (e.g., attentional modulation or “the preparedness to respond”) (Engel et al., 2001; Schoffelen et al., 2005). We find that fast responses to the velocity change are heralded by enhanced gamma activity before the cue. In the human, this result confirms LFP recordings from area V4 in monkeys showing that GBR predicts reaction time. In a focused attention task, reaction time covaried with GBR several hundred milliseconds before the task-relevant cue (Womelsdorf et al., 2006). Our results show a remarkably similar dynamic and spectral range of the GBR [compare Fig. 6A with Womelsdorf et al. (2006), their Figs. 2, 4C]. Moreover, GBR fluctuations before cue onset elicit a graduated vascular response supplying direct evidence that internal state-dependent GBR is detectable by vascular-based imaging techniques. This is noteworthy since synchronization driven by some distant network might not enhance the local metabolic demand. With regard to the optical imaging study in cats, we suggest that tightly coupled stimulus-independent GBR and hemodynamic response variability (Niessing et al., 2005) would correlate to internal state variables when tested. In animals and humans, the predictive value of gamma augmentation for behavior has been addressed in visuomotor and other tasks (Gonzalez Andino et al., 2005; Womelsdorf et al., 2006). An advantage of our approach is that stimulus salience (contrast) and task relevant cue (velocity) are independently modulated, allowing testing of both determinants for GBR variations within the same experiment. A recent study in monkeys investigated the relationship between LFP and multiunit activity in visual MT area and demonstrated that LFP is linked to “choice probability” in a speed detection task. This correlation only holds for higher frequencies (>40 Hz), whereas for lower frequencies an anticorrelation is found (Liu and Newsome, 2006). Although on a much coarser scale, we observe a similar predictive value of GBR in the human visual cortex and supply evidence for a close relationship between local neuronal activity and the vascular response. In an elegant design, it has been recently shown that nonattentional internal modulation, namely decision bias and awareness, are reflected by prestimulus gamma synchronization (Wyart and Tallon-Baudry, 2009). The temporal window of gamma-band activity (300–100 ms before the stimulus), which is shown to correlate with decision bias, is similar to the time window (200–100 ms before the velocity change) in which we find the state-dependent predictive gamma modulation for the behavioral response. Concerning the hemodynamic based imaging, another recent study (Hesselsmann et al., 2008) demonstrated that prestimulus BOLD changes in the fusiform face area correlate with the bias to perceive faces as opposed to a vase in a classical Rubin figure. The predictive value of prestimulus gamma synchronization and oxygenation changes in the present study may therefore encompass even more specific aspects of the behavioral response. Additional studies in combined neurovascular approaches are needed to clarify whether the here-proposed tight coupling between GBR and vascular response also holds true for differential ingredients of internal modulations.

**Cerebral origin of gamma-band response**

Our study supplies evidence for the coupling between GBR and vascular response. The recent heated discussion (Fries et al., 2008; Yuval-Greenberg et al., 2008; Melloni et al., 2009) as to the cerebral origin of GBR in noninvasive recordings is not a focus of the paper. Nonetheless, the cerebral origin of GBR changes is a prerequisite for the inference drawn from the results. The registered ocular electrodes revealed no systematic changes with contrast variation (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). Although we did not record miniature saccades by an eye-tracking system, we regard the measured gamma activity as a cerebral signal and do not consider them liable to stem from event-related microsaccades as has been shown for transient bursts of “induced” gamma (Yuval-Greenberg et al., 2008) since (1) GBR is sustained for the entire stimulation period (7 s) as opposed to the transient GBR response reported by a number of studies. Interestingly, in a pilot study, we found that GBR increase is stable over stimulation periods of up to 70 s (data not shown). (2) GBR increases parametrically with contrast strength at parieto-occipital electrodes, and (3) GBR is closely coupled to the regional hemodynamic response, which is
completely unsusceptible to ocular movement. Beyond that, our state-dependent GBR results are strikingly similar to results in monkeys (Womelsdorf et al., 2006) and confirm comparable contrast-dependent LFP modulations in the gamma range in other invasive studies (Henrie and Shapley, 2005).

Conclusion
Oscillations in the gamma range must be regarded as a multifunctional encoding mechanism forming the basis for visual feature integration (Gray and McCormick, 1996) and object recognition (Tallon-Baudry and Bertrand, 1999; Mima et al., 2001). Therein, higher cognitive factors such as attention (Gruber et al., 1999; Fries et al., 2001; Taylor et al., 2005) and anticipation (Lee, 2003; Fitzgibbon et al., 2004) have modulatory influences on the same encoding mechanism. Our data show that both exogenously and endogenously triggered fluctuations in the gamma range are mirrored in a vascular response and are hence accessible to vascular-based functional imaging.

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